Review

Lymphatic lipid transport: sewer or subway?

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The lymphatics began receiving attention in the scientific community as early as 1622, when Gasparo Aselli noted the appearance of milky-white vessels in the mesentery of a well-fed dog. Since this time, the lymphatic system has been historically regarded as the sewer of the vasculature, passively draining fluid and proteins from the interstitial spaces (along with lipid from the gut) into the blood. Recent reports, however, suggest that the lymphatic role in lipid transport is an active and intricate process, and that when lymphatic function is compromised, there are systemic consequences to lipid metabolism and transport. This review highlights these recent findings, and suggests future directions for understanding the interplay between lymphatic and lipid biology in health and disease.

Lymphatic Function

The lymphatic system (Figure 1) is found in most tissues in the body, and plays important roles in maintaining fluid balance [1], immune cell trafficking from the periphery to lymph nodes [2], and lipid transport from the intestine to the circulation [3]. The lymphatic vasculature comprises unique functional features that enable entry and transport of large proteins, immune cells, lipids and fluid against a pressure gradient (Figure 2). Specifically, the entry point of the lymphatic system is regulated by initial lymphatics (blind-ended microvessels lacking smooth muscle), which have specialized junctions that prevent backflow of fluid into the tissue after it has entered the vessel [4,5]. The initial lymphatics merge into larger collecting vessels composed of individually contracting units known as lymphangions. Each lymphangion is lined with a functionally unique form of smooth muscle that provides vessel tone and allows the vessel to contract down to as little as 20% of its resting diameter [6,7]. These contractions, when combined with the valve leaflets that separate each lymphangion [8], promote unidirectional propagation of flow [9]. This review specifically focuses on the role of the lymphatic vasculature in lipid metabolism and trafficking, highlighting recent work that suggests a connection between lymphatic dysfunction and lipid-related diseases such as obesity and hyperlipidemia.

Lymphatic development in the intestine

Significant strides have been made in our knowledge of lymphangiogenesis in both development and disease, and I refer the reader to a recent comprehensive review on the subject for details outside the development of the intestinal lymphatics [10].

Lymphangiogenesis initiates after the formation of the vasculature, with the endothelial cells of primitive lymphatics being of venous origin [11]. Vascular endothelial growth factor (VEGF)-C has been implicated as a primary growth factor involved in promoting lymphangiogenesis [12] and interstitial flow is an important biophysical phenomenon utilized to guide lymphangiogenesis by inducing a gradient of VEGF-C that directs vessel growth [13,14]. Recent work has outlined the development of the lymphatics in the intestine and indicated that these vessels have a unique set of molecular regulators involved in their development. Lymphatic vessels in the villi form around embryonic day (E)17.5 in the mouse, shortly after the blood vessels form, and are functionally ready to absorb lipids from milk at birth. Lacteals are not of mesodermal origin, unlike blood vessels, but rather form from the extension and branching of previously formed mesenteric lymphatics [15]. Norrmen and colleagues recently reported a comparative analysis of human intestinal and dermal lymphatics [16]. Through microarray analysis, they identified numerous genes that were differentially expressed between the two cell lines, with one gene in particular, liprin β1, being strongly expressed in intestinal lymphatics. Knockdown of this gene in a tadpole model resulted in dysfunctional lymphatics and development of edema. If intestinal lymphatics have unique or enhanced mechanisms for absorbing lipids, then similar studies should provide insight into the genetic regulation of these differences.

Blood and lymphatic vessels separate from one another in the embryo, and this separation is something that must be regulated beyond birth. This finding was recently supported through studies in fasting-induced adipose factor (*Fiaf*) knockout mice. *Fiaf* expression in the intestine rises immediately after birth and peaks at postnatal day 2. It appears that *Fiaf* is essential for maintaining lymphatic–venous separation, as *Fiaf* $^{-/-}$ mice, although exhibiting normal lymphatics at birth, present blood-filled lymphatics at around postnatal days 2–3. *Fiaf*$^{-/-}$ mice also exhibited, in the intestine, a three-fold reduction in prospero homeobox protein 1 (*Prox1*) expression, a homeobox gene expressed in lymphatics and important in the maintenance of lymphatic lineage [17], while maintaining normal *Prox1* expression in other tissues. However, the exact connection between *Prox1* and *Fiaf* remains elusive, as *Prox1*$^{-/-}$ mice

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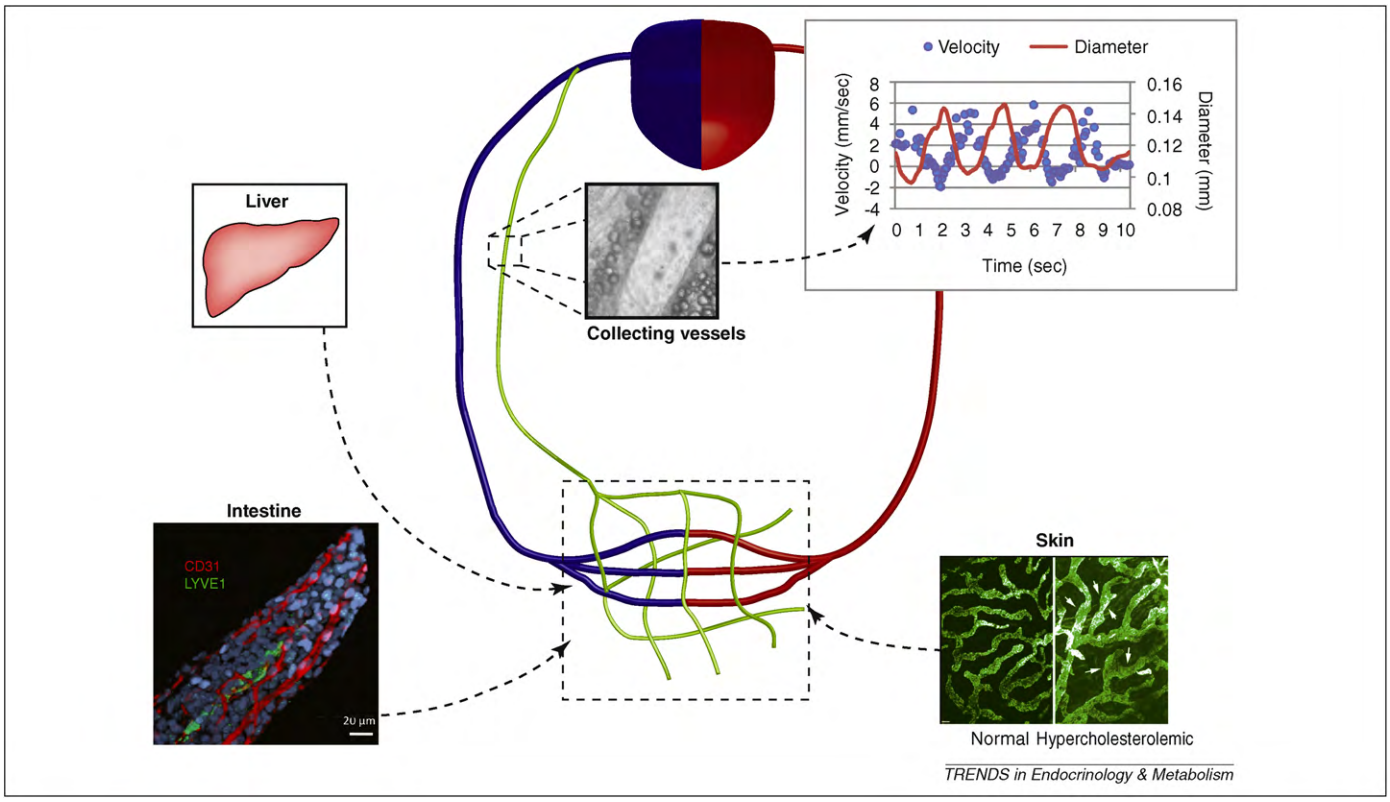

Figure 1. The lymphatic circulation in lipid transport.
The lymphatics pump fluid from the interstitial spaces throughout the body into the circulation by driving fluid velocity through the contraction of collecting lymphatics (graph adapted from [9]). Throughout the body, there are numerous locations in which the lymphatics exist alongside the vasculature and play a crucial role in lipid transport, such as the liver, the skin and the intestine (image adapted from [29]). Adipocytes form around most of the large collecting lymphatics (see image inset of collecting vessel) and skin lymphatics have been shown to remodel and enlarge in response to high levels of circulating LDL cholesterol (image adapted from [50]). Lacteal from a mouse intestine is stained with CD31 (red, blood vessels) and lymphatic vessel endothelial hyaluronan receptor LYVE-1 (green, lymphatic vessel) and 4′,6-diamidino-2-phenylindole (DAPI) (blue). Collecting vessel is a bright field image taken from a rat mesentery.

do not have lymphatics and are thus embryonic lethal [18] and *Prox1*$^{+/-}$ do not have lymphaticovenous connections [19]. *Prox1*$^{+/-}$ mice do have abnormalities in lipid transport, and develop obesity and high circulating levels of leptin and insulin due to leaky lymphatic vasculature, particularly in the mesentery, which results in chyle spilling out into the abdomen and promoting adipogenesis [19]. Similar in phenotype to *Fiaf*$^{-/-}$ mice, mice lacking a gene important for synthesis of core 1-derived O-glycans in endothelial and hematopoietic cells (*EHC T-syn*$^{-/-}$ mice) also develop blood-filled lymphatic vessels [20]. However, whereas *Fiaf* $^{-/-}$ mice do not exhibit their phenotype until after birth, *EHC T-syn*$^{-/-}$ mice show blood-filled lymphatics after E14.5 and have a 48% prenatal mortality rate. *EHC T-syn*$^{-/-}$ mice also have normal *Prox1* expression, but decreased expression of podoplanin, an O-glycoprotein that labels lymphatics and is important in lymphatic vessel development [21]. Using an inducible *T-syn*$^{-/-}$ mouse model, Fu *et al*. showed that endothelial O-glycans are required for the maintenance of blood and lymphatic partitioning, as these mice developed misconnections between blood and lymphatic vessels 6 months after gene deletion [20]. Interestingly, these mice developed fatty liver disease as chylomicrons (triglyceride-carrying particles) are allowed direct entry into the portal vein instead of being transported to the blood by the lymphatic system. Thus, although it has been known for some time that lymphatics are the primary transporter of lipid from the absorbing intestine to the blood, it is now clear that this function is essential for early survival, must be properly maintained throughout adulthood, and cannot be compensated for by portal blood absorption.

In addition to proper lumen formation, lymphatics must also develop functioning valves to prevent backflow. In a recent mouse model, it was shown that by deleting the gene that encodes for integrin α9 (*Itga9*$^{-/-}$), the development of lymphatic valves could be disrupted, allowing chyle to leak out from the vessel into the mesentery [8]. Given that all of the mouse models mentioned here (*Fiaf*$^{-/-}$, *Prox1*$^{+/-}$, *T-syn*$^{-/-}$ and *Itga9*$^{-/-}$) have distinct molecular mechanisms underlying their phenotypes, the presentation of these phenotypes as abnormalities in lipid transport emphasizes the importance of the lymphatic vasculature in lipid homeostasis and our current lack of knowledge as to how the lymphatic system is developed and regulated to absorb and transport lipids.

Stage1: lipid uptake into lacteals

Nearly all dietary lipid is absorbed by the enterocytes of the small intestine, packaged in chylomicrons, and transported from the intestine to the bloodstream via the lymphatic system [3]. In general, lymphatic transport of chylomicrons can be regarded as a two-stage process: (i) entry into the initial lymphatic vessel of the small intestine, known as a lacteal (Figure 3) and movement through the initial vessels via the intrinsic motion of

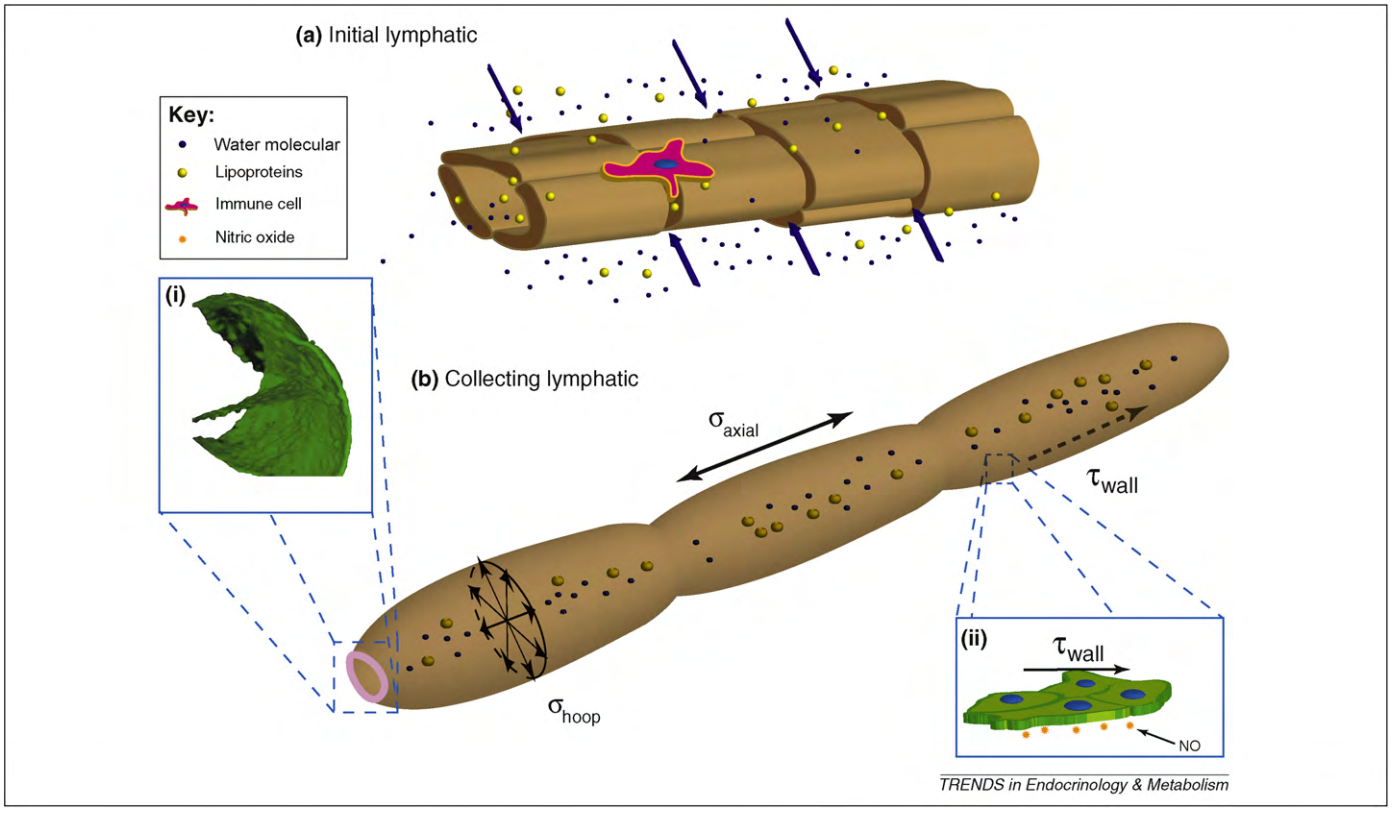

Figure 2. Morphology of initial and collecting lymphatics.
(a) Initial lymphatics are made of endothelial cells with specialized overlapping junctions that allow for easy entry of fluid, proteins and cells into the vessel. These lymphatics lack smooth muscle and therefore cannot contract. **(b)** Collecting lymphatics consist of individual contracting units known as lymphangions, which are lined with smooth muscle and separated by valves. **(i)** Confocal reconstruction of an isolated rat lymphatic vessel showing valve leaflets (courtesy of Dave Zawieja and Anitoliy Gashev). The collecting vessels are under a variety of mechanical loads: hoop stress (σ_{hoop}), axial stress (σ_{axial}) and wall shear stress (τ_{wall}). These forces have been shown to modulate contractile function. For example, an increase in **(ii)** wall shear stress through enhanced fluid flow has been shown to cause upregulation of eNOS and subsequent release of NO, which acts as a vasodilator on the smooth muscle and inhibits vessel contraction [38] Blue spheres are water molecules, yellow spheres are lipoproteins, pink are immune cells, orange stars are NO molecules.

intestinal peristalsis [22], and (ii) the subsequent movement of this lipid through the rest of the lymphatic system, driven by the contractile activity of the larger collecting lymphatics [9]. Recent elegant work illustrated the unique button-like molecular expression pattern of vascular endothelial (VE)-cadherin (one of the primary junctional molecules of endothelial cells) in the initial lymphatics in a mouse trachea, but it is unknown whether this particular pattern of expression exists in the lacteal [5]. In fact, most of our knowledge on the structure–function relationship of the lacteal is based upon early work in transmission electron microscopy (TEM) [23–27], and the conclusions of this work remain controversial. It is widely accepted that the ease of access by large molecules to the lymphatic vasculature is primarily a result of the specialized junctions of the initial lymphatics, which allow large particles to enter but not leave the lymphatic [4,28], and it seems likely that such a mechanism should also exist in the lacteal to allow for a rapid influx of chylomicrons, which can be up to 1000 nm in diameter [24]. Early TEM work also suggested this, as chylomicrons could occasionally be seen in between the junctions of endothelial cells in fixed sections from rat lacteals [23,24], and anchoring filaments similar to those observed in other initial lymphatics were noted [22]. However, Dobbins [25] later demonstrated in guinea pig and rat models that the majority of junctions on the lacteal remain tightly closed (< 1 nm) in animals fed lipid and in controls given saline. Dobbins found numerous lipid vesicles inside the endothelial cells (noted earlier by Casley-Smith [24]) and argued through these two pieces of evidence that vesicular transport through the endothelial cell was the primary route of chylomicron entry into the lacteal [25,26]. Others have confirmed Dobbins' findings both *in vivo* [27] and *in vitro* [29], yet the relative importance of these two mechanisms in chylomicron uptake into lymphatics in both health and disease remains controversial.

Interestingly, Van Dyck *et al*. reported on a transcription factor that reduced lacteal uptake of chylomicrons [30]. Pleomorphic adenoma gene-like 2 (*PlagL2*) knockout mice die of postnatal starvation as a result of poor fat absorption even though these mice have viable enterocytes that assemble and secrete chylomicrons. Upon histological examination, fat accumulation was seen primarily in the interstitium of the intestinal villi. Interstitial chylomicrons in controls were sparse, suggesting that lacteal uptake from the interstitium was impaired in *PlagL2*$^{-/-}$ mice. Because *PlagL2* expression in the small intestine appeared to be limited to enterocytes, the authors concluded that *PlagL2* must be important in some unknown modification of chylomicrons necessary for their uptake into lacteals. This observation would suggest that lymphatic uptake in the gut is an active process, as opposed to the notion of passive draining of any particulate in the interstitium that is too large to enter blood capillaries.

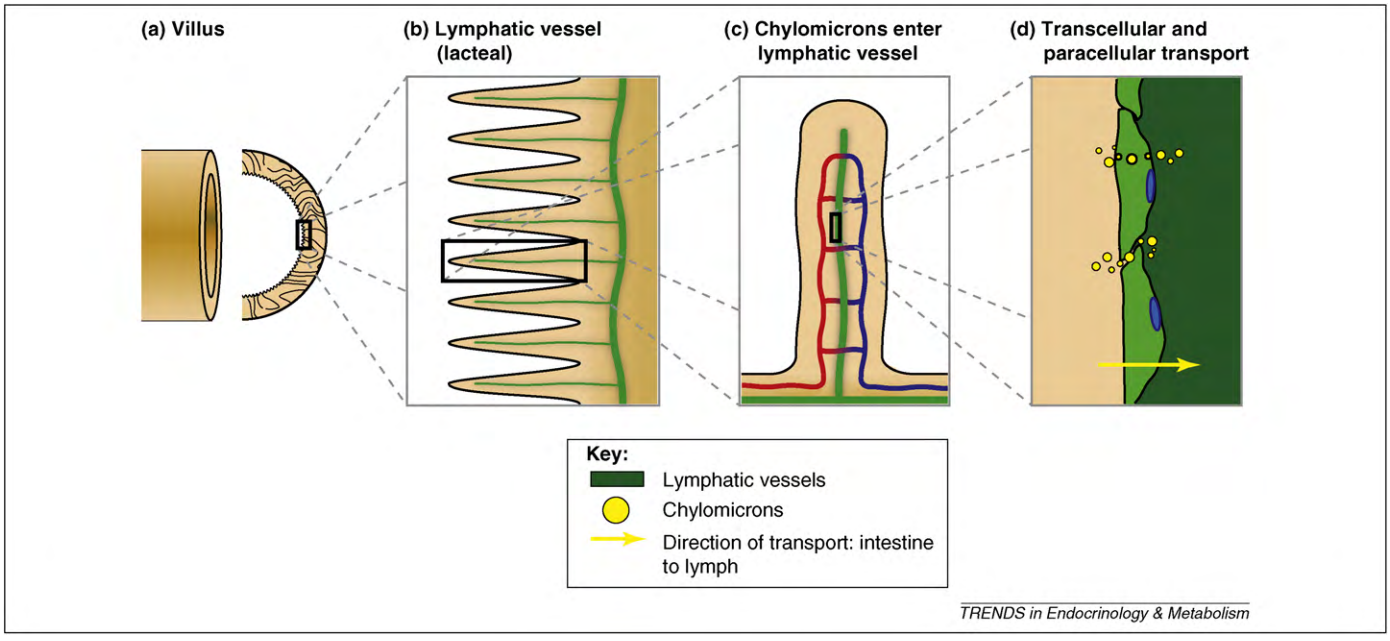

Figure 3. Mechanisms of chylomicron uptake into lacteals.
(a) Each villus of the small intestine has **(b)** a single lymphatic vessel (lacteal) running along the center of the villus. **(c)** Chylomicrons that are secreted by the intestinal epithelial cells (enterocytes) enter into the lymphatic vessel, not the blood. **(d)** TEM images have demonstrated both transcellular transport of chylomicrons through the endothelial cells in vesicles and paracellular transport between cell junctions, yet the relative importance of these two mechanisms remains unclear.

Other authors have shown morphological alterations in the structure of the lacteal in response to fasting and refeeding, again suggesting an active role of the lacteal in responding to lipid load [31]. However, specific molecular mechanisms regulating lymphatic function with regard to the uptake of chylomicrons into lacteals remain to be identified. One of the difficulties behind our current deficiencies in understanding lymphatic function in intestinal absorption is the lack of appropriate tools for separating out the different roles of lymphatic and enterocyte biology. However, several new tools have recently been developed that are allowing researchers to probe specific lymphatic functions *in vivo* and in isolated vessels (Box 1). These tools, when combined with the conventional lymph fistula technique [32], should provide new insight into lacteal function.

Stage 2: lymphatic pump function

After entry into the initial lymphatics, lymph must be transported against a pressure gradient, primarily through contractile lymphatics, by means of the periodic contraction of the lymphatic smooth muscle. Recent advances in isolated vessel preparation techniques [33] and *in vivo* imaging [34–36] are proving to be invaluable tools for understanding the molecular and physical cues that control lymphatic pump function as the vessel returns fluid, cells and particulate to the bloodstream. Given that lymph flow rate increases after lipid absorption [37], tissue hydration enhances lipid absorption in the gut [32] and lymphatic pump function is very sensitive to changes in mechanical load (Figure 2, Box 2), it is likely that changes in these loads on the lymphatic system of the gut are an important regulator in lipid transport.

In the presence of high flow rates under the same transmural pressure (i.e. same stretch but different shear stress), lymphatic contraction is inhibited by the upregulation of endothelial nitric oxide synthase (eNOS) and subsequent release of nitric oxide (NO) [38]. This is not surprising as NO is known to be a shear-released vasodilator in the blood vasculature [39,40]. In lymphatics, NO not only alters the

Box 1. Recently developed tools for accessing lymphatic function

(i) A tissue-engineered model of the human lacteal was developed in which polarized, selective transport of lipid along with transcytosis of lipoproteins through lymphatic endothelial cells was demonstrated [29]. With such a model, various transporters on the lymphatics alone can be selectively blocked and chylomicron uptake quantified.
(ii) A perfusion chamber for isolating a loop of small intestine *ex vivo* while permitting analysis of vascular, luminal, interstitial and lymphatic compartments allows researchers to quantify effects of inflammation on lymph transport; for example, in the context of other hemodynamic factors [74].
(iii) High speed intravital microscopy and automated image analysis of mesenteric lymphatic vessels *in situ* makes it possible to quantify the changes in lymphatic pumping after a specific biological stimulus, such as the postprandial release of lipid [9,34,35].
(iv) Photoacoustic lymph flow cytometry was recently developed for counting and categorizing circulating cells in lymph *in vivo* [75]. By using the inherent valve structure of the lymphatic vessel to 'focus' the cells into a channel, the photoacoustic signal of each cell could be measured and used to determine the cell type (i.e. lymphocyte versus melanoma cell).
(v) Noninvasive near-infrared imaging of lymphatic function in humans was recently reported [36]. Such a tool should prove to be an invaluable technique for accessing lymphatic function clinically in patients with lymphedema, with a much greater level of sensitivity than currently available techniques.
(vi) Gene transfection in isolated rat lymphatics was recently demonstrated in which vessels could be kept in culture for 3–12 days using adenovirus conjugated to green fluorescent protein to demonstrate targeted transfection of endothelial cells or smooth muscle cells [33]. Such work will allow quantification of the role of specific mechanism in pumping function by silencing the gene in the cultured lymphatic vessel.

Box 2. Lymphatic biomechanics

The vasculature experiences different mechanical loads in both normal and disease conditions. In general, these loads can be divided into three categories: (i) hoop stress (or circumferential stress), which is a result of the pressure inside of the vessel acting on the vessel wall; (ii) axial stress, which is caused by the axial loading of the vessel (i.e. the parts of the vessel upstream and downstream that pull the vessel longitudinally; and (iii) wall shear stress, a result of the shearing force that the fluid exerts on the vessel wall as it flows across the wall [76]. Various blood vessels have been shown to grow and remodel in response to prolonged changes in load (e.g. in hypertension); however, less is known about the growth and remodeling response in lymphatics as a function of changes in mechanical load. The lymphatics have been shown to be sensitive to temporal changes in mechanical load, as the lymphatic pump exhibits a rate-sensitivity to changes in hoop stress [77] and fluid shear stress [38,42].

tone (resting diameter) of the vessel, but also inhibits the contraction frequency and amplitude of the vessel, which results in a further increase in the time-averaged diameter. This mechanism is thought to regulate whether the vessel should behave as a pump or a conduit, as lymphatic contraction in the presence of an exogenous flow source (e.g. lymph formation) increases resistance to flow [41]. It is interesting to note that when comparing lymphatic vessels isolated from various regions in the rat, mesenteric lymphatics were the least sensitive to this flow inhibition [42]. This is particularly important in light of the fact that the changes in load experienced postprandialy by a mesenteric lymphatic are presumably substantial. However, our knowledge in this area is limited, as we do not have quantitative data on how lymphatic loads vary *in vivo* after a meal. However, NO regulation of flow seems likely, as it has been recently shown in mesenteric lymphatics *in vivo*. Specifically, endothelial cells located at the regions of highest shear stress within the lymphangion (i.e. near the valves) had the greatest eNOS upregulation, suggesting that this could be the primary shear-sensing site for the lymphatic vessel. In addition to this, NO release was shown to oscillate with the temporal changes in wall shear stress that occur during the contraction cycle, indicating that NO might be involved in coordinating individual contraction sequences [43]. Because high-density lipoprotein (HDL) has been shown to influence eNOS expression in blood endothelial cells [44], it is important to consider whether HDL or other lipoproteins could induce eNOS expression in lymphatics and how this fits into the context of the vessel response to changes in mechanical load. This is especially the case in the postprandial function of the lymphatic pump, as the lipoprotein (i.e. chylomicron) concentration changes drastically over a short time.

Lymphatic role in reverse cholesterol transport

Because one of the primary roles of the lymphatics is to provide a route of entry for large proteins to be removed from the interstitium and returned to the blood, it seems likely that extra vascular lipoproteins are returned to blood through the lymph. Lipoprotein concentrations in the lymph have been shown to correlate loosely with that in the blood, with the lymph lipoprotein concentration being approximately one-tenth of the blood lipoprotein concentration [45,46]. Through a careful analysis of the various lipoprotein fractions in human lymph, Nanjee and colleagues showed that the lymph concentration of HDL cholesterol was 30% greater than that of blood [47]. When comparing the size distribution of lipoproteins containing apolipoprotein (Apo)A-1 (a major component of HDL), fractions isolated from the lymph were shifted towards larger particles. With these data, the authors estimated a whole-body reverse cholesterol transport rate of 344 mg/day via the lymph. Therefore, given that lymph flow, and consequently lipoprotein transport, varies in lymphatics with posture and activity level [48], it is important to consider lymphatic transport when modeling lipoprotein kinetics [49]. This is especially important in pathologies in which lymphatic function is compromised. In a model of hypercholesterolemia, lymphatic function was shown to be severely compromised, as the lymphatics had reduced conductance, impaired dendritic cell migration, were hyperplastic, and had diminished smooth muscle coverage [50]. This is interesting because hyperlipidemia is known to cause inflammation [51], and inflammation has been shown to compromise the efficacy of the valve structure of the initial lymphatics that prevents leakage out of the vessel [28].

However, the exact mechanisms connecting lipid pathologies (e.g. hyperlipidemia, hypercholesterolemia, obesity and diabetes) with lymphatic physiology remain unknown. In a recent letter to the editor of *Atherosclerosis*, Horra *et al*. suggested that certain cases of familial hyperlipidemia could be magnified by ineffective drainage of interstitial lipoproteins via the lymphatic system; they showed downregulation of *Prox1* and *FoxC2* gene expression in adipose tissue compared with that in normolipidemic controls matched for body mass index [52]. However, it is unclear if the differences in expression levels are a cause or a consequence of the hyperlipidemia. The microcirculation is known to be intimately involved with the development of adipose tissue [53]. The role of the lymphatic vasculature in the development of adipose tissue remains ambiguous, as two different lymphatic growth factors, VEGF-C and VEGF-D, were not shown to correlate with or to modulate adipose tissue development [54,55]. However, serum concentrations of these two growth factors have been shown to be elevated in obese individuals [56]. Further studies need to be carried out to connect these phenomenological observations with the exact mechanisms of lymphatic-adipose crosstalk. Knowledge of such mechanisms might provide a lymphatic target for treating certain forms of obesity or hyperlipidemia.

Lymphedema and consequences for lipid metabolism

One of the most common forms of lymphatic dysfunction is lymphedema, characterized by the regional accumulation of interstitial fluid as a result of some form of compromised lymphatic drainage (Box 3). Impaired lymphatic drainage in a mouse model of secondary lymphedema resulted in lipid accumulation that persisted even after lymphatic drainage was restored [57]. Such observations have also been observed clinically in patients with secondary lymphedema, and obesity [58–60] and weight gain [58] are correlated with post-mastectomy lymphedema (caused by the surgical removal of lymphatic vessels). Few treatments have been proposed to restore flow and resolve the lym-

Box 3. Lymphedema

There are essentially two major classifications of lymphedema: primary and secondary (acquired). Primary lymphedema has been categorized into three groups, as determined by the age in which symptoms first appear. Congenital lymphedema is noticeable at birth or within the first 2 years of life; lymphedema praecox manifests itself during puberty; and lymphedema tarda appears typically after 35 years of age [61]. Some of the gene mutations that result in various primary lymphedemas have been identified, but there is still much that is unknown about the causes of the dysfunction. Secondary lymphedema occurs after disruption of the lymphatic system by surgery [66] or by lymphatic filariasis, a disease caused by a parasitic worm that invades the lymphatic system [78]. One of the common pathologies of all of these varying forms of lymphedema is that lipid transport appears to be compromised as the interstitial spaces accumulate large adipocyte deposits or chylous ascites.

phedema, with manual lymphatic drainage (MLD) to promote lymph flow being the most common [61,62]. However, the extent to which a patient will respond to MLD is unknown, and there are numerous patients with lymphedema whose condition remains untreatable. As suggested by the mouse model of secondary lymphedema [57], restoring lymphatic function alone might not be enough to reverse the gross remodeling of tissue and lipid accumulation that has occurred. This is supported clinically, as liposuction has proved to be effective in cases for which MLD has been inadequate in resolving lymphedema [63,64].

A recent animal study also suggests lipid deposition as a primary factor driving lymphedema severity, as two different mouse models of primary lymphedema exhibited significantly distinct pathologies [65]. Both mouse models (the *Chy* mouse and the *K14-VEGFR-3-Ig* mouse) lack dermal lymphatics and show signs of lymphedema. However, the *Chy* mouse exhibits collagen and fat deposition in the interstitium, which does not allow the tissue to compensate for its impaired lymphatic drainage with an increase in hydraulic conductivity. By contrast, the *K14* mouse lacks the lipid accumulation in the interstitium and has an increase in tissue hydraulic conductivity, which effectively limits the increase in tissue volume resulting from lymphatic dysfunction. These and similar animal models should help to resolve our current uncertainty of a patient's risk for developing lymphedema after mastectomy, thus allowing doctors to prescribe preventative measures that are dependent on this perceived risk [66].

Consequences of congenital lymphatic diseases on lipid metabolism

Malformations of the intestinal lymphatics clinically present as a variety of clinical pathologies (e.g. protein-losing enteropathy, intestinal lymphangiectasia, chyloperitoneum and chylothorax) [67,68]. Even before symptoms develop, patients have shown delayed transport of lipid from the intestine, suggesting that lymphatic lipid transport function is compromised at an early stage of the disease [67]. In primary intestinal lymphangiectasia, although edema is the main clinical feature (a result of low levels of serum albumin), patients also exhibit chylous reflux in the skin, and the intestinal lymphatics in the mucosa and submucosa appear dilated. The primary treatment for the disease is a low-fat diet with medium-chain triglycerides that can be absorbed directly into the portal vein, thus circumventing the compromised intestinal lymphatics. Very little is currently known about the underlying genetic causes of these diseases; however, the lymphatics do exist and do maintain some function, as the lack of a functional lymphatic system in mammals is embryonic lethal [18]. Both the molecular defects behind the inability of the intestinal lymphatics to transport lipid adequately from the intestine and the ways in which these defects might be reversed in these diseases remain unknown. Research on animal models of lymphatic dysfunction that survive past birth, such as the *Prox1*$^{+/-}$ mouse [19], might provide future insight into the mechanisms behind these diseases.

Lymphatic involvement in other lipid-related pathologies

In addition to the congenital defect already described, there are a few other noteworthy lipid-related pathologies in which targeted restoration or enhancement of lymphatic function might serve as an effective means of treatment. Crohn's disease (CD), a type of autoimmune inflammatory bowel disease, can present as several lymphatic pathologies [69]. Lymphatic contractile activity was shown to be impaired in an isolated vessel model of gut inflammation, suggesting that lymphatic function might be compromised in inflammatory diseases such as CD, which would lead to further edema and inflammation [70]. With recent progress in targeting lymphatics with orally delivered drugs [71], the oral delivery of a vasoactive substance known to promote lymphatic contraction might aid in the resolution of the inflammation by actively clearing the edema and inflammatory cytokines from the gut interstitium.

Lymphatic involvement in diabetes has also received some attention recently as lymph flow through the thoracic duct and transport of dextran from the footpad were greatly enhanced in a mouse model of diabetes. However, uptake of dextran to regional lymph nodes was reduced, suggesting that functional lymphatic transport is compromised [72]. Glucagon-like peptide 1, an intestinal hormone that plays an important role in glucose metabolism, was recently demonstrated to be transported through the lymphatic system from the gut to the blood immediately following a meal [73]. Whether lymphatic dysfunction plays a role in promoting the development of diabetes remains to be seen.

Conclusion

Progress in uncovering the molecular mechanisms involved in lymphatic development and function are rapidly accelerating the field of lymphatic research. With recent research implicating lymphatic involvement in a variety of diseases involving lipid transport and metabolism, the lymphatic vasculature is becoming an important target for understanding the progression of and developing treatments for such diseases. However, our current lack of knowledge of even the most basic mechanisms involved in lymphatic lipid transport emphasizes the need for further research in this area (Box 4). Such future knowledge

Box 4. Future questions and considerations

- The extent to which chylomicrons rely on transcellular versus paracellular mechanisms for their uptake into lacteals and the key molecular mechanisms involved in this process need to be elucidated.
- The developmental cues in intestinal lymphangiogenesis need to be further explored, particularly in the context of the unique capability of the lymphatic system to absorb chylomicrons from the intestine.
- The functional response of lymphatic contractility to the varying postprandial loads (and the extent to which these loads vary) in the mesenteric collecting lymphatics needs to be determined.
- The molecular mechanisms explaining the phenomenological correlation of lipid accumulation and lymphedema risk/severity need to be uncovered.
- The morphological response (i.e. changes in cell junctions, smooth muscle proliferation, vessel diameter) of both initial and collecting lymphatics in disease (i.e. lymphedema, hyperlipidemia) needs to be characterized, with the aim of preventing an unwanted remodeling response that might actually exacerbate rather than improve the disease condition.
- The implications of intestinal bowel disorders such as CD on lymphatic function, and particularly lymphatic lipid transport, need to be further explored.

should lead to more efficacious treatments of diseases such as lymphedema, CD and intestinal lymphangiectasia.

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