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Lymphatic Lipid Transport: Sewer or Subway?

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Abstract

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 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 is comprised of 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 much 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]. In this review we specifically focus on the lymphatic vasculature's role in lipid metabolism and trafficking, highlighting recent work that suggests a connection between lymphatic dysfunction and lipid-related diseases such as obesity and hyperlipidemia.

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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 of 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 C (VEGF-C) has been implicated as a primary growth factor involved in promoting lymphangiogenesis [12] and interstitial flow, by inducing a gradient of VEGF-C, an important biophysical phenomenon utilized to direct lymphangiogenesis [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 17.5 (E17.5) in the mouse, shortly after the blood vessels form, and are functionally ready to absorb lipids from milk at birth. Lacteals are not from mesodermal origin like 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 being highly expressed in intestinal lymphatics: liprin $\beta 1$. Knockdown of this gene in a tadpole model resulted in dysfunctional lymphatics and edema. If intestinal lymphatics have unique or enhanced mechanisms for absorbing lipids, then studies such as this should provide insight into the genetic regulation of these differences.

While blood and lymphatic vessels separate from one another in the embryo, 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, while exhibiting normal lymphatics at birth, present blood-filled lymphatics 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. The exact connection between *Prox1* and *Fiaf* remains elusive though, as *Prox1*^{-/-} mice do not have lymphatics and are thus embryonic lethal [18], and *Prox1*^{+/-} do not have lymphatic-venous connections [19]. *Prox1*^{+/-} mice do have abnormalities in lipid transport and develop obesity and high circulating levels of leptin and insulin due to a 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 mice developed misconnections between blood and lymphatic vessels six months after gene deletion [20]. Interestingly, these mice developed fatty liver disease as a consequence of direct chylomicron entry into the portal vein instead of being transported to the blood by the lymphatic system. Thus while 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 $\alpha 9$ (*Itga9*^{-/-}), one can disrupt the development of lymphatic valves. As a result, chyle leaks 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 underlying molecular mechanisms behind their phenotypes, the manifestation of these phenotypes into abnormalities in lipid transport underscores 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 triglyceride-carrying particles known as chylomicrons, and transported from the intestine to the blood stream via the lymphatic system [3]. In general one can regard lymphatic transport of chylomicrons as a two-stage process: 1) 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 intestinal peristalsis [22], and 2) the subsequent movement of this lipid through the rest of the lymphatic system by the contractile activity of the larger collecting lymphatics [9]. While recent elegant work illustrated the unique molecular expression pattern of vascular endothelial cadherin (VE-Cadherin), one of the primary junctional molecules of endothelial cells, of the initial lymphatics in a mouse trachea, it is unknown whether this button-like 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. Since it is widely accepted that the ease of access by large molecules to the lymphatic vasculature to large proteins is primarily due to the specialized junctions of the initial lymphatics which allow large particles to enter the lymphatic but not leave [4,28], it seems likely that such a mechanism would exist in the lacteal as well to allow for a rapid influx of chylomicrons, which can be up to 1000 nm in diameter [24]. Early TEM work suggested this as well, 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, a few years later Dobbins demonstrated in guinea pig and rat models that the majority of junctions on the lacteal remain tightly closed (less than 1 nm) during lipid feeding and saline controls. Dobbins showed numerous lipid vesicles inside the endothelial cells (noted earlier by Casley-Smith as well [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 reported on a transcription factor that reduced lacteal uptake of chylomicrons [30]. Pleomorphic adenoma gene-like 2 (*Plagl2*) knockout mice die of postnatal starvation due to poor fat absorption even though these mice have viable enterocytes that assembled and secreted 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. Since *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. Others 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 in regards 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], will provide new insight into lacteal function.

Box 1

Recently developed tools for accessing lymphatic function

1. 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 one can selectively block various transporters on the lymphatics alone and quantify chylomicron uptake.
2. 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].
3. 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 stimuli, such as the postprandial release of lipid [9,34,35].
4. 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 vs. melanoma cell).
5. Noninvasive NIR 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.
6. Gene transfection in isolated rat lymphatics was recently demonstrated in which vessels could be kept in culture for 3–12 days using adenoviral/GFP to demonstrate targeted transfection of endothelial cells or smooth muscle cells [33]. Such work will allow one to quantify the role of specific mechanism in pumping function by silencing the gene in the cultured lymphatic vessel.

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 blood stream. Given that lymph flow rate increases after lipid absorption [37], tissue hydration enhances lipid absorption in the gut [32], and lymphatic pump function is highly sensitive to changes in mechanical load (Figure 2 and Box 2), it is likely that changes in these loads on the lymphatic system of the gut are an important regulator in lipid transport.

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: 1) a hoop stress (or circumferential stress) that is a result of the pressure inside of the vessel acting on the vessel wall, 2) axial stress that is due to the axial loading of the vessel (i.e. parts of the vessel upstream and downstream that pull the vessel longitudinally; and 3) wall shear stress due to the shearing force that the fluid exerts on the vessel wall as it flows across it [76]. Various blood vessels have been shown to grow and remodel in response to prolonged changes in load (e.g. 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].

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 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) would actually increase 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 that a mesenteric lymphatic experiences postprandially are presumably quite 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 most 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]. Since HDL has been shown to influence eNOS expression in blood endothelial cells [44], it would be important to consider whether HDL or other lipoproteins could induce eNOS expression in lymphatics, and how this fits into the context of the vessels' 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 period of time.

Lymphatic role in reverse cholesterol transport

Since 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 would be returned to blood through the lymph. Lipoprotein concentrations in the lymph have been shown to loosely correlate with that in the blood, with the lymph concentration being approximately one tenth of the lipoprotein concentration in the blood [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 A-1 (ApoA-I, a major component of HDL), fractions isolated from the lymph were shifted towards larger particles. With this 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 where 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 since hyperlipidemia is known to cause inflammation [51], and inflammation has been shown to compromise the efficacy of the valve structure of the initial lymphatics and preventing leakage out of the vessel [28].

However, 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 suggested that certain cases of familial hyperlipidemia could be magnified by ineffective drainage of interstitial lipoproteins via the lymphatic system by showing a down-regulation of *Prox1* and *FoxC2* gene expression in adipose tissue as compared to BMI-matched, normolipidemic controls [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 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 done connecting these phenomenological observations with 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 to lipid metabolism

One of the most common forms of lymphatic dysfunction is lymphedema, characterized by the regional accumulation of interstitial fluid due to some form of compromised lymphatic drainage (Box 3). Impaired lymphatic drainage in a mouse model of secondary lymphedema results in lipid accumulation that persisted even after lymphatic drainage was restored [57]. Such observations have been observed clinically in secondary lymphedema patients as well, and obesity [58–60] and weight gain [58] have been correlated with post-mastectomy lymphedema (caused by the surgical removal of lymphatic vessels). Very few treatments have been proposed to restore flow and resolve the lymphedema, with manual lymphatic drainage (MLD) to promote lymph flow being the most common [61,62]. However, the extent that a patient will respond to MLD is unknown, and there are numerous lymphedema patients who are without any cure for resolving their condition. 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 where MLD has been inadequate in resolving lymphedema [63,64].

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 two years of life. Lymphedema precox manifests itself during puberty. 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 not known about the causes of the dysfunction. Secondary lymphedema occurs as a result of the disruption of the lymphatic system from surgery [66] or from lymphatic filariasis, 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.

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 (*Chy* mouse and *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. In contrast to this, 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 due to lymphatic dysfunction. These and similar animal models should provide future insight as to our current uncertainty of a mastectomy patient's risk for developing lymphedema, thus allowing doctors to prescribe preventative measures that are dependent on the patient's perceived risk to develop lymphedema [66].

Consequences of congenital lymphatic diseases on lipid metabolism

Malformations of the intestinal lymphatics clinically manifest in 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 early on in the disease [67]. In primary intestinal lymphangiectasia, while edema is the main clinical feature (due to 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 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 inability of the intestinal lymphatics to adequately transport lipid from the intestine and hypotheses as to how these defects might be reversed in these diseases remains unknown. Research with animal models of lymphatic dysfunction

that survive past birth, such as the *Prox1*^{+/-} mouse [19], could 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, a type of autoimmune inflammatory bowel disease, manifests itself with 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 Crohn's disease, which would lead to further edema and inflammation [70]. With the 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 and clearance of dextran were greatly enhanced while impairing the retention of dextran in lymph nodes in a rat model of diabetes [72]. Glucagon-like peptide 1 (GLP-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 underscores our need for further research in this area (Box 4). Such future knowledge will lead to more efficacious treatments of diseases such as lymphedema, Crohn's disease, and intestinal lymphangiectasia.

Box 4

Future Questions and considerations

- The extent that chylomicrons rely on transcellular vs. paracellular mechanisms for their uptake into lacteals and the key molecular mechanisms involved in this process needs to be elucidated.
- The developmental cues in intestinal lymphangiogenesis need to be further explored particularly in the context of the lymphatic system's unique capability to absorb chylomicrons from the intestine
- The functional response of lymphatic contractility to the varying postprandial loads (and the extent that 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 Crohn's disease on lymphatic function, and particularly lymphatic lipid transport, need to be further explored.

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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 where 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 insert 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 LYVE1 (green, lymphatic vessel) and DAPI (blue). Collecting vessel is a bright field image taken from a rat mesentery.

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 wall shear stress (ii) through enhanced fluid flow has been shown to cause upregulation of eNOS and subsequent release of nitric oxide (NO), which acts as a vasodilator on the smooth muscle and inhibits vessel contraction [38] (Key: blue spheres (water molecules), yellow spheres (lipoproteins), pink (immune cell), orange stars (nitric oxide molecules)).

Figure 3. Mechanisms of chylomicron uptake into lacteals

(a) Each villus of the small intestine has a (b) 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) Transmission electron microscopy 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.