LYMPHATIC RESEARCH AND BIOLOGY
Volume 7, Number 2, 2009

DOI: 10.1089/lrb.2009.0007

Proceedings of a Mini-Symposium: Lymphedema: An Overview of the Biology, Diagnosis, and Treatment of the Disease

Contractile Physiology of Lymphatics

David C. Zawieja, Ph.D.

Abstract

The lymphatic system has important roles in body fluid regulation, macromolecular homeostasis, lipid absorption, and immune function. To accomplish these roles, lymphatics must move fluid and its other contents (macromolecules, lipids/chylomicra, immune cells) from the interstitium through the lymphatics, across the nodes, and into the great veins. Thus, the principal task of the lymphatic vascular system is transport. The body must impart energy to the lymph via pumping mechanisms to propel it along the lymphatic network and use pumps and valves to generate lymph flow and prevent its backflow. The lymphatic system utilizes both extrinsic pumps, which rely on the cyclical compression and expansion of lymphatics by surrounding tissue forces, and intrinsic pumps, which rely on the intrinsic rapid/phasic contractions of lymphatic muscle. The intrinsic lymph pump function can be modulated by neural, humoral, and physical factors. Generally, increased lymph pressure/stretch of the muscular lymphatics activates the intrinsic lymph pump, while increased lymph flow/shear in the muscular lymphatics can either activate or inhibit the intrinsic lymph pump depending on the pattern and magnitude of the flow. To regulate lymph transport, lymphatic pumping and resistance must be controlled. A better understanding of these mechanisms could provide the basis for the development of better diagnostic and treatment modalities for lymphatic dysfunction.

The Lymphatic Transport System

The lymphatic system moves fluid from the interstitial spaces in the tissue parenchyma into the network of lymphatic vessels, through a series of lymph nodes into the postnodal lymph ducts that converge into the thoracic duct (for the lower half and upper left quadrant of the body) and right lymphatic duct (for the upper right quadrant of the body) before eventually emptying their lymph into the great veins. The unidirectional movement of lymph through this network is necessary for the transport of fluid, macromolecules, lipids, antigens, immune cells, and particulate matter. Thus, all of the important functions that the lymphatic system must accomplish to maintain body homeostasis depend on the controlled transport of lymph from the initial lymphatics to the great veins. Over the last 10 years, great strides have been made in the molecular and cellular processes that drive the formation and/or regeneration of the lymphatic vessels. This has greatly advanced our understanding of the developmental and remodeling processes that govern some of the structural considerations of the lymphatic system, particularly those in the initial lymphatic vessels where lymph is formed. Much less recent effort has been placed into the study of the lymphatic structures at any level beyond the most peripheral parts of the lymphatic network.

An understanding of the structure and function of the lymphatic architecture must go hand in hand if we are to develop a true appreciation of the impact of the lymphatic system in health and disease. When evaluating the lymphatic system it is crucial to remember that its principal purpose is the transport of lymph and it is by this regulated transport that ALL of the body's homeostatic functions that the lymphatic system participates in are served. Lastly but importantly, lymph transport includes not only the initial formation of lymph in the lymphatic capillaries but also the movement of lymph along the rest of the lymphatic network on its route to the veins.

Hydrodynamics of Lymph Transport

Under steady states, most interstitial fluid pressures are either near atmospheric (i.e., near zero cm H_2O relative pressure) or subatmospheric (-1 to -5 cm H_2O, i.e., negative

Department of Systems Biology and Translational Medicine, Cardiovascular Research Institute Division of Lymphatic Biology, Texas A&M Health Science Center College of Medicine, Temple, Texas.
This work was supported by grants from National Institutes of Health HL-07688, HL-75199, HL-080526, and HL085659.

relative pressures).[1] Because of the great difficulty in performing accurate measures of pressure in the initial and early collecting lymphatics, there are not many measurements of pressures in the initial lymphatics. There are many more measures of pressures in the immediate downstream components of the lymphatic vascular network, the early lymphatic collecting vessels. However, in most instances the pressures measured in these vessels are on average slightly positive.[2–7] Thus it would appear that the predominant fluid pressure gradient is one that does NOT favor fluid entering the initial lymphatic. So how does fluid enter the initial lymphatic structure to form lymph? There have been a few theories that have come about over the years to account for this process, but the data to support most of these theories have not been strong. One longstanding theory with good data to support it is that although the average prevailing pressure gradient between the interstitial fluid and the initial lymph opposes fluid entering the lymphatic, there are cyclical changes in both the interstitial fluid pressure and the lymph pressure in the initial lymphatic because of activities in either the tissue in which the lymphatic exists or the lymphatic itself that will produce a pressure gradient that transiently favors the formation of lymph.[8–13]

Anatomically, the initial lymphatics are composed of a layer of endothelial cells that have apparent transient gaps between parts of the adjacent endothelial cells. The endothelium of the initial lymphatics are unique in that they have valves not only across their lumen between the initial lymphatic bulb and the early collecting lymphatic plexus but also within in the walls of the initial lymphatics at these transient "gap sites" between adjacent lymphatic endothelial cells. Another unique characteristic is that the lymphatic endothelium is physically tethered into the surrounding tissue structure through the anchoring filaments.[14–16] These unique structural characteristics coupled with the changing physical conditions in active tissues allow lymph to be formed when interstitial pressure is higher than the lymph pressure in the initial lymphatic. This opens the initial lymphatic valves and fluid enters the initial lymphatics through these valves.[17–19]

As the pressure profile across the initial lymphatic wall changes because of either tissue or lymphatic activities, to one that opposes lymph formation, these initial lymphatic valves close to prevent the movement of fluid back out of the initial lymphatic. Thus one gets transient lymph formation as a result of activity that drives the pressure gradient across the lymphatic wall towards one that favors lymph formation. Activities known to be important to this process are cyclical tissue deformations such as skeletal muscle contraction, heart contraction, gastrointestinal muscle contraction, breathing, and intrinsic contraction of the initial lymphatic. These same activities are also known to be responsible for creating local pressure gradients along the early lymphatic network that move fluid out of the initial lymphatic into the collecting vessels.[10,11,13,20] Importantly, when either the intrinsic lymphatic contraction or the extrinsic tissue deformation enters a phase of relaxation or decompression, the initial lymphatic structure is "pulled open" because of the transmission of stored tissue energy through the anchoring filaments to the initial lymphatic wall. This can once again create a situation where the pressure gradients between the interstitium, the initial lymphatic, and the downstream collecting lymphatics transiently favor lymph formation.[8–11]

An analogous process is repeated along the lymphatic tree, moving lymph through the lymphatic network. However, since again the steady state fluid pressures along the lymphatic network are not conducive to the passive movement of fluid down a standing pressure gradient, as was shown decades ago in dogs (Table 1).[21] The average lymph pressures in the more peripheral parts of the lymphatic network have lower pressures than do the final outflow tracts (thoracic duct and right lymphatic duct) of the lymphatic network and the average lymph pressures in the final outflow tracts are lower than the venous circulation into which they empty. Confounding this problem in animals that spend a significant part of their life in the upright position is the influence of gravitational forces on the lymph pressures.[22,23] In the average height, upright human, there is a potential hydrostatic pressure gradient of -150 cm H_2O from the feet to the great veins of the neck. However, because this hydrostatic column of fluid in the lymphatic network is broken by the presence of the valves and nodal structures, the effective hydrostatic pressure gradient that opposes net central lymph flow is much less. Thus, lymph normally does not "drain" down the lymphatic network passively as is commonly described. Instead energy must be imparted to the lymph fluid in some fashion in order to transport it along the lymphatic network. In lower vertebrates, the lymphatic system utilizes a number of specialized "lymphatic hearts" situated in numerous locations within their bodies to drive lymph flow. Since humans and other mammals do not have the specialized lymphatic hearts that lower vertebrates do, we use a series of pumps and valves to overcome these prevailing pressure gradients and move lymph along the lymphatic network, through the nodes on to the great veins in the neck. Without the actions of these lymphatic pumps and valves, the proper function of the lymphatic system (i.e., transport) cannot occur. Consequently, to study the function/dysfunction of the lymphatic system, one must always consider the functions of the pumps and valves.

The Secondary Lymphatic Valve System

Once again, because the typical pressure gradients oppose central flow along the lymphatic network, to keep flow in the right direction, unidirectional valves are present.[24–31] These important structures are a hallmark of the lymphatic network and their proper function depends upon some unique properties of the lymphatic.[24,26,29,30,32,33] The function of the lymphatic valves is thought to be driven by the pressures and flow of fluid across them, given their unique structure.

Table 1. Hydrodynamics of Lymph Transport Along the Lymphatic Network. Mean Pressures in Different Lymphatic/Vascular Compartments in the Dog

Compartment	*Tissue*	*Pressure (cm H_2O)*
Upstream network sites	Femoral lymphatic	0.7
	Cardiac lymphatic	3.8
	Mesenteric lymphatic	4.7
Lymphatic outflow tracts	Thoracic duct	6.6
	Right lymphatic duct	2.8
Venous destination	Jugular vein	7.6

However, there is also some evidence for a possible role of specialized lymphatic muscle in the closure of these structures in some lymphatics.[34] The critical role of these unique valves in lymph transport is highlighted in the development of lymphedema seen as a result of genetic mutations of the FOXC2 gene in mice and humans. FOXC2 is a genetic determinant of valves and loss of its normal function leads to lymphatic valvular failure and the development of lymphedema.[35–37]

The motion of a secondary lymphatic valve in an isolated rat mesenteric lymphatic throughout the intrinsic lymphatic contraction cycle can be seen in Fig. 1 and the supplemental video. The images show the vessel at the site of a lymphatic valve with the input (more peripheral section) oriented at the top of the images, the valve structure cut across the two leaflets (left and right highlighted in black) and the next downstream lymphatic sinus. Panels A–D depict the changes in the lymphatic structure due to the constriction of the lymphatic muscle during lymphatic pumping over an approximate 3-sec interval. As seen in panel A, the lymphatic is at rest (lymphatic diastole), the diameter of the lymphatic is ~ 105 um at the arrow and the upper sections of the valve leaflets are sharply angled downward; the leaflets are partially open. Panel B is 1.1 sec later and demonstrate the strong, rapid phasic contraction (lymphatic systole); the diameter at the arrow is now $\sim 60\,\mu$m and the valve leaflets are still sharply angled downward and are open. Panel C is 0.78 sec later near the end of lymphatic systole; the diameter is similar in size to that in Panel B but the upper segments of the valve leaflets are "ballooned backwards" effectively closing the valve. Panel D represents the lymphatic 1.0 sec later when the lymphatic is once again in lymphatic diastole and the valve leaflets are reopening.

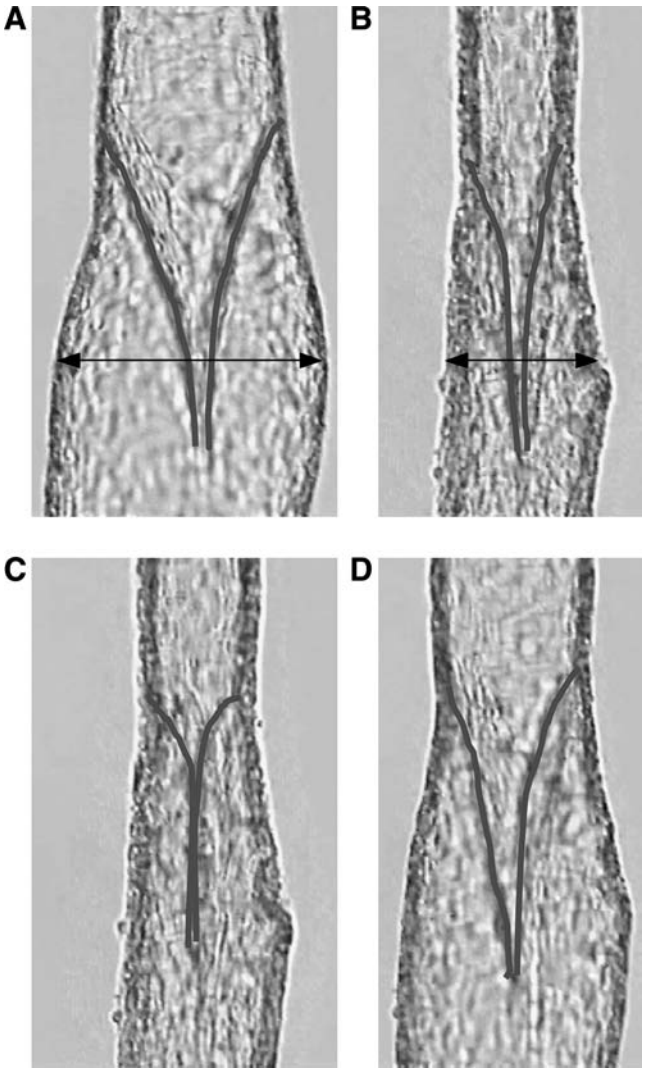

FIG. 1. Micrographs of an isolated rat mesenteric lymphatic at different stages of the intrinsic contractile cycle. The images depict a pre-valvular end of the lymphangion at the top of the image, the valve leaflets, and the post-valvular sinus of the next lymphangion. The valve leaflets are highlighted in black to show their position and orientation. (**A**) the end of diastole, (**B**) the vessel near the end ($\sim$ 0.2 s before the end of lymphatic systole), (**C**) the end of systole, (**D**) the middle of the subsequent diastole. See accompanying supplemental movie to show live action of the intrinsic pump and the valve leaflets. (Supplemental video online at www.liebertpub.com.)

A closer look at the lymphatic valve structure can be seen in Figure 2 and the supplemental video. These images were obtained in an isolated pressurized rat mesenteric lymphatic that was loaded with the intravital cellular fluorescent dye Cell Tracker Green. This dye will load into the cytoplasm of all living cells within the tissue. It was then imaged at a transmural pressure of 3 cm H_2O in calcium-free solution to stop the phasic contractions with a confocal/multiphoton microscope three-dimensionally at $\sim 0.2\,\mu$m intervals in the z axis and reconstructed in different orientations depicted in Panels A–D to display lymphatic structure. Panel A shows the vessel from the side with the more peripheral (input end) to the right. Panel B shows the lymphatic segment rotated 90 degrees with the input end facing the viewer; note the smooth transition to the lymphatic endothelial lined valve leaflets and the valvular opening into the sinus segment of the next lymphangion. The difference in cross-sectional areas of the end of the input segment, the outlet of the valve leaflets, and the beginning of the next lymphangion sinus is very dramatic ($\sim$ a 6-fold difference) and it is likely to play an important role in the hydrodynamics along the vessel. Panels C and D depict the segment rotated so as to show the opening of the output (next lymphangion sinus) and the backside of the valve leaflets also covered by lymphatic endothelial cells. Note the shape and structure of the valve leaflets with the slicker thicker trailing edges of the arch-shaped leaflets coming together to insert into the lymphatic wall (highlighted in the white rectangles). It is not known whether the cells that connect the insertion points of the valve leaflets into the muscular lymphatic wall are muscle or endothelial cells. The backside of the lymphatic leaflets appear to be the parts of the structure that bulge inward to oppose each other and form the seal of the valve at the end of the intrinsic lymphatic systole. In summary, the lymphatic valves are a hallmark of all lymphatic vessels that serve a number of important functions: They minimize lymph backflow when the pressure gradients are not conducive to central lymph flow. They help reduce the gravitational influence on lymph pressure by breaking up the hydrostatic lymph column. They also allow the sequential buildup (lymphangion by lymphangion) of lymph pressure to help overcome any opposing pressure gradients in a stepwise

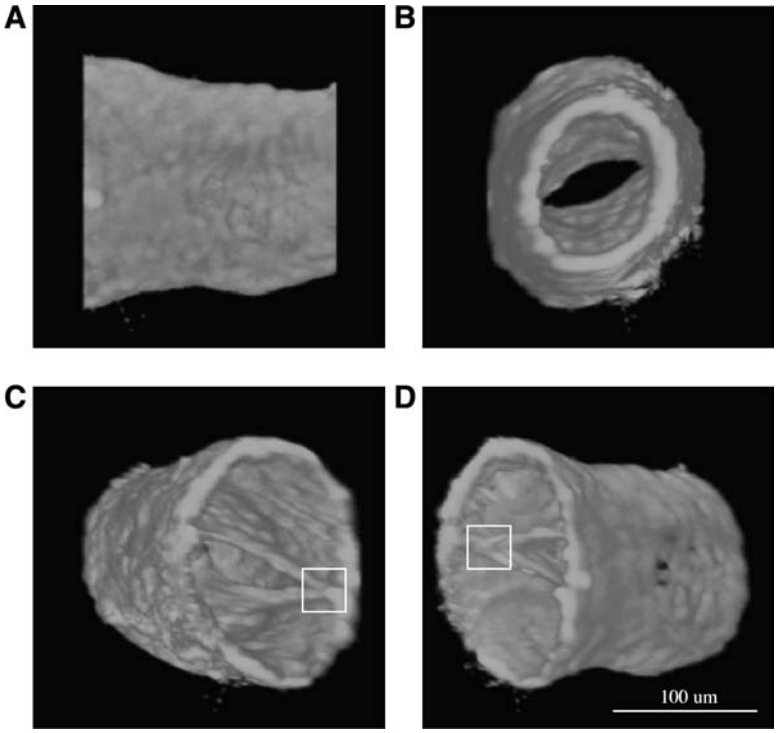

FIG. 2. Three-dimensional reconstruction of a stack of confocal images of an isolated rat mesenteric lymphatic loaded with the vital cell dye Cell-Tracker Green pressurized to 3 cm H_2O in calcium-free solution. Confocal images were taken at 0.2 μm intervals on a Leica confocal/multiphoton microscope in the z-axis and reconstructed in various orientations to show the vessel and valve leaflet microstructure. (**A**) the outside of the lymphatic surface with the inlet oriented towards the *right* and the outlet towards the *left*. (**B**) the vessel rotated to demonstrate the structure of the valve leaflets looking down the lumen of the lymphatic in the direction of net flow. (**C**) and (**D**) show the reconstruction rotated to depict the lumenal outflow end of the valve leaflets with their insertion points into the vessel wall. Note the unusual shaped, arched trailing edge of the valve leaflets, the leaflet insertion site (*white box*), and the relative size of the opening. (Supplemental video online at www.liebertpub.com.)

fashion. Lastly, they may be an anatomical site of fluid shear-sensation and therefore could play important roles in the production nitric oxide and the regulation of lymphatic contractions.

The Lymphatic Pumps

The lymphatic system uses lymph pumps (extrinsic and intrinsic) to provide the energy necessary to overcome the steady state opposing pressure gradients and propel lymph along the lymphatic network.[6,23,25,38–43] Different motive forces, in conjunction with lymphatic valves, move lymph centrally. These motive forces can be categorized into two types dependent upon their source of energy; 1) the "intrinsic" lymph pump relies on the intrinsic rapid/phasic contractions of the lymphatic muscle to generate the needed forces, and 2) the "extrinsic" lymph pump that relies on the cyclical compression and expansion of lymphatics by surrounding extrinsic tissue forces to generate the pressures gradients to move lymph centrally. The extrinsic pumps are thought to predominate in the lymphatics of the heart, skeletal muscle, thorax, and the gut wall, while the intrinsic pumps are essential for lymph flow in most other lymphatic beds.

Lymphatic Contractility and the Intrinsic Lymph Pump

The collecting, transport, and some initial lymphatics possess layers of smooth muscle cells in their outer walls to generate and control the movement of lymph along the lymphatic network. The functional units within the muscular lymphatic vessels, called lymphangions, are arranged in series and separated by highly competent valves.[40,44] This muscle layer is responsible for the regulation of lymphatic diameter and thus its compliance and resistance to generate and control lymph flow. Lymphatics that are not in a tissue that undergoes regular periodic changes in tissue pressure typically demonstrate phasic pumping contractions that generate the pressures that transiently drive lymph flow through the lymphatic network. Therefore, to regulate lymph transport function, the lymphatic contractions that drive pumping (strong, fast, brief contractions) and that alter flow-resistance (moderate, slow, long-lasting contractions) must both be controlled. Since in essence the lymphatics must work as both pumps and conduits, they have characteristics of both hearts and blood vessels.[45–48] To achieve these divergent functions, local, neural, and humoral factors can modulate flow by altering the outflow resistance via tonic contraction/relaxation of the lymphatic muscle. Neural and humoral agents such as a-adrenergic agonists, prostanoids, natriuretic factors, bradykinin, substance P, and others modulate lymphatic tone, flow resistance, and thus lymphatic function.[4,49–73] In addition, local physical factors such as stretch/pressure and shear/flow can also modulate lymphatic tone and function. It has been shown that lymphatics possess myogenic activity and that tonic contraction strength is

modulated by stretch both in its magnitude and its temporal pattern.[6,25,27,39,74–76] In all the lymphatic vessels we have studied in the rat, an increase in transmural pressure (i.e., increased stretch) results in a decrease in the strength of the tonic contraction when compared to the passive diameter.[47]

The intrinsic lymph pump generates lymph flow via the coordinated rapid strong contractions fashions and of lymphatic muscle cells.[40,75,77–80] This type of contraction results in a rapid reduction of the lymphatic diameter, a decrease in lymphatic compliance, an increase in the local lymph pressure, closure of the upstream valve, opening of the downstream valve, and the ejection of lymph into the next downstream lymphangion. The phasic contractions are initiated by electrical pacemaker activity. While it is not yet clear exactly what type of cell the pacemaker is, it is located within the muscle layer of the lymphatic wall.[81–88] While the electrophysiological properties of lymphatic muscle are key cellular regulators of the lymphatic contractile function, that subject is not the focus of this report but has recently been reviewed elsewhere.[89] The intrinsic lymph pump can be analyzed using a cardiac cycle analogy[42] where the phasic contractile cycle is divided into lymphatic diastolic and systolic periods. Lymphatic pumping function can then be evaluated using the phasic contraction frequency, ejection fraction, stroke volume, and lymph pump flow as can be seen in the lymphatic diameter tracing from a rat mesenteric lymphatic in Figure 3.[7,42] Extending the cardiac pump analogy, the intrinsic lymph pump can be modulated via inotropic (i.e., changes in the strength of contraction) and/or chronotropic (i.e., mediated by changes in the contraction frequency) fashions and physical, neural, and humoral influences.[4,6,25,39,50,56,63,65,67,90–98]

Our laboratory group has focused much of our efforts analyzing the physical effects of stretch and shear on the intrinsic lymph pump and other contractile mechanisms. Elevated lymph pressure acting via an increase in the stretch of the lymphatic vessel is a classic activator of the lymph pump.

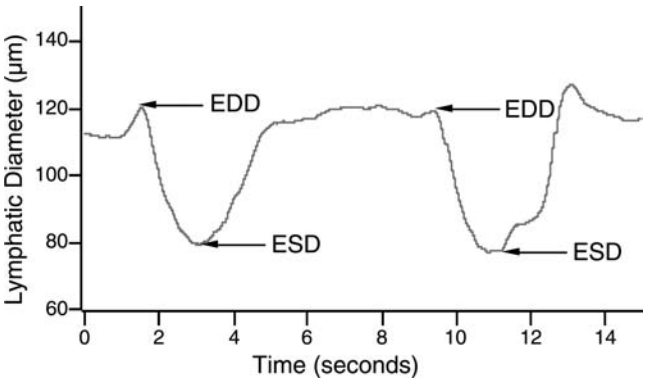

FIG. 3. The typical temporal tracing of the diameter of an isolated lymphatic throughout the intrinsic lymphatic contraction cycle. Lymphatic pumping parameters are defined in a fashion similar to that in the heart. EDD, end diastolic diameter (μm); ESD, end systolic diameter (μm); contraction frequency (CF) = contractions/minute; stroke volume (SV) = end diastolic volume − end systolic volume; ejection fraction (EF) = SV/end diastolic volume; lymph pump flow (LPF) = CF×SF; fractional pump flow (fractional volumes pumped per minute) = CF×EF.

Stretch of the lymphatic increases the lymphatic contraction frequency and initially increases the phasic contraction strength.[6,25,44] However, further increases in pressure/stretch eventually produces a fall in the phasic contraction strength (inotropy), presumably as the ability to increase active contractile force begins to fail. This effect on inotropy is differentially expressed in lymphatics from different tissues [47] and presumably is dependent on the typical tissue characteristics in that species and region. Although lymphatics from different species and tissues will achieve their maximum ability for intrinsic pumping at somewhat different pressures, they are all comparatively low, between 3 and 20 cm H_2O. The influence of pressure/stretch on the intrinsic lymph pump in rat mesenteric lymphatics, a more upstream peripheral lymphatic and rat thoracic duct, the final outflow tract of the lymphatic network, can be seen in Fig. 4 and the supplemental videos. For the rat thoracic duct, the peak intrinsic pump productivity was observed at a transmural pressure of ~3 cm H_2O, with no significant differences in pumping over a range of transmural pressures of 2–4 cm. Transmural pressures beyond ~2–4 cm H_2O produced a weakening of the intrinsic pump. For the rat mesenteric lymphatics, maximum pumping occurred at a pressure of 5 cm H_2O, with no significant differences in pumping over the range of transmural pressures of 2–7 cm. Inotropy of the rat mesenteric lymph pump declined at transmural pressures greater than 5–7 cm H_2O. These data indicate that optimal pumping in more peripheral lymphatics occurs at somewhat higher transmural pressures, presumably a reflection of the greater outflow resistance against which they must pump. For example, in these two lymphatics, the highest fractional pump flow was seen in the mesenteric lymphatics (6–8 volumes/min) at the optimal pressure levels and the lowest fractional pump flow (~2 volumes/min) was found in the thoracic duct.

Somewhat less well-studied in lymphatics are the effects of the other dominant physical factor—flow/shear. The magnitude and pattern of lymph flow is the result of numerous factors, lymph formation, extrinsic and intrinsic pumping, etc. Lymph flow will create shear forces that act on the lymphatic wall. These shear forces may alter lymphatic contractility in a fashion similar to those well-defined actions in blood vessels.[99–101] Isolated bovine mesenteric lymphatics exposed to an imposed axial pressure gradient exhibited an inhibition of the intrinsic phasic lymph pump even at the low axial pressure gradients of ~3 cm H_2O, with further increases in the axial pressure gradient producing a complete inhibition of active pumping.[102] However, the transmural pressure was not maintained constant in these experiments as the axial positive was increased, confounding the action of the physical forces. We have shown that increased lymph flow/shear in the muscular lymphatics can modulate intrinsic lymphatic pumping in a more controlled experimental protocol.[91] We used isolated and perfused lymphatics from different regions of the body from the rat. In these experiments, we altered the imposed axial flow while maintaining a constant transmural pressure by changing the input and output pressures simultaneously to the same degree but in opposite directions. The imposed flow produced a strong inhibition of the active lymph pump in mesenteric lymphatics and especially the thoracic duct.[91] These effects are shown in Table 2 and the accompanying supplemental videos. Shear due to the imposed flow gradient caused a loss of basal tonic contraction

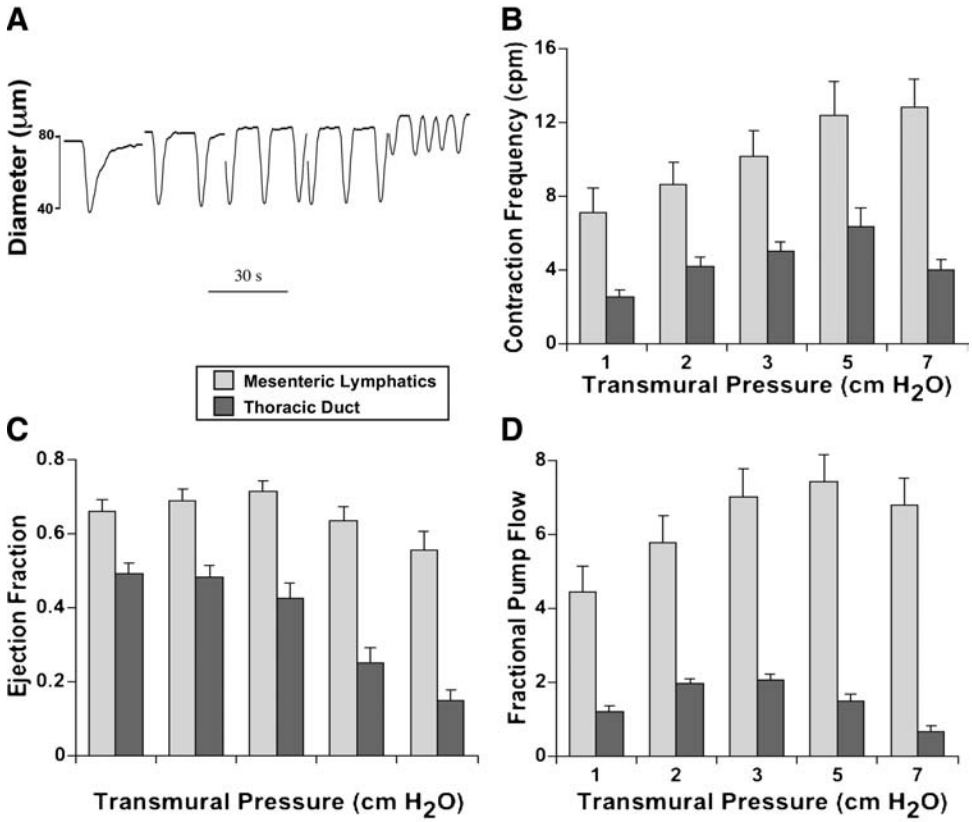

FIG. 4. The effects of transmural pressure (stretch) on lymphatic pumping functions in isolated rat mesenteric lymphatics and rat thoracic ducts. (**A**) shows an example of a lymphatic diameter temporal tracing during increasing transmural pressure in 30 s intervals (*left to right*). Note the consistent increase in diameter and contraction frequency as pressure is elevated. (**B**) depicts the effect of stretch on intrinsic contraction frequency (cpm) in mesenteric and thoracic lymphatics. Note the increased sensitivity to stretch in the mesenteric compared to thoracic duct. (**C**) demonstrates the changes in ejection fraction (EF) in this group of lymphatics. Note the slight rise and plateau in EF with a subsequent decline above 5 cm H_2O in rat mesenteric lymphatics. (**D**) shows the effects of pressure on the fractional pump flow as a combination of frequency and EF. Note the rise in FPF in mesenteric lymphatics until 5 cm H_2O in mesenteric lymphatics, whereas the thoracic duct is a significantly weaker pump that shows a slight increase in FPF from 1–3 cm H_2O but then a decline above 3 cm H_2O. (Supplemental video online at www.liebertpub.com.)

strength, reductions in the contraction frequency, amplitude of the phasic lymphatic contractions, ejection fraction, and fractional pump flow. The inhibition in the intrinsic pumping by imposed flow was significantly greater in the thoracic duct when compared to the mesenteric lymphatic. In the thoracic duct, an imposed flow gradient of 3–5 cm H_2O caused essentially complete cessation of the pumping activity. We hypothesize that this enhanced shear-sensitivity of the thoracic duct is due to its location in the lymphatic network—the final outflow path, where other pumps upstream (intrinsic and extrinsic) can drive lymph flow. We know that the inhibition of the intrinsic lymph pump by a relatively high steady state flow is dependent on the lymphatic endothelium, since removal of the endothelium eliminates this effect. We have also shown that this effect in these tissues is predominantly due to the production of nitric oxide (NO) since blockade of NO synthase (NOS) almost completely blocked this effect.[91]

Table 2. Effects of Imposed Flow on the Lymph Pump

Parameters	*ML control*	*ML flow (7 cm H_2O)*	*TD control*	*TD flow (5 cm H_2O)*
SD (μm)	63 ± 7	89 ± 8*	456 ± 27	562 ± 24*
DD (μm)	99 ± 6	102 ± 6	548 ± 24	567 ± 23
CF (min−1)	9.0 ± 1.6	3.1 ± 1.4*	4.6 ± 0.6	0.1 ± 0.1*
EF	0.59 ± 0.05	0.22 ± 0.05*	0.31 ± 0.03	0.02 ± 0.02*
FPF (s-1)	5.1 ± 1.0	1.0 ± 0.5*	1.4 ± 0.2	0.01 ± 0.01*

However, one complication in these experiments was the issue of what level of imposed flow/shear should be used in these isolated vessel? Since at the time there were no measures of the lymph flow in these vessels *in situ,* we had to estimate the pressure gradient to apply based on our previous measures of the lymph pressure gradients associated with the intrinsic contractions. Later we developed and implemented methods whereby we could measure lymph flow *in situ* in the rat mesenteric lymphatics using hi-speed video lymphocyte-tracking techniques.[103–105] By measuring lymphocyte velocity and lymphatic diameter throughout the lymphatic contractile cycle, we could better estimate the flow and shear profiles that these lymphatics are exposed to under normal and pathophysiological conditions. An example of the lymphocyte velocity and diameter profiles seen during intrinsic lymph pumping in situ in the rat mesenteric lymphatics can be seen in Fig. 5. The mean diameter of these lymphatics was

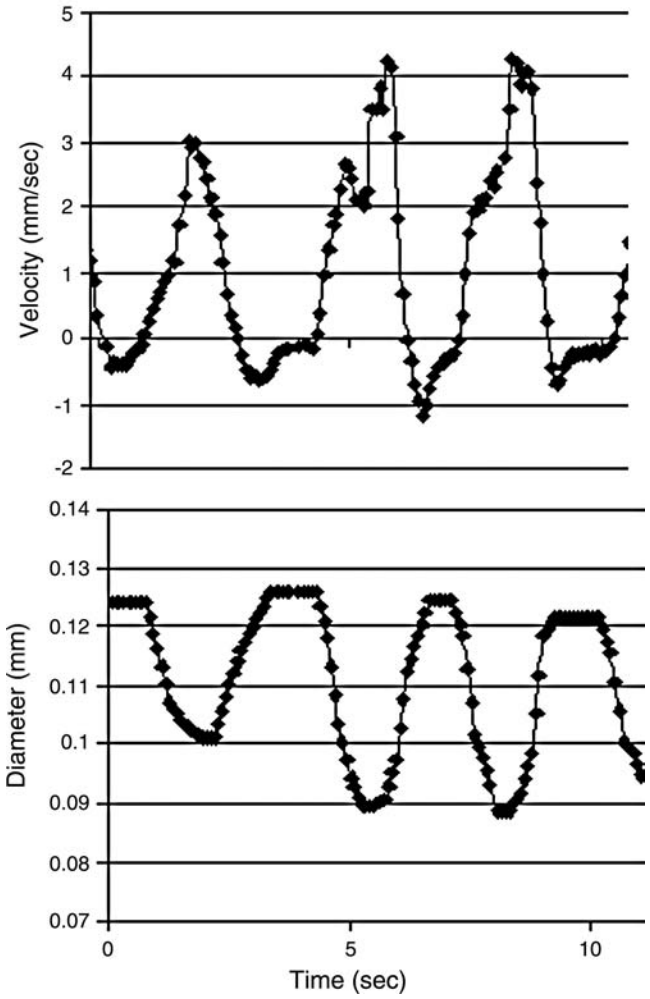

FIG. 5. Traces of lymph velocity and diameter over time throughout 3.5 contractile cycles from an *in situ* experiment measuring lymph flow using lymphocyte-tracking techniques. Note the phase delay between the diameter changes and velocity, the periodic velocities, and the small but significant backflow that occurs before valve closure.

$\sim$105 μm with phasic contractions amplitude of $\sim$30%–40%. The lymphocyte density varied $\sim$300–35,000 cells/μL with a flux $\sim$100–8000 cells/minute. Based on simple Poiseuille flow models, the average lymph velocity was $\sim$0.9 mm/s with transient peaks up to 10-fold higher 2–9 mm/s. The lymph velocity is $\sim$180° out of phase with the phasic diameter changes with periods of flow reversal preceding each valve closure. The average calculated lymph flow is $\sim$14 μL/hour with average lymph shear stress of $\sim$0.4–0.6 dynes/cm2 and with shear stress peaks of 3–10 dynes/cm^2. Overall, this is a much more complex flow/shear pattern than what is typically exhibited in similar sized arterioles, with the lymphatic having greatly reduced averaged flow/shear but with much greater degree of change in flow/shear both in its magnitude and direction. Comparing these measured *in situ* values to what one would expect using the imposed axial pressure gradients in the isolated lymphatic experiments gives very similar peak velocities.[104] However, one significant difference in the flow generated by an imposed axial pressure gradient in the isolated lymphatic experiments is that it is a much more of a steady flow/shear profile when compared to those measured *in situ*. How these flow/shear-induced effects may change when the magnitude and profile of flow/shear is altered is the focus of our current studies.

Additionally, the direction and magnitude of the pump modulation is dependent on the pattern and magnitude of the flow/shear as well as the sensitivity of the particular lymphatic region. We conducted a series of studies in rat thoracic duct using the blockade of eNOS with LNAME in isolated thoracic duct segments that were not exposed to any imposed axial pressure gradients.[90] Thus, the only flow through these isolated lymphatics came about because of the intrinsic pumping activity. Blockade of NO in the thoracic duct increased the contraction frequency as we had seen earlier. The NO blockade also increased basal tone by 30%–50%, which altered lymphatic diastolic compliance and thus decreased lusitropy. This resulted in a decrease in the phasic contraction amplitude and ejection fraction. This implies that there is inherent NO activity in these vessels due to the flow generated by the intrinsic pumping and the inherent sensitivity of the vessel to shear. The phasic relatively low-level shear patterns generated by the intrinsic pump modulates intrinsic lymph pumping and increases the efficiency of the pump via NO-dependent mechanisms in distinction to the imposed steady flows.[90] Thus it appears that there are substantial differences between the effects on the NO generated via a relatively high steady-state imposed flow versus the lower magnitude, oscillatory patterns of NO generated by flow via the intrinsic lymph pump. We propose that the imposed flow-dependent inhibition of the active lymph pump is a physiological mechanism that saves energy by decreasing or stopping intrinsic pumping when the lymphatics do not need to generate lymph flow because some other mechanism upstream is doing so. Inhibition of the intrinsic lymph pump under these conditions will also reduce lymph outflow resistance as a result of the net increase in average lymphatic diameter that occurs when strong phasic contractions are inhibited. This decrease in outflow resistance would ease the removal of fluid from the tissue producing the high lymph flows, thus facilitating the resolution of edema with a reduced energy cost. Thus, the influence of flow/shear on lymphatic pumping and contractile activity is complex and heavily intertwined in the accompanying changes in lymph pressure/stretch. Together, changes in these physical factors are critical regulators of lymphatic function. However, our understanding of these processes, as well as those of other neural and humoral processes, depends on our knowledge of BOTH the activities of the lymphatic endothelial cells and muscle cells and how these interactions generate/regulate lymphatic muscle contractions and thus function.

Conclusions

Impairment of lymph flow can result in a wide range of pathologies, including lymphedema, depressed immune function, impaired lipid metabolism, etc. Globally, lymphedema is on the World Health Organization's top ten list of debilitating diseases to conquer, with hundreds of millions patients affected by the lymphedema caused by parasitic lymphatic filariasis. In the United States, the most common lymphatic disease diagnosed is secondary lymphedema

resulting from mastectomy, congestive heart failure, or reconstructive surgery, with millions of patients suffering from secondary lymphedema after surgical node resection alone.[106] Most importantly, there are likely many other pathologies in which lymphatic dysfunction has important roles that have not yet been defined. Furthermore, our understanding of the relative roles of lymphatic structure versus function in lymphatic dysfunction is still minimal. Thus, although studies have provided some information on lymphatic pumping activity, the basic physical, cellular, and molecular regulation of lymphatic muscle contraction is still not well understood. In particular, our understanding of the molecular processes that regulate the tonic and phasic lymphatic muscle contractions is incomplete. A better understanding of these processes would provide the basis for the development of better diagnostic and treatment modalities for lymphatic disease.

Acknowledgments

The author would like to thank the following individuals who have helped conduct many of the studies described in this report: Eric Bridenbaugh, Gerry Cote, Ph.D., Michael Davis, Ph.D., Brandon Dixon, Ph.D. Anatoliy Gashev, M.D., Ph.D., Olga Gasheva, M.D., Jimmy Moore, Ph.D., Mariappan Muthuchamy, Ph.D., and Wei Wang, M.D.

Disclosures

Dr. Zwaieja has no conflicts of interest or financial ties to disclose.

References

1. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. Physiol Rev 1993;73:1–78.
2. Lee J. Distension pressure on subserosal and mesenteric lymph pressures of rat jejunum. Am J Physiol 1986;251: G611–G614.
3. Lee J. Tissue fluid pressure, lymph pressure, and fluid transport in rat intestinal villi. Microvasc Res 1986;31:170–183.
4. McHale NG, Roddie IC, Thornbury KD. Nervous modulation of spontaneous contractions in bovine mesenteric lymphatics. J Physiol 1980;309:461–472.
5. Ohhashi T, Azuma T, Sakaguchi M. Active and passive mechanical characteristics of bovine mesenteric lymphatics. Am J Physiol 1980;39:H88–95.
6. Hargens AR, Zweifach BW. Contractile stimuli in collecting lymph vessels. Am J Physiol 1977;233:H57–65.
7. Benoit JN, Zawieja DC, Goodman AH, Granger HJ. Characterization of intact mesenteric lymphatic pump and its responsiveness to acute edemagenic stress. Am J Physiol 1989;257:H2059–2069.
8. Negrini D, Moriondo A, Grimaldi A, Sciacca L. Transdiaphragmatic lymphatic pathways in spontaneously breathing rats. FASEB J 2006;20:A274.
9. Grimaldi A, Moriondo A, Sciacca L, MGuidali ML, Tettamanti G, Negrini D. Functional arrangement of rat diaphragmatic initial lymphatic network. Am J Physiol Heart Circ Physiol 2006;291:H876–885.
10. Moriondo A, Mukenge S, Negrini D. Transmural pressure in rat initial subpleural lymphatics during spontaneous or mechanical ventilation. Am J Physiol Heart Circ Physiol 2005;289:H263–269.
11. Negrini D, Moriondo A, Mukenge S. Transmural pressure during cardiogenic oscillations in rodent diaphragmatic lymphatic vessels. Lymphat Res Biol 2004;2:69–81.
12. Miserocchi G, Negrini D, Passi A, De Luca G. Development of lung edema: Interstitial fluid dynamics and molecular structure. News Physiol Sci 2001;16:66–71.
13. Negrini D, Fabbro MD. Subatmospheric pressure in the rabbit pleural lymphatic network. J Physiol 1999;520:761–769.
14. Leak L, Burke J. Ultrastructural studies on the lymphatic anchoring filaments. J Cell Biol 1968;36:129–149.
15. Collin H. The ultrastructure of conjunctival lymphatic anchoring filaments. Exp Eye Res 1969;8:102–105.
16. Casley–Smith J. Are the initial lymphatics normally pulled open by the anchoring filaments? Lymphology 1980;13:120–129.
17. Lynch PM, Delano FA, Schmid–Schonbein GW. The primary valves in the initial lymphatics during inflammation. Lymphat Res Biol 2007;5:3–10.
18. Mendoza E, Schmid–Schonbein GW. A model for mechanics of primary lymphatic valves. J Biomech Eng 2003;125:407–414.
19. Trzewik J, Mallipattu SK, Artmann GM, Delano FA, Schmid–Schonbein GW. Evidence for a second valve system in lymphatics: Endothelial microvalves. FASEB J 2001;15: 1711–1717.
20. Negrini D, Ballard ST, Benoit JN. Contribution of lymphatic myogenic activity and respiratory movements to pleural lymph flow. J Appl Physiol 1994;76:2267–2274.
21. Szabo G, Magyar Z. Pressure measurements in various parts of the lymphatic system. Acta Med Academ Scient Hungaricae 1967;23:237–241.
22. Olszewski WL, Engeset A, Sokolowski J. Lymph flow and protein in the normal male leg during lying, getting up, and walking. Lymphology 1977;10:178–183.
23. Olszewski WL, Engeset A. Intrinsic contractility of prenodal lymph vessels and lymph flow in human leg. Am J Physiol Heart Circ Physiol 1980;239:H775–783.
24. Albertine K, Fox L, O'Morchoe C. The morphology of canine lymphatics valves. Anat Rec 1982;202:453–461.
25. McHale NG, Roddie IC. The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. J Physiol 1976;261:255–269.
26. Gnepp D. The bicuspid nature of the valves of the peripheral collecting lymphatic vessels of the dog. Lymphology 1976; 9:75–77.
27. Zweifach B, Prather J. Micromanipulation of pressure in terminal lymphatics in the mesentery. Am J Physiol 1975; 228:1326–1335.
28. Papp M, Viragh S, Ungvary G. Structure of cutaneous lymphatics propelling lymph. Acta Med Acad Sci Hung 1975;32:311–320.
29. Takada M. The ultrastructure of lymphatic valves in rabbits and mice. Amer J Anat 1971;32:207–217.
30. Lauweryns JM. Stereomicroscope funnel-like architecture of pulmonary lymphatic valves. Lymphology 1971;4:125–132.
31. 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.
32. Mazzoni MC, Skalak TC, Schmid–Schonbein GW. Structure of lymphatic valves in the spinotrapezius muscle of the rat. Blood Vessels 1987;24:304–312.
33. Gnepp D, Green F. Scanning electron microscopic study of canine lymphatic vessels and their valves. Lymphology 1980;13:91–99.

34. Petrenko VM. [Conceptions on the structural organization of the active lymph flow between the neighboring lymphangions]. Morfologiia 2007;132:87–92.
35. Kriederman BM, Myloyde TL, Witte MH, Dagenais SL, Witte CL, Rennels M, Bernas MJ, Lynch MT, Erickson RP, Caulder MS, Miura N, Jackson D, Brooksand BP, Glover TW. Foxc2 haplo insufficient mice are a model for human autosomal dominant lymphedema–distichiasis syndrome. Hum Mol Genet 2003;12:1179–1185.
36. Petrova TV, Karpanen T, Norrmen C, Mellor R, Tamakoshi T, Finegold D, Ferrell R, Kerjaschki D, Mortimer P, Yla–Herttuala S, Miura N, Alitalo K. Defective valves and abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis. Nat Med 2004;10:974–981.
37. Mellor RH, Brice G, Stanton AWB, French J, Smith A, Jeffery S, Levick JR, Burnand KG, Mortimer PS. Mutations in foxc2 are strongly associated with primary valve failure in veins of the lower limb. Circulation 2007;115:1912–1920.
38. Szegvari M, ALakos A, Szontagh F, Foldi M. Spontaneous contractions of lymphatic vessels in man. Lancet 1963;7294: 1329.
39. Mislin H, Schipp R. Structural and functional relations of the lymph vessels. Progress in Lymphology. Proc Intl Symp Lymphol Zurich, Switzerland 1966, 360–365.
40. Mislin H. Active contractility of the lymphangion and coordination of lymphangionchains. Experientia 1976;32:820–822.
41. Smith R. Lymphatic contractility. A possible intrinsic mechanism of lymphatic vessels for the transport of lymph. J Exp Med 1949;90:497–509.
42. Granger HJ, Kovalcheck S, Zweifach BW, Barnes GE. Quantitative analysis of active lymphatic pumping. In: Proceedings of the VII Summer Computer Simulation Conference. La Jolla, CA, Simulation Council; 1977: 562–565.
43. Orlov RS, Borisova RP. Spontaneous and evoked contractile activity of lymphatic vessel smooth muscle. Dokl Akad Nauk SSSR 1974;215:1013–1015.
44. Mislin H. Structural and functional relations of the mesenteric lymph vessels. New Trends in Basic Lymphology; Experientia 1966;14:87–96.
45. Gashev AA and Zawieja DC. Lymph transport and lymphatic system. In: Encyclopedic Reference of Immunotoxicology. Heidelberg, Germany, Springer-Verlag; 2004; 400–403.
46. Muthuchamy M, Gashev A, Boswell N, Dawson N, Zawieja D. Molecular and functional analyses of the contractile apparatus in lymphatic muscle. FASEB J, 2003; 17: 920–922.
47. Gashev AA, Davis MJ, Delp MD, Zawieja DC. Regional variations of contractile activity in isolated rat lymphatics. Microcirculation 2004;11:477–492.
48. Quick CM, Venugopal AM, Gashev AA, Zawieja DC, Stewart RH. Intrinsic pump-conduit behavior of lymphangions. Am J Physiol Regul Integr Comp Physiol 2007;292:R1510–1518.
49. Orlov RS, Borisova RP. [inhibition of spontaneous lymph vessel contraction evoked by transmural stimulation]. Fiziol Zh SSSR Im I M Sechenova, 1976;62:1500–1505.
50. Ohhashi T, Kawai Y, Azuma T. The response of lymphatic smooth muscles to vasoactive substances. Pflugers Arch 1978;375:183–188.
51. Alessandrini C, Gerli R, Sacchi G, Ibba L, Pucci AM, Fruschelli C. Cholinergic and adrenergic innervation of mesenterial lymph vessels in guinea pig. Lymphology 1981;14:1–6.
52. Allen JM, Iggulden HL, McHale NG. Beta-adrenergic inhibition of bovine mesenteric lymphatics. J Physiol 1986;374: 401–411.
53. Dabney JM, Buehn MJ, Dobbins DE. Constriction of lymphatics by catecholamines, carotid occlusion, or hemorrhage. Am J Physiol 1988;255:H514–524.
54. McHale NG. Lymphatic innervation. Blood Vessels 1990;27: 127–136.
55. Benoit JN. Effects of alpha-adrenergic stimuli on mesenteric collecting lymphatics in the rat. Am J Physiol 1997;273: R331–336.
56. Yokoyama S, Benoit JN. Effects of bradykinin on lymphatic pumping in rat mesentery. Am J Physiol 1996;270:G752–756.
57. Ferguson MK Modulation of lymphatic smooth muscle contractile responses by the endothelium. J Surg Res 1992;52: 359–363.
58. Dabney JM, Soika CY, Dobbins DE. Evidence for constriction of canine prenodal lymphatic vessels by vasoactive agents and carotid occlusion. Microcirc Endothel Lymphat 1985;2:55–65.
59. Azuma T, Ohhashi T, Roddie IC. Bradykinin-induced contractions of bovine mesenteric lymphatics. J Physiol 1983;342: 217–227.
60. Johnston MG, Hay JB, Movat HZ. Kinetics of prostaglandin production in various inflammatory lesions, measured in draining lymph. Am J Pathol 1979;95:225–238.
61. Johnston MG, Hay JB, Movat HZ. The distribution of prostaglandins in afferent and efferent lymph from inflammatory sites. Am J Pathol 1980;99:695–714.
62. Mannheimer E, Sinzinger H, Oppolzer R, Silberbauer K. Prostacyclin synthesis in human lymphatics. Lymphology 1980;13:44–46.
63. Johnston MG, Gordon JL. Regulation of lymphatic contractility by arachidonate metabolites. Nature 1981;293:294–297.
64. Mizuno R, Koller A, Kaley G. Regulation of the vasomotor activity of lymph microvessels by nitric oxide and prostaglandins. Am J Physiol1998;274:R790–796.
65. Zawieja DC, Greiner ST, Davis KL, Hinds WM, Granger HJ. Reactive oxygen metabolites inhibit spontaneous lymphatic contractions. Am J Physiol 1991;260:H1935–1943.
66. Davis MJ, Lane MM, Davis AM, Durtschi D, Zawieja DC, Muthuchamy M, Gashev AA. Modulation of lymphatic muscle contractility by the neuropeptide substance P. Am J Physiol Heart Circ Physiol 2008;295:H587–597.
67. Amerini S, Ziche M, Greiner ST, Zawieja DC. Effects of substance P on mesenteric lymphatic contractility in the rat. Lymphat Res Biol 2004;2:2–10.
68. Rayner SE, Van Helden DF. Evidence that the substance P-induced enhancement of pacemaking in lymphatics of the guinea-pig mesentery occurs through endothelial release of thromboxane a2. Br J Pharmacol 1997;121:1589–1596.
69. Zawieja DC. Lymphatic microcirculation. Microcirculation 1996;3:241–243.
70. Hukkanen M, Konttinen Y, Terenghi G, Polak J. Peptide-containing innervation of rat femoral lymphatic vessels. Microvasc Res 1992;43:7–19.
71. Guarna M, Pucci A, Alessandrini C, Volpi N, Fruschelli M, D'Antona D, Fruschelli C. Peptidergic innervation of mesenteric lymphatics in guinea pigs: An immunocytochemical and pharmacological study. Lymphology 1991;24:161–167.
72. Ito Y, Magari S, Sakanaka M. Immunoelectron-microscopic localization of peptidergic nerve fibers around lymphatic capillaries in the rat liver. Arch Histol Cytol 1990;53:199–208.

73. Foy W, Allen J, McKillop J, Goldsmith J, Johnston C, Buchanan K. Substance P and gastrin releasing peptide in bovine mesenteric lymphatic vessels: Chemical characterization and action. Peptides 1989;10:533–537.
74. Zweifach B. Micropressure measurements in the terminal lymphatics. 7th Europ Conf Microcirculation, Aberdeen, Part II Bibl Anat; editors J. Ditzel & D.H. Lewis, 1972; no. 12: 3615.
75. McHale NG, Roddie IC. Pumping activity in isolated segments of bovine mesenteric lymphatics. J Physiol 1975;244: 70P–72P.
76. Lobov GI, Orlov RS, Kostikova MA. Active and passive mechanical properties of the wall of the lymphangion. Fiziologicheskii Zhurnal SSSR Imeni I, 1989;75:218–226.
77. JAllen JM, McHale NG. Neuromuscular transmission in bovine mesenteric lymphatics. Microvasc Res 1986;31:77–83.
78. Zawieja DC, Davis KL, Schuster R, Hinds WM, Granger HJ. Distribution, propagation, and coordination of contractile activity in lymphatics. Am J Physiol 1993;264:1283–1291.
79. McHale NG, Meharg MK. Co-ordination of pumping in isolated bovine lymphatic vessels. J Physiol 1992;450:503–512.
80. Crowe MJ, von der Weid PY, Brock JA, Van Helden DF. Coordination of contractile activity in guinea-pig mesenteric lymphatics. J Physiol 1997;500:235–244.
81. Orlov RS, Borigora RP, Mundriko ES. Investigation of contractile and electrical activity of smooth muscle of lymphatic vessels. in *Physiology of Smooth Muscle*, M. F. Bulbring EaS, Editor. 1976, Ranon: New-York. p. 147–152.
82. Ohhashi T, Azuma T. Electrical activity and ultrastructure of bovine mesenteric lymphatics. Lymphology 1979;12:4–6.
83. Lobov GI, Orlov RS. [electrical and contractile activity of the lymphangions of the mesenteric lymphatic vessels]. Fiziol Zh SSSR Im I M Sechenova, 1983;69:1614–1620.
84. Ward SM, Sanders KM, Thornbury KD, McHale NG. Spontaneous electrical activity in isolated bovine lymphatics recorded by intracellular microelectrodes. J Physiol 1991; 438:168.
85. McCloskey KD, Toland HM, Hollywood MA, Thornbury KD, McHale NG. Hyperpolarisation-activated inward current in isolated sheep mesenteric lymphatic smooth muscle. J Physiol 1999;521:201–211.
86. Toland HM, McCloskey KD, Thornbury KD, McHale NG, Hollywood MA. Ca2+ -activated cl-current in sheep lymphatic smooth muscle. Am J Physiol Cell Physiol 2000;279: C1327–1335.
87. Van Helden DF. Pacemaker potentials in lymphatic smooth muscle of the guinea-pig mesentery. J Physiol 1993;471:465–479.
88. von der Weid PY, Crowe MJ, Van Helden DF. Endothelium-dependent modulation of pacemaking in lymphatic vessels of the guinea-pig mesentery. J Physiol 1996;493:563–575.
89. von der Weid P. Review article: Lymphatic vessel pumping and inflammation—the role of spontaneous constrictions and underlying electrical pacemaker potentials. Aliment Pharmacol Ther 2001;15:1115–1129.
90. Gasheva OY, Zawieja DC, Gashev, AA. Contraction-initiated no-dependent lymphatic relaxation: A self-regulatory mechanism in rat thoracic duct. J Physiol 2006;575:821–832.
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;540:1023–1037.
92. Ferguson MK, DeFilippi VJ. Nitric oxide and endothelium-dependent relaxationin tracheobronchial lymph vessels. Microvasc Res 1994;47:308–317.
93. Schmid–Schonbein GW. Mechanisms causing initial lymphatics to expand and compress to promote lymph flow. Arch Histol Cytol 1990;53:107–114.
94. Ferguson MK, Shahinian HJ, Michelassi F. Lymphatic smooth muscle responses to leukotrienes, histamine and platelet activating factor. J Surg Res 1988;44:172–177.
95. Elias RM, Johnston MG, Hayashi A, Nelson W. Decreased lymphatic pumping after intravenous endotoxin administration in sheep. Am J Physiol 1987;253:H1349–1357.
96. Hogan R, Unthank J. Mechanical control of initial lymphatic contractile behavior in bat's wing. Am J Physiol 1986;251: H357–363.
97. McHale NG, Roddie IC. The effects of catecholamines on pumping activity in isolated bovine mesenteric lymphatics. J Physiol 1983;338:527–536.
98. Orlov RS, Lobacheva TA. Intravascular pressure and spontaneous lymph vessels contractions. [russian]. Bull Exp Biol Med 1977;83:392–394.
99. Busse R, Fleming I. Pulsatile stretch and shear stress: Physical stimuli determining the production of endothelium-derived relaxing factors. J Vasc Res 1998;35:73–84.
100. Bevan JA, Henrion D. Pharmacological implications of the flow-dependence of vascular smooth muscle tone. Annu Rev Pharmacol Toxicol 1994;34:173–190.
101. Johnsson E, Folkow B, Karlstrom G. Myogenic responsiveness in rat hindquarter vessels during constant-flow and constant-pressure perfusion in vitro; effects of various potassium concentrations and of endothelial nitrous oxide blockade. Acta Physiol Scand 1991;142:319–328.
102. Gashev AA. The pump function of the lymphangion and the effect on it of different hydrostatic conditions. [russian]. Fiziologicheskii Zhurnal SSSR Imeni I. M. Sechenova 1989; 75:1737–1743.
103. Dixon JB, Zawieja DC, Gashev AA, Cote GL. Measuring microlymphatic flow using fast video microscopy. J Biomed Opt 2005;10:064016-1–064016-7.
104. Dixon J, B., Greiner BST, Gashev AA, Cote G, Moore J, Zawieja DC. Lymph flow, shear stress, and lymphocyte velocity in rat mesenteric prenodal lymphatics. Microcirculation 2006;13:597–610.
105. Dixon JB, Gashev AA, Zawieja DC, Moore JE, Jr, Cote GL. Image correlation algorithm for measuring lymphocyte velocity and diameter changes in contracting microlymphatics. Ann Biomed Eng 2007;35:387–396.
106. Rockson SG. A population-based assessment of the problem of lymphatic disease. Lymphat Res Biol 2008;6:1–2.

Address correspondence to:
David C. Zawieja, Ph.D.
Department of Systems Biology and Translational Medicine
Cardiovascular Research Institute Division of Lymphatic Biology
Texas A&M Health Science Center College of Medicine
702 SW HK Dodgen Loop
Temple, Texas 77843-1114

E-mail: dcz@tamu.edu