Physiology: Lymph Flow

Anish Mukherjee, Joshua Hooks, and J. Brandon Dixon

B.-B. Lee et al. (eds.), *Lymphedema*, https://doi.org/10.1007/978-3-319-52423-8_8

Summary of Basic Concepts

The specialized structure of the lymphatic vasculature enables it to propel lymph through the body and back into blood circulation. The process starts with the extravasation of fluid from the blood capillaries. This excess fluid is taken up by the initial lymphatics and then propelled by the collective action of the initial and collecting lymphatic vessels back into the blood circulation at the subclavian veins.

- The interstitium is composed of structural proteins and glycosaminoglycans that control its properties.
- Extravasation of fluid into the interstitium occurs from the blood capillaries and is governed by Starling's law.
- The initial lymphatic vessels found throughout interstitial tissue are blind-ended structures composed only of endothelial cells and no basement membrane.
- Fluid entering the lymphatic system is termed «lymph» and is prevented from escaping into the interstitium by the unique morphology of the initial lymphatics which form «primary lymphatic valves.»
- The initial lymphatics play an important role in the uptake of lipid from the intestines and transport of macrophages to the lymph nodes.
- Initial lymphatic function gets disrupted during chronic inflammation and lymphedema.
- Collecting lymphatic vessels are formed of contractile units called lymphangions that actively pump lymph through the lymphatic network.
- Lymphatic valves are present between the lymphangions that prevent the backflow of lymph.
- Production of vasoactive substances, such as neurogenic and hormonal stimuli, allows the body to manipulate lymph flow rates by impacting lymphangion function and structure.
- Mechanical factors, such as intramural pressure, stretch, and shear stress, can modulate and coordinate the function of the lymphatic muscle cells.
- The presence of pacemaking cells has been suggested in collecting lymphatic vessels that might dictate the spontaneous contractility of the lymphangions.
- Extrinsic factors like skeletal muscular contraction, arterial pulsations, and breathing can also contribute to the lymph flow.
- Collecting lymphatic vessels might be compromised during lymphedema due to remodeling of the vessel wall and chronic pathological stimulation.
- The lymph flows into lymph nodes through afferent collecting lymphatic vessels and exits through efferent collecting lymphatic vessels.
- The lymph not only flows through the lymph node, but some of it is reabsorbed into the blood circulation at the lymph nodes.

8.1 Interstitium

8.1.1 Composition of the Interstitium and Maintenance of Interstitial Volume

The source of all lymphatic flow is in the interstitium, where the fluid and soluble interstitial proteins that are not absorbed by the venous circulation are returned to blood circulation by the lymphatic vasculature. The interstitial space is primarily composed of elastic fibers, collagens, and glycosaminoglycans (GAG) that combine to form larger protein complexes called proteoglycans. Proteoglycans are large negatively charged molecules that are essential for the structural properties of the interstitium. Their large negative charge makes them essential for maintaining interstitial volume by providing the interstitium with the capacity to resist compressive forces. Furthermore, the negative charge attracts other diffusible species which helps to maintain osmotic pressure and thus the fluid volume within the tissue. The osmotic pressure of the interstitium plays a significant role in determining the hydration of the tissue and effective porosity of the matrix and hence transport processes like convection, diffusion, etc. The porosity of the matrix and the extent that this porosity can be dynamically altered with mechanical loading depend also on the composition of the interstitium. This in turn dictates the hydraulic conductivity under various physiologic states. Interstitial properties are also highly variable between different parts of the body [6, 7]. Hence, the rates of interstitial fluid formation and drainage, and therefore the local demand on the lymphatic vessels, vary throughout the body.

8.1.2 Physics of Interstitial Fluid Formation

The circulatory system and the lymphatic system form a loop, regulating the amount of interstitial fluid by a continuous process of extravasation and reabsorption [8–10]. This process starts in the blood capillaries. Blood capillaries are composed of a single layer of endothelium with an almost nonexistent basement membrane, which makes it conducive to the exchange of fluid across its walls. The exact mechanism by which fluid is transferred across the blood capillary endothelium was first explained by physiologist Ernest Starling in what has come to be known as «Starling's law».

Starling's law, seen below, is an equation which states that the net rate of fluid transfer across a membrane is the weighted sum of the hydrostatic pressure difference across the membrane and the osmotic pressure of the solutes.

$$J_v = L_p \frac{S}{V}\left(\Delta P + \sigma \Delta \pi\right)$$

where J_v is the fluid flux across the vessel wall (in or out), L_p is the permeability, S is the surface area, V is the volume, ΔP is the hydrostatic pressure difference, $\Delta\pi$ is the osmotic pressure difference, and σ is the capillary osmotic reflection coefficient. In the context of fluid transport across blood capillaries, the blood capillary wall acts as a porous membrane, with blood on one side and interstitial fluid on the other side. There are two opposing forces acting on the blood capillary walls. Hydrostatic pressure drives fluid out of capillaries, while osmotic pressure drives fluid back in.

The rate of uptake of solutes by the blood capillaries is size and charge dependent, and it is found that small molecules and crystalloids are preferentially taken up, while transport of larger proteins is not permitted [11]. This is reflected as an osmotic reflection coefficient that is less than 1 in Starling's law. This value means that there is a higher concentration of proteins within the blood capillaries than the interstitium. As the osmotic pressure is created primarily by the proteins, it is referred to as the oncotic pressure gradient. Fluid moves in the opposite direction of the oncotic pressure gradient, and therefore, the net effect is the movement of fluid from the interstitium into the blood capillaries. The mean capillary hydrostatic pressure is much greater than the mean interstitial hydrostatic pressure, which has the net effect of pushing fluid out of the capillaries [6, 9, 12].

The following example will help to better visualize this continuous exchange process. Let us start with an equilibrium where the contribution due to the hydrostatic pressure difference balances the contribution due to the oncotic pressure difference. Now consider an increase in the hydrostatic pressure in the capillary. This shift will cause movement of fluid out of the capillary. Under physiological conditions, the greater hydrostatic pressure of the capillary would lead to excess fluid flow into the interstitium. Fortunately, any reduction in capillary fluid increases the concentration of protein within the capillary, therefore increasing capillary oncotic pressure. This increase in oncotic pressure will cause movement of fluid into the capillary until an equilibrium is reached again.

In general, the rate at which fluid is squeezed out is slightly greater than the rate at which fluid is pushed back in. Thus, there is a net buildup of fluid in the interstitial space that needs to be drained in order to maintain fluid homeostasis in the interstitium. Additionally, it is essential that the lymphatics drain large proteins from the interstitial space to keep the protein concentration low and thus maintain an effective oncotic pressure gradient that favors fluid retention within the microcirculation. That is where the lymphatic system comes in, acting as a drainage system for the excess fluid or solutes while also serving many other important functions in the body like transportation of lipids from the intestine and the trafficking of immune cells to the lymph nodes [6, 7, 10, 13].

8.2 Initial Lymphatics

8.2.1 Structure

The initial lymphatics, also referred to as lymphatic capillaries, are blind-ended vessels characterized by endothelial cells with an attenuated cytoplasm, discontinuities in the basement lamina, and specialized junctions. The initial lymphatic endothelium is char-

acterized by cells that are apposed to each other, forming valve-like structures that are commonly referred to as «primary lymphatic valves» since functionally they have been observed to allow fluid and large proteins to enter easily when the pressure gradient is favorable for lymphatic uptake and yet provide a large resistance to fluid and molecules leaving the capillary when lymphatic capillary pressure exceeds interstitial fluid pressure [1, 14–18]. This unique functional capacity of the initial lymphatics is thought to occur due to the specialized junctions formed by a unique configuration of VE-cadherin and CD31 commonly referred to as «buttons». The lymphatic endothelial cells form oak leaf-shaped structures that are characterized by discontinuous button-like junctions that form flap-like structures. These are characteristics of mature initial lymphatics, since immature initial lymphatics such as those observed during lymphangiogenesis induced by acute inflammation have zipper-like junctions [1]. The button-like junctions provide enough structural integrity to the initial lymphatics by being anchored at the side of the flaps while also forming points of entry at the tip of the flaps. Thus, the integrity of the initial lymphatic endothelium is maintained while also forming the valves that are necessary for entry of fluid into the lymphatics. While the size of many macromolecules and cells would restrict them from typically entering capillaries, the unique and 'large' entry junctions of the initial lymphatics enable passage into the lymphatics. The primary lymphatic valves are hence involved during the filling up of the lymphatic vessels with interstitial fluid and also prevent backflow into the interstitium. The initial lymphatic endothelial cells are connected to the surrounding connective tissues with anchoring filaments that play an important role in keeping the initial lymphatics from collapsing. Additionally, these filaments help in opening the primary lymphatic valves, leading to the formation of «lymph» [9, 14]. The structure of the initial lymphatic network and anatomy of the initial lymphatic vessels and valves are portrayed in Fig. 8.1.

8.2.2 Functional Role of Initial Lymphatics

Role in Lymph Formation and Transport

The initial lymphatics are the first stage of the lymphatic network that takes up the excess interstitial fluid. The average pressures in the interstitium and the initial lymphatics are similar. In fact, the average pressure in the interstitial space is usually slightly negative, thus being subatmospheric, while the average pressure in the initial lymphatic lumen is slightly positive. These measurements might indicate that the prevailing hydrodynamic forces are not conducive for the flow of interstitial fluid into the initial lymphatics. However, these are average pressures and do not account for the transient fluctuations in the interstitial and initial lymphatic pressures because of extrinsic and intrinsic factors. Extrinsic factors include skeletal muscle contractions, heart contractions, gastrointestinal muscle contractions, respiration, and arterial pulsations; intrinsic factors refer to the spontaneous contractions of the lymphatic network which will be described in greater detail later [13, 20].

During these cyclical pressure fluctuations, when the initial lymphatic pressure is lower than the interstitial pressure, the fluid flows into the initial lymphatics, thereby being referred to as «lymph.» The intrinsic properties of the initial lymphatics, such as anchoring filaments and «primary valves,» play a vital role here. An accumulation of fluid

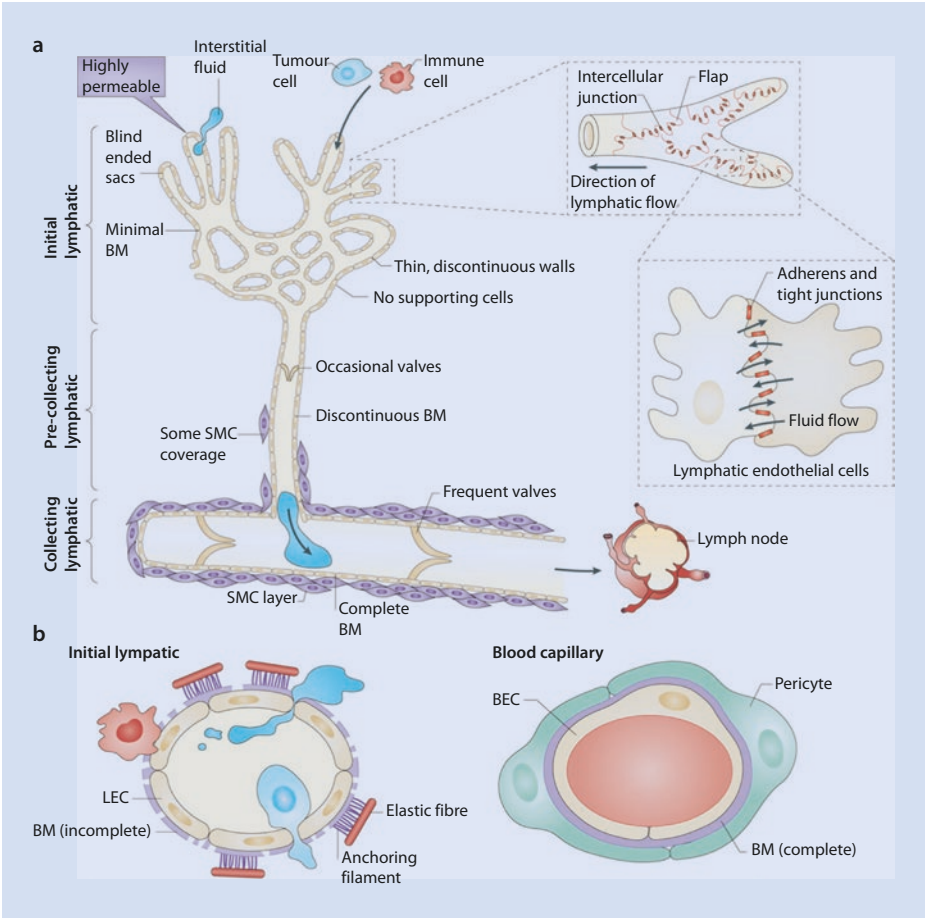

Fig. 8.1 **a** Structure of the initial lymphatic network and how it connects to the collecting lymphatic network is shown. The blowouts show the detailed structure of the initial lymphatic vessels, with the button-like junctions that form the flaps that act like valves. **b** The initial lymphatic vessels exhibit a discontinuous basement membrane and loose endothelial cell junctions, in contrast to blood capillaries that have a continuous basement membrane. This specialized structure of the initial lymphatics allows the paracellular transport of cells and proteins into the initial lymphatic lumen, but transport also happens through transcellular pathways. Anchoring filaments, which are responsible for pulling the initial lymphatic vessel open during the filling up stage, are also shown [19]

in the interstitium leads to swelling, which causes a tension in the anchoring filaments. This tension forces the primary lymphatic valves to open, allowing inflow. Once pressure inside the initial lymphatics is greater than the interstitial pressure, the primary valves in the initial lymphatics close, preventing the backflow of lymph into the interstitium [6, 20–23]. Recent research also suggests that apart from the aforementioned mechanism, transport of solutes can also occur through transcellular pathways which might be important in lymphatic solute transport [24]. Thus, the unique structure of the initial lymphatics coupled with the extrinsic and intrinsic factors leads to the formation of lymph.

Role in Lipid Transport

A special kind of initial lymphatic vessel is the lacteal. Lacteals are the initial lymphatic vessels found in the small intestine at the center of each villus. The main function of the lacteals, apart from the removal of water absorbed across the mucosal membrane [25], is the uptake of absorbed lipids in the form of high-density lipoproteins and chylomicrons from the small intestine. They are also shown to allow the migration of immune cells from the interstitium across the endothelium [26]. The lacteals then act as a conduit toward the mesenteric collecting lymphatic vessels, which then transports this unique, lipid-rich lymph to the systemic circulation [27]. For this reason, the lacteals also serve as an important channel for the uptake of lipophilic drugs that are orally ingested [28]. This uptake of lipoproteins and cells can happen paracellularly across the lymphatic endothelial cell junctions and also through transcellular pathways [29–33].

The lacteals are unique, because they show spontaneous contractility in conjunction with adjacent smooth muscles. This was first discovered by Howard Florey in 1927 [34, 35]. This unique physiology makes them exhibit properties of both the initial lymphatics in that they possess «button-like» junctions and also of the collecting lymphatics in that they possess spontaneous contractility that is regulated by the autonomic nervous system [36]. The function of lacteals, and hence the uptake of chylomicrons, is highly dependent on the prevailing forces in the interstitium, as lacteals have been observed as undergoing morphological changes in response to fasting and feeding and they also show sensitivity to lymph flow [37]. Experiments with mice having abnormal lymphatic lineage commitment due to a heterozygous mutations in the Prox-1 gene develop obesity due to compromised leaky lymphatic vessels [38]. VEGF-C, an important factor in lymphatic endothelial cell growth and proliferation, has been found to be extremely important in the maintenance of the function of the lacteals, as knockout of VEGF-C leads to atrophied intestinal lymphatics and impaired lipid transport [39].

Role in Transport of Immune Cells

The lymphatic vessels play a very important role in immune cell trafficking. However, the transmigration of leukocytes across the initial lymphatic endothelium is a matter of ongoing investigation. In the presence of pro-inflammatory cytokines, cultured lymphatic endothelial cells have been shown to upregulate the expression of leukocyte adhesion molecules [40]. Carbohydrate-binding proteins like galectin have also been implicated in this transmigration. The binding of the leukocytes to the adhesion molecules on the lymphatic endothelial cells causes them to crawl toward the cell junctions, where they interact with other adhesion molecules to promote entry into the initial lymphatic lumen. For example, gradients of the chemokine CCL21 have been implicated in the crawling of dendritic cells (DCs) across the initial lymphatic junctions [41, 42]. The transmigration can occur via both the button-like junctions and through the endothelial cells, although the exact point of entry is unresolved [1]. The amount of lymph drainage through the lymphatic network has also been implicated as a factor in regulating the transmigration of dendritic cells in the initial lymphatics [43]. There is also evidence for bidirectional transmigration of the leukocytes, which might be an important factor in effective immune surveillance [44].

8.2.3 Initial Lymphatic Disruption in Inflammatory Diseases and Lymphedema

Inflammation involves a series of responses initiated by the body in response to harmful stimuli like pathogens or external irritants causing damage to cells. Inflammation has several effects on the circulation, including increased permeability of the blood vasculature, leading to extravasation of fluid and tissue swelling. This increased permeability also allows leukocytes to enter the interstitium by extravasation. In the case of initial lymphatics, the effect of inflammation is similar, with an increase in permeability of the vessels. One probable cause for this increase in permeability might be the opening of the primary lymphatic valves due to the swelling of the tissue, which pulls on the anchoring filaments and holds the valves open. Other reasons proposed include the formation of pores in the lymphatic endothelium and rearrangement and loosening of the tight cell-cell junctions that are occasionally present in the initial lymphatic endothelium [1, 40]. It is hypothesized that there is a reduction in the effective functioning of the lymphatics during chronic inflammation because of compromised initial lymphatic junctions.

Inflammation and lymphedema influence each other in a complex manner, and this can be traced to altered function of the intial lymphatic system. Lymphedema induced in rodent models by artificial lymph stasis has been shown to induce inflammation and fibrosis [45, 46]. There is an overabundance of pro-inflammatory cells like macrophages, dendritic cells, and lymphocytes in the interstitium, leading to chronic inflammation and fibrosis [40, 44, 47]. Fibrosis caused by chronic inflammation can also worsen lymphedemic conditions [48, 49]. Lymphangiogenesis (the formation of new lymphatic vessels from pre-existing ones) induced by inflammation is thought to be involved in lymphedema, although whether the effect is benign or pathological is still a matter of contention [50, 51]. In the context of primary lymphedema, mutations in VEGFR-3 that lead to impaired lymphangiogenesis have been shown to induce lymphedema [52–54]. Hence, the use of lymphangiogenesis as a mean of therapeutics during lymphedema has also been suggested [55, 56]. However, lymphangiogenesis might not always have a benign effect on lymphedema. It has been shown that $CD4^+$ T cells can interact with macrophages to produce VEGF-C which is essential for lymphangiogenesis. Inhibiting the interaction can lead to reduced lymphangiogenesis as well as reduced chances of later onset of secondary lymphedema [51]. The upregulation of VEGF-C during inflammation may also lead to loss of collecting lymphatic pumping function, although there are contradictory reports on the same [57, 58].

8.3 Collecting Lymphatic Vessels

8.3.1 Structure

As initial lymphatics connect downstream, they transition into a region of the lymphatic network known as the pre-collecting lymphatic vessels. Pre-collecting vessels are distinguished from the initial lymphatics by the presence of a basement membrane surrounding the lymphatic endothelial cells and the discontinuous arrangement of lymphatic

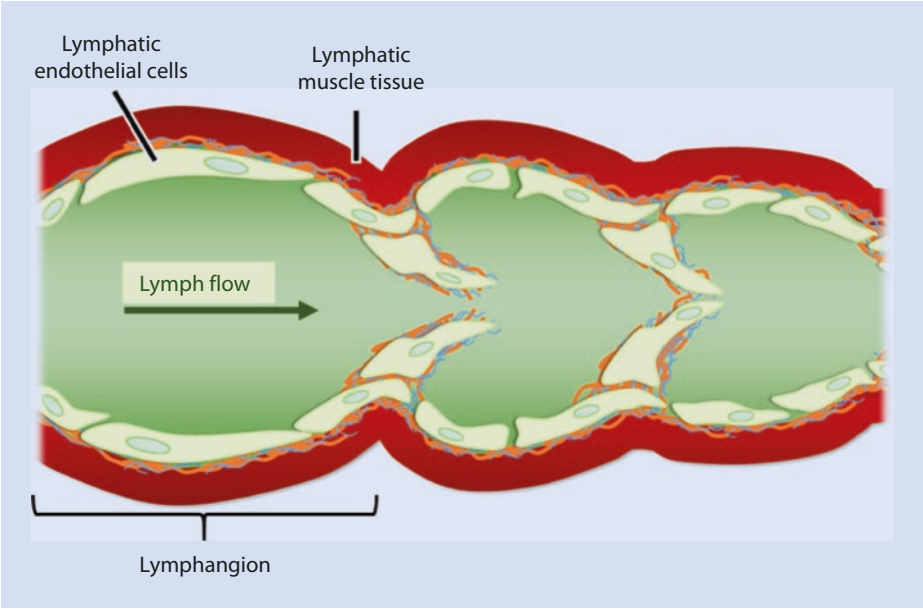

Fig. 8.2 The mature lymphatic collecting vessel. The lymphatic endothelial cells (LECs) are supported by a layer of basement membrane composed primarily of extracellular fibrils of collagen and elastin (seen in *orange* and *blue*). These fibers also provide structural support to the bileaflet valves. The entire collecting vessel is surrounded by a layer of lymphatic muscle cells which contract to propel lymph flow downstream

smooth muscle cells [59, 60]. Pre-collectors have the continued presence of anchoring filaments, presumably to assist in lymph absorption and transport [59].

Pre-collecting lymphatic vessels transition into mature collecting lymphatic vessels. These collecting vessels are characterized by fully developed basement membranes, full coverage by lymphatic smooth muscle cells, and the presence of a second type of lymphatic valve. The basement membrane between the LEC monolayer and the muscle is primarily composed of collagen and elastin fibers which contribute significantly to the mechanical properties of the vessel. These two extracellular protein fibers also provide structural support to the bileaflet, unidirectional valves. The valves separate the collecting vessel into discrete segments known as lymphangions as seen in Fig. 8.2. These valves physically prevent backflow between lymphangions and allow for the directional flow of lymph toward the distal lymph nodes [15, 61]. The smooth muscle cells surrounding the collecting lymphatic vessels are highly specialized and are commonly referred to as lymphatic muscle cells (LMCs). Lymphatic muscle cells are capable of rapid, phasic contractions that are well coordinated with neighboring cells [2].

Unlike blood vasculature, there is no central pump to drive the flow of lymph through the lymphatic system. Lymph transport through the collecting vessels is therefore entirely dependent on the intrinsic contractility of the lymphatic muscle and extrinsic factors such as skeletal muscular contraction, arterial pulsation, passive movement, and respiration.

Lymphatic Valves

Functioning lymphatic valves within the collecting vessels are critical for functional lymph propulsion by either intrinsic or extrinsic factors. In humans, these valves are typically spaced every 1–3 mm [62]. To properly function, these valves must open and close under flow rates and pressure that are relatively low compared to pressure in the blood vasculature. Work with isolated vessels, where the inlet and outlet pressures can be manipulated independently, demonstrated that the pressure gradient across the valve needed for the valve to open is fairly consistent, but closing pressure gradient is dependent on transmural pressure of the adjacent lymphangion. In addition, there appears to be a slight bias in the valve to remain in the open position [3]. This likely explains why a small amount of retrograde flow is typically observed in the contraction cycle before valve closure [4].

While largely efficient, some regurgitation of lymph into an upstream lymphangion may occur before valve fully closes, specifically given their open bias [63]. Eliminating backflow is particularly critical at lymphovenous valves; unique valves present at the intersection of major collecting vessels, known as ducts, and the left or right subclavian vein where lymph is returned to the blood vasculature. At these locations, adverse pressure gradients can be significant, and several studies in mice demonstrate that pathologies of the lymphovenous valves lead to blood flowing into the lymphatic system [64]. Activation of platelets by specific receptors on the surface of lymphatic endothelial cells seems to play a critical role in preventing blood flow into the lymphatics particularly at these locations [65].

8.3.2 Intrinsic Contractility and Active Lymph Propagation

As previously stated, the lymphatic collecting vessel is composed of a chain of lymphangions, separated by unidirectional valves. These lymphangions are considered the contractile units of the vessel, working as a series of pumps to propel lymph. The rapid, phasic nature of this contraction is unique to LMCs and made possible by their unique molecular composition. While the LMCs are nonstriated muscle cells, they share contractile proteins that are otherwise limited to cardiomyocytes, which in part account for their unique pumping phenotype [2, 66]. This pumping mechanism in lymphatics is described using language consistent with the heart. For example, as a heart undergoes systole and diastole, so does the lymphangion. Systolic diameter of a lymphangions describes the minimum diameter in the lymphangion at the peak of a contraction, while the diastolic diameter is the maximal diameter between contractions. Ejection fraction refers to the fraction of end-diastolic volume ejected during lymphatic contraction.

The contractile frequency of a lymphangion is usually on the order of five to ten contractions per minute with amplitudes of up to 50% of the resting diameter for a major collecting vessel, but there is a significant amount of regional variability due to mechanical loading on the vessel [4, 67–69]. These relatively strong, regularly occurring contractions are critical for the collecting vessel to pump fluid against an adverse pressure gradient. In large animal models, experimental work has shown that short segments of isolated lymphatic vessels can overcome adverse pressure gradients of 10–30 cmH_2O [70]. To overcome greater pressure gradients, it is important to remember that collecting

lymphatic vessels work not as individual units, but as chains of pumps arranged in series. The extent that the coordination of the pumping activity of the chains regulates lymph flow is not well established, but computational modeling suggests that coordination could improve pumping [69], and it has also been observed experimentally [71]. Chains of multiple lymphangions are better suited to overcome larger adverse pressure gradients and appear to coordinate contractility to reduce pressure downstream before additional fluid arrives from upstream segments [72]. A chain of lymphatic vessels is able to overcome a rather large pressure gradient, with measurement in vivo reporting pumping pressures in excess of 30 mmHg (~40 cmH_2O) in human legs [73], 40 mm Hg (~54 cmH_2O) in human arms [74], and 35 mmHg (~47.5 cmH_2O) in rats [75].

In addition to executing active phasic contractions, LMCs must modulate the vessel diameter in order to adjust the resistance to lymph flow through the lymphatics. Similar to the blood vasculature, regional changes in the lymphatic vessels' diastolic diameter can locally regulate the quantity and velocity of transported lymph. This is often at odds with the vessels' role as a pump, as relaxing the LMCs often impairs active contractility. Furthermore, active pumping by LMCs may increase the resistance to passive flow by transiently constricting the diameter of the vessel. Therefore, it is important that LMCs react to a variety of environmental cues that regulate their role as either an active pump or a passive conduit to lymph flow [76]. Factors that impact the coordination of these roles are outlined below.

Innervation and Sensitivity to Vasoactive Substances

Collecting lymphatic vessels have long been shown to be responsive to a variety of vasoactive peptides [77]. Noradrenergic, purinergic, cholinergic, and peptidergic neurons can be found throughout collecting vessel walls and manipulate various aspects of contractility [78]. Innervation of the lymphatics leads to altered function in response to a number of neurogenic and humoral stimuli that have been extensively researched [77–83]. Lymphatic regulation through by these vasoactive substances have been shown to influence resistance to flow, ejection rate, rate of formation of lymph, and lymphatic vessel permeability.

Recent work has explored the role of various vasoactive substances on the permeability of collecting vessels. Atrial and brain natriuretic peptides, hormones that are released to reduce stress on the heart, both reduce collecting vessel permeability. This may have the effect of temporarily reducing the reabsorption of lymph into blood circulation [84]. Similarly, immune cells that interact closely with or within the walls of the collecting lymphatic vessel play a significant role in modulating collecting vessel permeability and function [85]. Within adipose deposits, modulation of collecting vessel permeability has been shown to regulate local inflammation [86]. As a whole, the aforementioned studies in combination with others have demonstrated that excessive collecting vessel permeability impairs the ability of lymph, rich in antigens and dendritic cells, to reach downstream lymph nodes, significantly weakening the adaptive immune response [87].

Vasoactive substances released during inflammation appear to play competing roles in regulating lymph flow. Vasoactive substances released by myeloid cells during inflammation, such as nitric oxide and histamine, seem to both dilate collecting vessel walls and reduce frequency of spontaneous contractions. Furthermore, these substances

seem to assist in DC trafficking to lymph nodes [88–90]. Other inflammatory cytokines, such as substance P, actually increase contractile frequency when incubated with isolated vessels [79]. How immune cells coordinate the release of these potentially competing vasoactive substances throughout the progression of inflammation is still poorly characterized.

Mechano-Regulation

As with the blood vasculature, shear stress elevated above a certain threshold due to fluid flow along the lymphatic lumen induces vasodilation of the collecting lymphatic vessels, reducing or fully inhibiting contractility [91]. The mechanism for this response is believed to be largely based on nitric oxide generation by LECs [92, 93], but recent research has implicated other vasodilators, such as histamine [94]. The shear stress magnitude within the lymphatic vasculature is much lower than that seen in blood vasculature and highly oscillatory [4]. A recent study demonstrated that the magnitude of shear needed to inhibit spontaneous contractions is much lower than that seen in the blood vasculature, depends on distention due to transmural pressure [95], and involves Ca2+ release by LEC [96, 97]. It is not clear what the molecular mechanisms are that allow lymphatic endothelial cells to respond to lower shear stresses than blood endothelial cells; however, recent work has suggested that this enhanced sensitivity involves VEGFR3, possibly through a mechanosensory complex formed at the cell junction [98]. Furthermore, shear stress along the wall appears to contribute to the coordination of contractility. Simply put, as lymph ejected from an upstream lymphangion moves into a downstream lymphangion, the increased shear stress along the wall of the downstream lymphangion dilates the diameter, reducing the resistance to the incoming flux of lymph [95, 99].

Contractility of the collecting vessel demonstrates a sensitivity to wall distension in accordance to the Frank-Starling's law [63]. The length-tension relationship of lymphatic muscle cells determines the tension within the LMC at which force generation is maximal. It was determined in human thoracic ducts that average peak active wall tension was 6.24 ± 0.75 N/m at a corresponding transmural pressure of 47.3 ± 4.7 mmHg [100]. Increased transmural pressure increases wall tension, causing greater force generation by the LMCs, leading to an increase in pumping efficiency (e.g., the ejection fraction increases) up until a critical pressure is reached. Beyond this point, pumping efficiency diminishes as the LMCs, presumably, cannot generate more force [101, 102]. An early theory for contraction coordination along the collecting vessel was that the volume increase after a lymphangion takes on a bolus of lymph was the sole trigger for the contraction of locally distended muscle cells. This theory has been shown to be an oversimplification, as lymphangions can contract in the absence of lymph formation [103] and in the absence of apparent intraluminal distending force [104].

Electrophysiology and Pacemaking

Contractility of the collecting lymphatics in the absence of vessel distention indicates that contractile coordination depends, in part, on electrical mechanisms. Multiple ionic channels have been well characterized in LMCs [105–107]. Outward currents include calcium-dependent potassium channels and delayed rectifier potassium channels. Inward currents include fast sodium channels [108] and both L- and T-type calcium channels.

The exact expression of these channels on LMCs seems to be heterogeneous, and it is hypothesized that different channels are responsible for specialized aspects of contractility. For example, work by Roizes and von der Weid demonstrated that the inhibition of L-type calcium channels primarily reduced the force generation during a contraction, while T-type calcium channel inhibition caused irregular contractile frequency [109]. Furthermore, a relationship between the resting membrane potential of LMCs and isometric tension has been established in animal models, further demonstrating the interplay between these regulatory factors [110].

The pacemaking behavior of lymphatic vessels was well demonstrated for decades, but the mechanisms by which this is possible were difficult to elucidate [111]. Are all LMCs capable of pacemaker-like spontaneous depolarization, or are there specialized pacemaker cells embedded within the vessel? More recently, it has been shown that a small population, approximately 5%, of LMCs isolated from sheep lymphatic collecting vessel walls may produce an inward current after hyperpolarization, similar to pacemaker cardiomyocytes. This inward current is similar to the «funny» pacemaker current seen in cardiac pacemaker cells [112]. Interstitial cells of Cajal are a cell type that is known to be responsible for pacemaking in the smooth muscle cells of the gut. Work by Boedtkjer has confirmed the presence of interstitial Cajal-like cells in human thoracic ducts through a variety of imaging modalities, but their exact functional roles need to be further investigated [113]. Earlier work seems to indicate that these pacemaking cells can coordinate over distances of at least 80 mm [114]. The mechanism behind this coordination and the differentiation of these specialized pacemakers within a developing collecting vessel are areas of active research.

8.3.3 Extrinsic Factors that Contribute to Lymph Flow

Under healthy conditions, external factors seem to improve lymph flow and interstitial fluid clearance by the lymphatics. Notably, exercise and manual massaging improve the rate of clearance of tracers injected within the interstitium via the lymphatics and the quantity of lymph flowing through the collecting vessels [115–117]. The exact means by which the extrinsic factors improve these metrics are contested. Amplitudes of pressure changes within subcutaneous lower leg lymphatic collecting vessels (3.2–4.7 mmHg) were unchanged by exercise [118]. In addition, one study demonstrated that muscular contractions did not independently generate flow, but did increase lymph flow during intrinsically generated contractions but muscular contractions did not independently generate flow [119].

Rates of lymph formation and reliance on extrinsic factors vary for collecting vessels found in different regions of the skin, subcutaneous tissue, fascia, or muscle. Lymphatics within highly mobile tissue such as skeletal muscle, lungs, and the heart have undergone special adaptations to adapt to their local environments. A review article by Negrini and Mariondo nicely summarizes some of these adaptations [23]. For instance, their research demonstrates how both the organization and properties of diaphragmatic lymphatics take advantage of the stresses exerted by the muscle fibers. Lymphatic orientation, both perpendicular and parallel to skeletal muscle fibers, allowed lymphatic vessels to exploit the full contractile cycle of lungs for lymph generation and propulsion. In

addition, variations in the stiffness of diaphragmatic lymphatic walls allow compliant regions to act as a reservoir for lymph, while stiffer walled regions better allow local tissue displacement, leading to lymph propulsion [120]. The extent to which lymphatic orientation assists lymph propulsion in other tissue beds (e.g., cardiac tissue) is less known.

8.3.4 Collecting Lymphatics in Lymphedema

During lymphedema, lymph flow through the collectors appears to become increasingly dependent on extrinsic factors. Several studies demonstrate that intraluminal lymphatic pressure increases during lymphedema [121] and becomes more sensitive to contractions of surrounding skeletal muscle. The elevated sensitivity of lymphedemic limbs to manual massaging further highlights the reliance on extrinsic factors for lymph propulsion after the onset of lymphedema. Interstitial tissue pressures can increase to over 100 mmHg after application of manual massaging, creating a favorable pressure gradient for fluid to flow into the lymphatic system [122].

Structurally, it has been shown that the wall thickness of the lymphatic collecting vessel is strongly correlated with lymphedema disease severity. Rapid expansion of smooth muscle actin-positive cells and increased deposition of extracellular matrix elements such as collagen are hallmarks of vessels isolated from lymphedemic regions [123, 124]. While modeling indicates that these changes in vessel mechanics and dimensions would impact contractility [125], there is little work looking at functional pumping metrics of these remodeled vessels in vivo or in vitro. Many animal studies demonstrate that healthy collecting vessels exposed to edematous conditions, such as elevated pressures, have improved pumping metrics and lymph flow [70, 126, 127]. The extent to which these results translate to remodeled collecting vessels is unclear. In addition, it is difficult to determine if the increased lymph flow rates during edematous conditions are due to improved pumping of the collecting vessel or simply elevated rates of lymph formation.

Recent work by Mortimer et al. has shown that women with elevated lymphatic pumping are at a significantly higher risk of developing lymphedema later [74, 128]. They demonstrated that lymphatic pumping pressures before breast cancer surgery were 1.7-fold greater in patients who later developed lymphedema than those who did not. Clearance rates of tracers by the lymphatic system were consistently shown to be elevated by about 2.2-fold in patients who later developed lymphedema. The exact cause of this phenomena is still under investigation.

8.4 Lymph Nodes

8.4.1 Structure

Lymph moving through collecting vessels passes through at least one but often a series of lymph nodes. The human body contains hundreds of lymph nodes, which vary in size from 1 mm to 10 mm. Lymph nodes are vascularized for the exchange of nutrients and

immune cells [5, 6]. Lymph, arriving from multiple afferent collecting vessels, contains a variety of antigen-presenting cells, cytokines, antigens, and exosomes from upstream tissue beds, which play a critical role in the priming and activation of the immune system. Lymph nodes are comprised of multiple «compartments» which house various lymphocytes, and the route and quantity of lymph that arrives in each compartment may potentially impact immune response. Lymph first arrives into the subcapsular sinus and can then be directed through either a «central» or «peripheral» path to the medulla sinus [129]. The path by which solutes travel within the lymph node is dictated in part by hydraulic conductivity, with different conduits within the node having variable densities. Therefore, size alone of arriving solutes can dictate their route through the lymph node [130]. While the peripheral path takes lymph directly to the medulla sinus, the central path has lymph travelling through B cell follicles and the T cell cortex. Within the T cell cortex are high endothelial venules (HEVs) which allow circulating T cells to enter the lymph node.

8.4.2 Lymph Flow and Fluid Exchange

Recent models estimate that about 90% of the fluid that is carried from the afferent collecting vessel into the lymph node flows through paths along the periphery of the lymph node [53, 131, 133]. The remaining fluid that travels along the central path, deeper into the lymph node, is reabsorbed into blood circulation by parenchymal HEVs. While the amount of fluid reabsorbed into circulation at an individual node is relatively small, given the hundreds of lymph nodes within the body, it is estimated that an additional 4 L of fluid is returned to circulation at lymph nodes in basal conditions. This doubles the amount of fluid the lymphatics is responsible for clearing from the interstitial tissue space from previous estimates, which were solely based on the flow rates out of the major lymphatic ducts at lymphovenous junctions. This fluid exchange has been shown to be dictated by Starling's forces and is sensitive to changes in oncotic pressure of lymph or blood pressure at the HEVs [132]. Importantly, this fluid exchange within the node can alter the flow rate of lymph through the node as well as the composition of the efferent lymph. This alteration of lymph composition and flow implicates that the fluid exchange at the node not only regulates fluid recirculation but also activation of adaptive immunity.

Highlighted References

1. Baluk P, Fuxe J, Hashizume H, Romano T, Lashnits E, Butz S, et al. Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med [Internet]. 2007;204(10):2349–62. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2118470&tool=pmcentrez&rendertype=abstract.
2. Muthuchamy M, Gashev A, Boswell N. Molecular and functional analyses of the contractile apparatus in lymphatic muscle. FASEB J. 2003;17(8):920–2.
3. Davis MJ, Rahbar E, Gashev AA, Zawieja DC, Moore JE. Determinants of valve gating in collecting lymphatic vessels from rat mesentery. Am J Physiol Heart Circ Physiol. 2011;301(1):48–60.
4. Dixon JB, Greiner ST, Gashev AA, Cote GL, Moore JE, Zawieja DC. Lymph flow, shear stress, and lymphocyte velocity in rat mesenteric prenodal lymphatics. Microcirculation. 2006;13(7):597–610.
5. Schmid-Schonbein GW. Microlymphatics and lymph flow. Physiol Rev. 1990;70(4):987 LP–1028.

References

6. Swartz MA. The physiology of the lymphatic system. Adv Drug Deliv Rev. 2001;50(1–2):3–20.
7. Granger HJ. Role of the interstitial matrix and lymphatic pump in regulation of transcapillary fluid balance. Microvasc Res. 1979;18(2):209–16.
8. Levick JR. Flow through interstitium and other fibrous matrices. Q J Exp Physiol [Internet]. 1987; 72(4):409–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3321140.
9. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. Physiol Rev [Internet]. 1993;73(1):1 LP–78. Available from: http://physrev.physiology.org/content/73/1/1.abstract.
10. Olszewski WL. The lymphatic system in body homeostasis. Lymphat Res Biol. 2003;1(1):11–21.
11. Yoffey JM, Courtice FC. Lymphatics, lymph and the lymphomyeloid complex [Internet]. Academic Press. 1970. Available from: https://books.google.com/books?id=ROVqAAAAMAAJ.
12. Nipper ME, Dixon JB. Engineering the lymphatic system. Cardiovasc Eng Technol [Internet]. 2011;2(4):296–308. Available from: http://dx.doi.org/10.1007/s13239-011-0054-6.
13. Swartz MA, Kaipainen A, Netti P, Brekken C, Boucher Y, Grodzinsky A, et al. Mechanics of interstitial-lymphatic fluid transport: theoretical foundation and experimental validation. J Biomech. 1999;32(12):1297–307.
14. Leak L. Electron microscopic observations on lymphatic capillaries and the structural components of the connective tissue-lymph interface. Microvasc Res. 1970;2(4):361–91.
15. Bazigou E, Wilson JT, Moore JE. Primary and secondary lymphatic valve development: molecular, functional and mechanical insights. Microvasc Res. 2014;96(5):38–45.
16. Leak LV, Burke JF. Fine structure of the lymphatic capillary and the adjoining connective tissue area. Am J Anat United States. 1966;118(3):785–809.
17. Leak LV, Burke JF. Electron microscopic study of lymphatic capillaries in the removal of connective tissue fluids and particulate substances. Lymphology United States. 1968;1(2):39–52.
18. Trzewik J, Mallipattu SK, Artmann GM, Delano FA, Schmid-Schonbein GW. Evidence for a second valve system in lymphatics: endothelial microvalves. FASEB J Off Publ Fed Am Soc Exp Biol United States. 2001 Aug;15(10):1711–7.
19. Stacker SA, Williams SP, Karnezis T, Shayan R, Fox SB, Achen MG. Lymphangiogenesis and lymphatic vessel remodelling in cancer. Nat Rev Cancer [Internet] Nature Publishing Group. 2014;14(3):159–72. Available from: http://www.nature.com/doifinder/10.1038/nrc3677.
20. Gashev AA. Physiological aspects of lymphatic contractile function. Ann N Y Acad Sci. 2002;979:178–87.
21. Schmid-Schonbein GW. Mechanisms causing initial lymphatics to expand and compress to promote lymph flow. Arch Histol Cytol Japan. 1990;53(Suppl):107–14.
22. Casley-Smith JR. Are the initial lymphatics normally pulled open by the anchoring filaments? Lymphology [Internet]. 1980;13(3):120–9. Available from: http://europepmc.org/abstract/MED/7442305.
23. Negrini D, Moriondo A. Lymphatic anatomy and biomechanics. J Physiol. 2011;589(Pt 12):2927–34.
24. Triacca V, Guc E, Kilarski WW, Pisano M, Swartz MA. Transcellular pathways in lymphatic endothelial cells regulate changes in solute transport by fluid stress. Circ Res [Internet]. 2017. Available from: http://circres.ahajournals.org/content/early/2017/01/25/CIRCRESAHA.116.309828.abstract.
25. Kvietys PR, Granger DN. Role of intestinal lymphatics in interstitial volume regulation and transmucosal water transport. Ann N Y Acad Sci [Internet]. Blackwell Publishing Inc. 2010;1207:E29–43. Available from: http://dx.doi.org/10.1111/j.1749-6632.2010.05709.x.
26. Azzali G. The passage of macrophages and lymphocytes from the interstitium across the lymphatic endothelium of rat lacteals. Cell Tissue Res [Internet]. 1990;262(1):191–3. Available from: http://dx.doi.org/10.1007/BF00327761.
27. Randolph GJ, Miller NE. Lymphatic transport of high-density lipoproteins and chylomicrons. J Clin Invest [Internet] The American Society for Clinical Investigation. 2014;124(3):929–35. Available from: https://doi.org/10.1172/JCI71610.
28. Florence AT, Hussain N. Transcytosis of nanoparticle and dendrimer delivery systems: evolving vistas. Adv Drug Deliv Rev [Internet]. 2001;50(Supple):S69–89. Available from: www.sciencedirect.com/science/article/pii/S0169409X01001843.
29. Reed AL, Rowson SA, Dixon JB. Demonstration of ATP dependent, transcellular transport of lipid across the lymphatic endothelium using an in vitro model of the lacteal. Pharm Res [Internet].

2013;30(12). doi:10.1007/s11095-013-1218-x. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3849718/.
30. Dixon JB. Mechanisms of chylomicron uptake into lacteals. Ann N Y Acad Sci [Internet] Blackwell Publishing Inc. 2010;1207:E52–7. Available from: http://dx.doi.org/10.1111/j.1749-6632.2010.05716.x.
31. Lee JS. Tissue fluid pressure, lymph pressure, and fluid transport in rat intestinal villi. Microvasc Res. 1986;31(2):170–83.
32. Dixon JB. Lymphatic lipid transport: sewer or Subway? Trends Endocrinol Metab [Internet]. 2010;21(8):480–7. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2914116/.
33. Dobbins WO. Intestinal mucosal lacteal in transport of macromolecules and chylomicrons. Am J Clin Nutr [Internet]. 1971;24(1):77–90. Available from: http://ajcn.nutrition.org/content/24/1/77.abstract.
34. Florey H. Observations on the contractility of lacteals. J Physiol [Internet]. 1927;62(3):267–72. Available from: http://dx.doi.org/10.1113/jphysiol.1927.sp002357.
35. Florey H. Observations on the contractility of lacteals: part II. J Physiol [Internet]. 1927;63(1):1–18. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1514918/.
36. Choe K, Jang JY, Park I, Kim Y, Ahn S, Park DY, et al. Intravital imaging of intestinal lacteals unveils lipid drainage through contractility. J Clin Invest. 2015;125(11):4042–52.
37. Tso P, Pitts V, Granger D. Role of lymph flow in intestinal chylomicron transport. Am J Phys. 1985;249(1):G21–8.
38. Dixon JB, Raghunathan S, Swartz MA. A tissue-engineered model of the intestinal lacteal for evaluating lipid transport by lymphatics. Biotechnol Bioeng. 2009;103(6):1224–35.
39. Nurmi H, Saharinen P, Zarkada G, Zheng W, Robciuc MR, Alitalo K. VEGF-C is required for intestinal lymphatic vessel maintenance and lipid absorption. EMBO Mol Med [Internet]. 2015. Available from: http://embomolmed.embopress.org/content/early/2015/10/09/emmm.201505731.abstract.
40. Lynch PM, Delano FA, Schmid-Schonbein GW. The primary valves in the initial lymphatics during inflammation. Lymphat Res Biol [Internet]. 2007;5(1):3–10. Available from: www.ncbi.nlm.nih.gov/pubmed/17508898%5Cnhttp://online.liebertpub.com/doi/pdfplus/10.1089/lrb.2007.5102.
41. Russo E, Teijeira A, Vaahtomeri K, Willrodt A-H, Bloch JS, Nitschké M, et al. Intralymphatic CCL21 promotes tissue egress of dendritic cells through afferent lymphatic vessels. Cell Rep [Internet] Elsevier. 2017;14(7):1723–34. Available from: http://dx.doi.org/10.1016/j.celrep.2016.01.048.
42. Weber M, Hauschild R, Schwarz J, Moussion C, de Vries I, Legler DF, et al. Interstitial dendritic cell guidance by haptotactic chemokine gradients. Science [Internet]. 2013;339(6117):328 LP–332. Available from: http://science.sciencemag.org/content/339/6117/328.abstract.
43. Miteva DO, Rutkowski JM, Dixon JB, Kilarski W, Shields JD, Swartz MA. Transmural flow modulates cell and fluid transport functions of lymphatic endothelium. Circ Res [Internet]. 2010;106(5):920 LP–931. Available from: http://circres.ahajournals.org/content/106/5/920.abstract.
44. Johnson LA, Clasper S, Holt AP, Lalor PF, Baban D, Jackson DG. An inflammation-induced mechanism for leukocyte transmigration across lymphatic vessel endothelium. J Exp Med [Internet]. 2006;203(12):2763–77. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2118156&tool=pmcentrez&rendertype=abstract.
45. Zampell JC, Aschen S, Weitman ES, Yan A, Elhadad S, De Brot AM, et al. Regulation of adipogenesis by lymphatic fluid stasis part I: adipogenesis, fibrosis, and inflammation. Plast Reconstr Surg [Internet]. 2012;129(4):825–34. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3433726/.
46. Rockson SG. The lymphatics and the inflammatory response: lessons learned from human lymphedema. Lymphat Res Biol. 2013;11(3):117–20. Available from: http://dx.doi.org/10.1089/lrb.2013.1132.
47. Von Der Weid PY. Review article: lymphatic vessel pumping and inflammation – the role of spontaneous constrictions and underlying electrical pacemaker potentials. Aliment Pharmacol Ther. 2001;15(8):1115–29.
48. Lynch LL, Mendez U, Waller AB, Gillette AA, Guillory RJ, Goldman J. Fibrosis worsens chronic lymphedema in rodent tissues. Am J Physiol Heart Circ Physiol. 2015;308(10):H1229–36.
49. Olszewski WL, Jain P, Ambujam G, Zaleska M, Cakala M, Gradalski T. Tissue fluid pressure and flow during pneumatic compression in lymphedema of lower limbs. Lymphat Res Biol. 2011;9(2):77–83.
50. Kim H, Kataru RP, Koh GY. Inflammation-associated lymphangiogenesis: a double-edged sword? J Clin Invest [Internet] (The American Society for Clinical Investigation). 2014;124(3):936–42. Available from: https://doi.org/10.1172/JCI71607.

51. Ogata F, Fujiu K, Matsumoto S, Nakayama Y, Shibata M, Oike Y, et al. Excess Lymphangiogenesis cooperatively induced by macrophages and CD4+ T cells drives the pathogenesis of lymphedema. J Invest Dermatol [Internet]. 2016;136(3):706–14. Available from: www.sciencedirect.com/science/article/pii/S0022202X15000925.
52. Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. Nature [Internet]. 2005;438(7070):946–53. Available from: http://dx.doi.org/10.1038/nature04480.
53. Karkkainen MJ, Ferrell RE, Lawrence EC, Kimak MA, Levinson KL, McTigue MA, et al. Missense mutations interfere with VEGFR-3 signalling in primary lymphoedema. Nat Genet [Internet]. 2000;25(2):153–9. Available from: http://dx.doi.org/10.1038/75997.
54. Makinen T, Jussila L, Veikkola T, Karpanen T, Kettunen MI, Pulkkanen KJ, et al. Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. Nat Med [Internet]. 2001;7(2):199–205. Available from: http://dx.doi.org/10.1038/84651.
55. Jeltsch M, Tammela T, Alitalo K, Wilting J. Genesis and pathogenesis of lymphatic vessels. Cell Tissue Res [Internet]. 2003;314(1):69–84. Available from: http://dx.doi.org/10.1007/s00441-003-0777-2.
56. Saito Y, Nakagami H, Kaneda Y, Morishita R. Lymphedema and therapeutic lymphangiogenesis. Biomed Res Int. 2013;2013:804675.
57. Rutkowski JM, Moya M, Johannes J, Goldman J, Swartz MA. Secondary lymphedema in the mouse tail: lymphatic hyperplasia, VEGF-C upregulation, and the protective role of MMP-9. Microvasc Res [Internet]. 2006;72(3):161–71. Available from: http://www.sciencedirect.com/science/article/pii/S0026286206000720.
58. Liao S, von der Weid P-Y. Inflammation-induced lymphangiogenesis and lymphatic dysfunction. Angiogenesis [Internet]. 2014;17(2):325–34. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3981917/.
59. Scavelli C, Weber E, Aglianò M, Cirulli T, Nico B, Vacca A, et al. Lymphatics at the crossroads of angiogenesis and lymphangiogenesis. J Anat. 2004;204(6):433–49.
60. Sacchi G, Weber E, Agliano M, Raffaelli N, Comparini L. The structure of superficial lymphatics in the human thigh: precollectors. Anat Rec. 1997;247(1):53–62.
61. Albertine KH, Fox LM, O'Morchoe CCC. The morphology of canine lymphatic valves. Anat Rec. 1982;202(4):453–61.
62. Pan W-R, le Roux CM, Levy SM, Briggs CA. The morphology of the human lymphatic vessels in the head and neck. Clin Anat. 2010;23(March):654–61.
63. McHale NG, Roddie IC. The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. J Physiol. 1976;261(2):255–69.
64. Geng X, Cha B, Mahamud MR, Lim KC, Silasi-Mansat R, Uddin MKM, et al. Multiple mouse models of primary lymphedema exhibit distinct defects in lymphovenous valve development. Dev Biol Elsevier. 2016;409(1):218–33.
65. Hess PR, Rawnsley DR, Jakus Z, Yang Y, Sweet DT, Fu J, et al. Platelets mediate lymphovenous hemostasis to maintain blood-lymphatic separation throughout life. J Clin Invest. 2014;124(1):273–84.
66. Muthuchamy M, Zawieja D. Molecular regulation of lymphatic contractility. Ann N Y Acad Sci. 2008;1131:89–99.
67. Gashev AA, Davis MJ, Delp MD, Zawieja DC. Regional variations of contractile activity in isolated rat lymphatics. Microcirculation. 2004;11(6):477–92.
68. McHale NG, Roddie IC. Pumping activity in isolated segments of bovine mesenteric lymphatics. J Physiol. 1975;261(1):70–2.
69. Jamalian S, Davis MJ, Zawieja DC, Moore JE. Network scale modeling of lymph transport and its effective pumping parameters. PLoS One. 2016;11(2):1–18.
70. Davis MJ, Scallan JP, Wolpers JH, Muthuchamy M, Gashev AA, Zawieja DC. Intrinsic increase in lymphangion muscle contractility in response to elevated afterload. Am J Physiol Heart Circ Physiol. 2012;303(7):H795–808.
71. Zawieja DC, Davis KL, Schuster R, Hinds WM, Granger HJ. Distribution, propagation, and coordination of contractile activity in lymphatics. Am J Phys. 1993;264(4 Pt 2):H1283–91.
72. Eisenhoffer J, Kagal A, Klein T, Johnston MG. Importance of valves and lymphangion contractions in determining pressure gradients in isolated lymphatics exposed to elevations in outflow pressure. Microvasc Res. 1995;49:97–110.

73. Unno N, Nishiyama M, Suzuki M, Tanaka H, Yamamoto N, Sagara D, et al. A novel method of measuring human lymphatic pumping using indocyanine green fluorescence lymphography. J Vasc Surg Elsevier Inc. 2010;52(4):946–52.
74. Cintolesi V, Stanton AW, BCh M, Bains SK, Cousins E, Michael Peters A, et al. Constitutively enhanced lymphatic pumping in the upper limbs of women who later develop breast cancer-related lymphedema. Lymphat Res Biol. 2016;14(2):50–61.
75. Nelson TS, Akin RE, Weiler MJ, Kassis T, Kornuta JA, Dixon JB. Minimally invasive method for determining the effective lymphatic pumping pressure in rats using near-infrared imaging. Am J Physiol Regul Integr Comp Physiol. 2014;306(5):R281–90.
76. Von Der WP, Zawieja DC. Lymphatic smooth muscle: the motor unit of lymph drainage. Int J Biochem Cell Biol. 2004;36:1147–53.
77. Ohhashi T. The response of lymphatic smooth muscles to vasoactive substances. Pflugers Arch. 1986;c:341–7.
78. McHale N. Nervous control of the lymphatic system. Vasc Med Rev. 1993;4:307–19.
79. Amerini S, Ziche M, Greiner ST, Zawieja DC. Effects of substance P on mesenteric lymphatic contractility in the rat. Lymphat Res Biol. 2004;2(1):2–10.
80. Allen JM, McHale NG, Rooney BM. Effect of norepinephrine on contractility of isolated mesenteric lymphatics. Am J Physiol Heart Circ Physiol. 1983;244(4):H479 LP–H486.
81. Allen JM, Iggulden HL, McHale NG. Beta-adrenergic inhibition of bovine mesenteric lymphatics. J Physiol. 1986;374:401–11.
82. ALLEN JM, BURKE EP, JOHNSTON MG, NG MH. The inhibitory effect of aspirin on lymphatic contractility. Br J Pharmacol. 1984;82(2):509–14.
83. Allen JM, McHale NG. Neuromuscular transmission in bovine mesenteric lymphatics. Microvasc Res. 1986;31(1):77–83.
84. Scallan JP, Davis MJ, Huxley VH. Permeability and contractile responses of collecting lymphatic vessels elicited by atrial and brain natriuretic peptides. J Physiol. 2013;591(Pt 20):5071–81.
85. Ivanov S, Scallan JP, Kim KW, Werth K, Johnson MW, Saunders BT, et al. CCR7 and IRF4-dependent dendritic cells regulate lymphatic collecting vessel permeability. J Clin Invest. 2016;126(4):1581–91.
86. Kuan EL, Ivanov S, Bridenbaugh EA, Victora G, Wang W, Childs EW, et al. Collecting lymphatic vessel permeability facilitates adipose tissue inflammation and distribution of antigen to lymph node–homing adipose tissue dendritic cells. J Immunol. 2015;194(11):5200–10.
87. Fonseca DMD, Hand TW, Han SJ, Gerner MY, Zaretsky AG, Byrd AL, et al. Microbiota-dependent Sequelae of acute infection compromise tissue-specific immunity. Cell Elsevier Inc. 2015;163(2):354–66.
88. Chatterjee V, Gashev AA. Aging-associated shifts in functional status of mast cells located by adult and aged mesenteric lymphatic vessels. AJP Hear Circ Physiol. 2012;303(6):H693–702.
89. Jawdat DM, Albert EJ, Rowden G, Haidl ID, Marshall JS. IgE-mediated mast cell activation induces Langerhans cell migration in vivo. J Immunol. 2004;173(8):5275–82.
90. Liao S, Cheng G, Conner DA, Huang Y, Kucherlapati RS, Munn LL, et al. Impaired lymphatic contraction associated with immunosuppression. Proc Natl Acad Sci U S A. 2011;108(46):18784–9.
91. Gashev AA, Davis MJ, Zawieja DC. Inhibition of the active lymph pump by flow in rat mesenteric lymphatics and thoracic duct. J Physiol. 2002;540(Pt 3):1023–37.
92. von der Weid PY, Crowe MJ, Van Helden DF. Endothelium-dependent modulation of pacemaking in lymphatic vessels of the guinea-pig mesentery. J Physiol [Internet]. 1996;493(Pt 2):563–75. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1158938&tool=pmcentrez&rendertype=abstract.
93. Schmid-Schönbein GW. Nitric oxide (NO) side of lymphatic flow and immune surveillance. Proc Natl Acad Sci U S A. 2012;109(1):3–4.
94. Nizamutdinova IT, Maejima D, Nagai T, Bridenbaugh E, Thangaswamy S, Chatterjee V, et al. Involvement of histamine in endothelium-dependent relaxation of mesenteric lymphatic vessels. Microcirculation. 2014;21(7):640–8.
95. Kornuta JA, Nepiyushchikh Z, Gasheva OY, Mukherjee A, Zawieja DC, Dixon JB, et al. Effects of dynamic shear and transmural pressure on wall shear stress sensitivity in collecting lymphatic vessels. Am J Physiol Regul Integr Comp Physiol. 2015;309(9):R1122–34.

96. Kassis T, Yarlagadda SC, Kohan AB, Tso P, Breedveld V, Dixon JB. Post-prandial lymphatic pump function after a high-fat meal: a characterization of contractility, flow and viscosity. Am J Physiol Gastrointest Liver Physiol. 2016;310(10). doi:10.1152/ajpgi.00318.2015.
97. Jafarnejad M, Cromer WE, Kaunas RR, Zhang SL, Zawieja DC, Moore JE, et al. Measurement of shear stress-mediated intracellular calcium dynamics in human dermal lymphatic endothelial cells. Am J Physiol Heart Circ Physiol. 2015;308(7):H697–706.
98. Baeyens N, Nicoli S, Coon BG, Ross TD, Van Den Dries K, Han J, et al. Vascular remodeling is governed by a vegfr3-dependent fluid shear stress set point. elife. 2015;2015(4):1–35.
99. Munn LL. Mechanobiology of lymphatic contractions. Semin Cell Dev Biol Elsevier Ltd. 2015;38:67–74.
100. Telinius N, Drewsen N, Pilegaard H, Kold-Petersen H, de Leval M, Aalkjaer C, et al. Human thoracic duct in vitro: diameter-tension properties, spontaneous and evoked contractile activity. Am J Physiol Heart Circ Physiol. 2010;299(3):H811–8.
101. Gashev AA, Zhang RZ, Muthuchamy M, Zawieja DC, Davis MJ. Regional heterogeneity of length-tension relationships in rat lymph vessels. Lymphat Res Biol. 2012;10(1):14–9.
102. Gashev AA, Zawieja DC. Hydrodynamic regulation of lymphatic transport and the impact of aging. Pathophysiology [Internet] Elsevier Ireland Ltd. 2010;17(4):277–87. Available from: http://dx.doi.org/10.1016/j.pathophys.2009.09.002.
103. McHale NG, Thornbury K. A method for studying lymphatic pumping activity in conscious and anaesthetized sheep. J Physiol. 1986;378(1):109–18.
104. Mawhinney H, Roddie IC. Spontaneous activity in isolated bovine mesenteric lymphatics. J Physiol. 1973;229(2):339–48.
105. Cotton KD, Hollywood MA, McHale NG, Thornbury KD. Outward currents in smooth muscle cells isolated from sheep mesenteric lymphatics. J Physiol. 1997;503(1):1–11.
106. Beckett EAH, Hollywood MA, Thornbury KD, McHale NG. Spontaneous electrical activity in sheep mesenteric lymphatics. Lymphat Res Biol. 2007;5(1):29–43.
107. Imtiaz MS, Zhao J, Hosaka K, von der Weid P-Y, Crowe M, van Helden DF. Pacemaking through Ca2+ stores interacting as coupled oscillators via membrane depolarization. Biophys J [Internet]. Elsevier. 2007;92(11):3843–61. Available from: http://dx.doi.org/10.1529/biophysj.106.095687.
108. Telinius N, Majgaard J, Kim S, Katballe N, Pahle E, Nielsen J, et al. Voltage-gated sodium channels contribute to action potentials and spontaneous contractility in isolated human lymphatic vessels. J Physiol. 2015;593(14):3109–22.
109. Lee S, Roizes S, von der Weid P-Y. Distinct roles of L- and T-type voltage-dependent Ca 2+ channels in regulation of lymphatic vessel contractile activity. J Physiol. 2014;592(24):5409–27.
110. Von Der Weid P-Y, Lee S, Imtiaz MS, Zawieja DC, Davis MJ. Electrophysiological properties of rat mesenteric lymphatic vessels and their regulation by stretch. Lymphat Res Biol. 2014;12:66–75.
111. Van HD. Pacemaker potentials in lymphatic smooth muscle of the guinea-pig mesentery. J Physiol [Internet]. 1993;471:465–79. Available from: http://jp.physoc.org/content/471/1/465.short.
112. McCloskey KD, Toland HM, Hollywood MA, Thornbury KD, McHale NG. Hyperpolarisation-activated inward current in isolated sheep mesenteric lymphatic smooth muscle. J Physiol. 1999;521:Pt 1:201–11.
113. Briggs Boedtkjer D, Rumessen J, Baandrup U, Skov Mikkelsen M, Telinius N, Pilegaard H, et al. Identification of interstitial cajal-like cells in the human thoracic duct. Cells Tissues Organs. 2013;197(2):145–8.
114. McHale NG, Meharg MK. Co-ordination of pumping in isolated bovine lymphatic vessels. J Physiol. 1992;450:503–12.
115. Survivors B. Exercise and the lymphatic system. Sports Med. 2005;35(6):461–71.
116. Coates G, O'Brodovich H, Goeree G. Hindlimb and lung lymph flows during prolonged exercise. J Appl Physiol. 1993 Aug;75(2):633 LP–638.
117. Havas E, Parviainen T, Vuorela J, Toivanen J, Nikula T, Vihko V. Lymph flow dynamics in exercising human skeletal muscle as detected by scintography. J Physiol. 1997;504(1):233–9.
118. Sjöberg T, Norgren L, Steen S. Contractility of human leg lymphatics during exercise. Pdf. Lymphology. 1989;22(4):186–93.
119. Olszewski WL, Engeset A. Intrinsic contractility of prenodal lymph vessels and lymph flow in human leg. Am J Physiol Heart Circ Physiol. 1980 Dec;239(6):H775 LP–H783.

120. Moriondo A, Solari E, Marcozzi C, Negrini D. Diaphragmatic lymphatic vessel behavior during local skeletal muscle contraction. Am J Physiol Heart Circ Physiol. 2015;308(3):pH193–205.
121. Gretener SB, Läuchli S, Leu AJ, Koppensteiner R, Franzeck UK. Effect of venous and lymphatic congestion on lymph capillary pressure of the skin in healthy volunteers and patients with lymph edema. J Vasc Res. 2000;37(1):61–7.
122. Olszewski WL, Jain P, Ambujam G, Zaleska M, Cakala M, Gradalski T. Tissue fluid pressure and flow in the subcutaneous tissue in lymphedema – hints for manual and pneumatic compression therapy. Phlebolymphology. 2010;17(3):144–50.
123. Ogata F, Fujiu K, Koshim I, Nagai R, Manabe I, Manabe I. Phenotypic modulation of smooth muscle cells in lymphedema. Br J Dermatol. 2013;172(5):1286–93.
124. Mihara M, Hara H, Hayashi Y, Narushima M, Yamamoto T, Todokoro T, et al. Pathological steps of cancer-related lymphedema: histological changes in the collecting lymphatic vessels after lymphadenectomy. PLoS One. 2012;7(7):1–10.
125. Caulk AW, Dixon JB, Gleason RL. A lumped parameter model of mechanically mediated acute and long-term adaptations of contractility and geometry in lymphatics for characterization of lymphedema. Biomech Model Mechanobiol. 2016;5(6):1601–18.
126. Dongaonkar RM, Nguyen TL, Quick CM, Hardy J, Laine GA, Wilson E, et al. Adaptation of mesenteric lymphatic vessels to prolongedchanges in transmural pressure. Am J Physiol Heart Circ Physiol. 2013;305(9):203–10.
127. Rahbar E, Akl T, Coté GL, Moore JE, Zawieja DC. Lymph transport in rat mesenteric lymphatics experiencing edemagenic stress. Microcirculation. 2014;21(5):359–67.
128. Bains SK, Stanton AWB, Cintolesi V, Ballinger J, Allen S, Zammit C, et al. A constitutional predisposition to breast cancer-related lymphoedema and effect of axillary lymph node surgery on forearm muscle lymph flow. Breast Elsevier Ltd. 2015;24(1):68–74.
129. Heath TJ, Kerlin RL, Spalding HJ. Afferent pathways of lymph flow within the popliteal node in sheep. J Anat. 1986;149:65–75.
130. Roozendaal R, Mempel TR, Pitcher LA, Santiago F, Verschoor A, Mebius RE, et al. Conduits mediate transport of low molecular weight antigen to lymph node follicles. Immunity. 2009;30(2):264–76.
131. Adair TH, Guyton AC. Modification of lymph by lymph nodes. II. Effect of increased lymph node venous blood pressure. Am J Physiol Heart Circ Physiol. 1983;245(4):H616 LP–H622.
132. Adair TH, Guyton AC. Modification of lymph by lymph nodes. III. Effect of increased lymph hydrostatic pressure. Am J Physiol Heart Circ Physiol. 1985;249(4):H777 LP–H782.
133. Jafarnejad M, Woodruff MC, Zawieja DC, Carroll MC, Moore JE. Modeling lymph flow and fluid exchange with blood vessels in lymph nodes. Lymphat Res Biol. 2015;13(4):234–47.