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Mechanisms of chylomicron uptake into lacteals

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Abstract

Right from birth, the lymphatics play a crucial role in dietary functions. A majority of the lipid absorbed from the newborn's lipid-rich diet enters the blood circulation through the lymphatic system, which transports triglyceride-loaded particles known as chylomicrons from the villi of the small intestine to the venous circulation near the heart. In light of the significance of this role, as well as the fact that lipid transport from the gut was one of the earliest discovered functions of the lymphatic vasculature, it is surprising that so little is known about how chylomicrons initially gain access to the lymphatic vessel. This review will focus on the current mechanisms thought to be important in this process and highlight important questions that need to be answered in the future.

Keywords

chylomicron; lymphatic; lipid; lymph; transcellular; paracellular

Introduction

The lymphatics have been observed to play an essential role in lipid transport for many years, as early work on the lymphatic system in the mid-1600s relied on this function to observe them, since the vessels could be easily identified from the white, lipid-rich fluid that filled them after a meal.¹ However, our understanding of the molecular mechanisms that regulate the functional transport of lipid by the lymphatics is significantly behind our knowledge of other lymphatic functions such as fluid balance and lymphatic involvement in cancer metastasis. Nearly all dietary lipid is transported in chylomicrons from the gut to the blood through the lymphatic system by entering specialized lymphatic vessels, referred to as lacteals, in the villi of the intestine (Fig. 1). In doing so, the lymphatic vasculature allows postprandial lipid to be available for storage and energy throughout the body before it arrives at the liver.² This unique property of the lymphatics has received recent attention from the pharmaceutical community, as it would be advantageous for an orally delivered drug to enter the lymphatic system utilizing similar mechanisms.^{3,4} However, since the mechanisms regulating uptake into lacteals remain unknown, most of the work in this area

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Conflicts of interest

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has focused on targeting the enterocyte to incorporate the drug into a chylomicron, to then be carried into the circulation.⁵ A more complete understanding of how chylomicrons enter into the lacteal would thus not only enhance our knowledge of the fundamental mechanisms that initiate lymphatic-lipid transport but could also provide new strategies for targeting lymphatics with orally delivered drugs or vaccines. While lymphatic involvement in lipid transport and adipose tissue has received recent attention in other reviews,^{6–8} this review will focus on our current understanding of how chylomicrons enter into the lacteals after secretion by enterocytes and will highlight some important areas of future research.

Lymphatic uptake

The lymphatic vasculature has been known for some time to have the capacity to take up and transport particulate that is too large to enter the blood circulation. This unique property of the lymphatics has been utilized to image the lymphatic vasculature and assess function by either injecting labeled molecules, such as albumin,⁹ dextran,^{10,11} nanoparticles,¹² or other large proteins,¹³ or by tracking immune cells that have entered the lymphatic vessels.^{14–16} Recent advances in molecular imaging have shown that initial lymphatics have a unique junctional structure that enables them to efficiently take up fluid and large proteins from the interstitial spaces.^{17,18} In addition, early transmission electron microscopy (TEM) work has shown that lymphatic endothelial cells (LEC) of initial lymphatics have anchoring filaments that attach the vessel to the tissue space and assist in opening up the junctions to allow for fluid entry.¹⁹ While quantifying the uptake of various tracers has proved useful in understanding the size selectivity and functionality of the lymphatic vasculature in various tissues, such methods are not very helpful for studying lacteal uptake, as the tracer must first get past the barrier provided by the epithelial cells of the intestine. Recently, investigators have reported the use of an orally delivered, fluorescently labeled, fatty acid analogue, Bodipy C16, to image lymphatic uptake from the intestine.^{20,21} However, the extent to which such a technique can provide a quantitative measurement of lacteal function remains to be seen. Thus, our current understanding of the mechanisms involved in lymphatic uptake into the central lacteal of the villus is dependent entirely on observations made from TEM images taken of fixed, postprandial intestinal tissue sections.

Chylomicron uptake: transcellular versus paracellular

When comparing most lymphatic and gastrointestinal physiology texts that go into the details of chylomicron uptake in the lacteal, the general consensus is that paracellular transport through the opening of intercellular gaps is the primary mechanism through which chylomicrons enter the lumen.^{1,22,23} However, a closer look at the literature reveals a large degree of uncertainty regarding this issue. TEM images of a lacteal containing chylomicrons were first reported by Palay.²⁴ While the focus of this work was on the internal structure of the lipid-absorbing enterocyte, the authors displayed an image of a lacteal in which chylomicrons could be seen in the lumen as well as half a dozen chylomicrons entering between two overlapping junctions. Shortly thereafter, two different papers reported numerous TEM images demonstrating potential mechanisms of chylomicron entry into the lacteal.^{25,26} In these works, both open junctions containing chylomicron particles, as well as numerous endothelial vesicles with chylomicrons inside them were demonstrated. In hypothesizing which mechanism of chylomicron entry is more important, Casley-Smith acknowledged that it is difficult to know for certain the extent that chylomicrons can cross the vessel wall in vesicles. In summarizing his findings he stated that, “It would seem then that it is the open junctions which give the lacteals their great permeability. Thus, it is likely that much material passes through the patent lacteal junctions rather than through the endothelial cells.” Several other reports concurred with Casley-Smith’s original findings,

being unable to rule out the importance of transcytosis, but at the same time concluding that paracellular transport was probably of primary importance.^{27–32}

However, a few years later, Dobbins challenged this idea by demonstrating numerous vesicles containing chylomicrons and showing that the majority of the junctions between cells remained tightly closed.^{33,34} While he had shown in earlier studies that very few junctions are open in the lacteal³⁵ even when the vessel was extremely distended in a person with intestinal lymphangiectasia, a common characteristic of the disease,³⁶ this was the first study showing quantitative data on the distribution of junctions and vesicles in over 500 TEM images of chylomicron-containing lacteals.³⁴ Since only 6 of the 149 junctions identified in the lacteals of mice and guinea pigs were open (having a gap greater than 40 Å) and since vesicles occupied approximately 15% of the cytoplasmic area within the cell, Dobbins concluded that transcellular transport must be of primary importance in the uptake of chylomicrons into the lacteal. Dobbins suggested that numerous gaps between junctions demonstrated in previous studies might in fact have been artificially created because of the delicate process of creating tissue sections for TEM imaging. The controversy over the two mechanisms of transport continued through the next decade, with some supporting Dobbins' hypothesis that a transcellular mechanism is the primary route of uptake into the initial lymphatics of the gut.^{37–40} However, Collan demonstrated through TEM images that while numerous junctions were tightly connected and overlapping (which he referred to as "complicated joint areas"), there appeared to be junctions interspersed that contained a "simple joint area" between the junctions, which he suggested would be the primary entry point for chylomicrons.⁴¹ Others suggested junctional entry of chylomicrons through the tip of the villus,^{42–44} and still others have continued to recognize the uncertainty regarding the relative importance of the two mechanisms.²

Chylomicron transport

After initial entry into the lacteal, the chylomicrons must be transported through the lymphatic system to the blood. This appears to be done in two stages. Flow starts through the initial lymphatics, which lack the smooth muscle found on collecting lymphatics, by utilizing the peristaltic motion of the intestinal wall, first described by Florey.⁴⁵ Collan later showed through TEM images that there resided muscle cells within close proximity of the lacteal, which he suggested could provide some pumping activity to the villus.⁴⁶ Lymph formation, which is increased through an increase in the intraluminal pressure in the gut, also directly correlates with lymph flow through the initial lymphatics and can provide a driving force to promote lymph flow.⁴⁷ Once in the initial lymphatics, it was recognized early on that the intrinsic contractility of the collecting lymphatics was essential in driving lymph flow.^{45,48} This pumping activity is responsive to changes in mechanical loads such as wall shear stress^{49,50} and transmural pressure.^{49–52} However, it is currently unknown how these mechano-sensitive features of the lymphatic vasculature are utilized in optimizing postprandial lymph transport and how such a phenomenon correlates with the observed increase in contraction frequency that occurs after olive oil administration.⁵³

Models of lacteal uptake

One of the most impactful techniques that has been essential to our understanding of lipid absorption in the gut is the lymph fistula model. By isolating and collecting lymph before lipid reaches the blood, investigators have been able to uncover many of the mechanisms regulating chylomicron assembly⁵⁴ as well as show the importance of lymph flow on lipid absorption.⁵⁵ However, the challenge associated with using this technique to study lymphatic uptake of lipid is that, while a general idea of the kinetics of the process of lipid transport by lymphatics can be deduced, it is difficult to separate out certain effects of the

lymphatic vasculature (e.g., pump function, lacteal permeability, LEC vesicular uptake) from that of the enterocyte. We recently reported on a tissue-engineered model of the lacteal in which chylomicron uptake across the lymphatic endothelium could be visualized and quantified.²¹ By supplementing the media supplied to an enterocyte cell model with oleic acid, a fluorescently labeled fatty acid, and a bile salt, we were able to get the cell to synthesize and secrete fluorescently labeled lipoproteins, which could then be used to track and quantify lipid transport across the lymphatic endothelium. Through the use of confocal and TEM imaging we demonstrated transcytosis of lipoproteins across the LEC and showed that the model recapitulated the desired characteristics of *in vivo* lipid transport in the small intestine. One of the advantages of this model is that the contributions to lipid transport of the enterocyte and the lymphatic can be separated, something that is difficult to do with the *in vivo* lymph fistula model. This *in vitro* model will prove useful in determining the relative importance of vesicular-driven transport of chylomicrons into the lacteal and the molecular mechanisms behind this process. In another recent paper, a tissue preparation system was discussed in which a loop of an isolated small intestine along with the blood and lymphatic vessels that support it could be cannulated and kept functioning.⁵⁶ Such a model could be used to determine the effect of hemodynamics on lacteal function by providing a means to directly control and monitor hemodynamic parameters independently (e.g., arterial pressure, venous pressure, interstitial fluid pressure, lymphatic pressure) while at the same time controlling the contents of the small intestine and the rate at which they are delivered.

Lacteal dysfunction

While our fundamental understanding of chylomicron uptake by lymphatics is still premature, there are several noteworthy studies of late that have reported disease models of the intestinal lymphatics and the resulting consequences to lipid absorption in the gut. In a seminal paper, Harvey described a mouse model of leaky intestinal lymphatics in a heterozygous mouse model effecting a gene important to lymphatic lineage commitment (*Prox1*^{+/-}), which resulted in adult onset obesity due to the adipogenic nature of the chyle leaking from the vessel.²⁰ Whether lymphatic dysfunction is an underlying cause of certain clinical cases of regional abdominal obesity remains to be seen; however, the lymphatics have been implicated in chyle leakage in other diseases such as intestinal lymphangiectasia.⁵⁷ Several other genes have also been indicated in maintaining a functional lymphatic network for lipid absorption in the gut and while the underlying mechanisms causing the dysfunction are different in each report, the primary manifestation of the dysfunction is a phenotype of abnormal lipid transport and metabolism.⁵⁸⁻⁶⁰ In wild-type animals, lacteal morphology has been shown to vary dramatically with dietary status, as the lacteal drastically retracts into the lamina propria during fasting, and can be quickly restored (within 3 days) upon refeeding.⁶¹ Finally, Van Dyck reported on a transcription factor, whose functions remain largely unknown, that when deleted, results in a mouse that dies of postnatal starvation.⁶² Interestingly, starvation was not due to poor enterocyte absorption of lipid or chylomicron synthesis, but rather was a result of the accumulation of chylomicrons in the lamina propria due to their not being transported properly into the lacteal. Taken together, these data all indicate that lymphatic involvement in lipid transport is much more active than previously thought, and is not merely the passive draining of fluid and chylomicrons from the gut through large gaps between cells.

Future directions

With the advances in lymphatic molecular biology and imaging techniques, it is an exciting time to be involved in lymphatic research. The emergence of recent reports implicating the lymphatic vasculature in certain lipid disorders highlights the importance of understanding the fundamental biology that regulates lipid transport by lymphatics. Fundamental to this

process is the initial uptake of chylomicrons secreted by enterocytes into the lumen of the lacteal, yet it is still unclear exactly how these rather large particles rapidly gain access to the lymphatic vessel. Hopefully these new tools and molecular approaches will allow insight into this significant process in the near future.

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Figure 1. Optical slice taken on a confocal microscope of a whole-mount mouse intestine showing a villi with a lymphatic vessel running down the center. Cell nuclei are stained with DAPI (blue) and LEC are stained with LYVE-1 (red).