Lymphatic vessels transition to state of summation above a critical contraction frequency

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Lymphatic vessels play an essential role in fat metabolism, immune function, and interstitial homeostasis by transporting lymph from the interstitium to the central venous circulation (3). Unidirectional valves divide lymphatic vessels into a series of functional compartments, called lymphangions. Lymphangions can exhibit rhythmic spontaneous contractions that actively pump lymph (7). Whereas lymphangions respond to higher lymph pressures by increasing frequency and strength of contraction (21, 30, 43), they respond to increased flow by decreasing frequency and strength of contraction (17, 22). These two responses are believed to work synergistically to maintain interstitial fluid balance (7).

Because the responses of lymphatic vessels to increased pressure and increased flow are similar to behaviors first observed in ventricles and blood vessels, many investigators adopted existing mechanical characterizations. Viewed as pumping chambers, lymphangions exhibit identifiable systolic and diastolic periods (6, 24, 30). Similar to the heart, the strength of lymphatic pumping increases with preload (15, 30, 43) (Frank-Starling effect), the rate of contraction increases with stretch (21, 30, 43) (Bainbridge effect), and the force of lymphatic contraction is limited by the velocity of shortening (9) (Hill effect). However, similar to blood vessels, lymphatic vessels regulate basal tone in diastole (7, 34) and release nitric oxide (NO), causing dilation in response to flow (17, 22, 45). This behavioral dualism is reflected in structural dualism: cardiac and vascular muscle myosin heavy chain and α-actin isoforms have been found in lymphatic muscle (35).

Cardiac and vascular behaviors require fundamentally different contractile properties. Although traditionally associated with skeletal muscle tetany, mechanical summation is not specific to skeletal muscle. In ventricles, incomplete relaxation is associated with pathological behavior (20, 50). Rapid relaxation in ventricles, coupled with an extended refractory period, minimizes summation, however, and generally allows for complete relaxation between beats. In stark contrast, sustained basal tone is required for normal behavior in blood vessels. Slow contractile myofilaments, coupled with the capacity for graded depolarization of membrane potential, allow for maintenance and regulation of tonic constriction (4, 8).

Although the phasic and tonic contractile properties of lymphatic vessels are common to ventricles and blood vessels, their interaction may result in unique behavior in lymphatic vessels. In terms of unique summation behavior, although lymphatic vessels possess a refractory period that prevents tetany, the effective refractory period is less than total contraction time, ending at ~50% relaxation (25). Therefore, the possibility arises that lymphatic vessels do not fully relax between contractions when contraction frequencies are high; however, evidence for mechanical summation has not been previously reported.

Examination of such behavior by biomechanical characterization of lymphatic vessels would require a union of biomechanical testing regimens that have traditionally been applied to study ventricular or blood vessel mechanics. Cardiac biomechanical studies conventionally use isovolumic preparations to control for the Hill effect (46) and to characterize the ability of the heart to relax (lusitropy) (48). Isolated blood vessel biomechanical studies, in contrast, conventionally apply controlled-flow conditions to characterize shear-mediated release of NO and isobaric conditions to characterize basal tone (41).
Combination of these disparate approaches to study lymphatic vessel biomechanics yields four unique challenges. 1) Because the lymphatic vessel mechanics are sensitive to pressure (21, 30, 43), flow (17, 22), and wall motion (9), a single experimental preparation would have to accommodate isobaric, controlled-flow, and isovolumetric tests on the same vessel. 2) Because the temporal variability of contractions is significant, it would be necessary to analyze numerous contraction cycles, yet minimize total experimental time. 3) Because nonuniform contraction would lead to muscle shortening, vessel segments would have to be kept short enough to ensure uniform contraction, yet long enough to preserve vessel geometry between cannulated ends. 4) Because metabolites and variations in luminal wall shear stress may confound results, an experimental preparation must periodically provide a luminal flow high enough to eliminate metabolites, yet small enough to prevent significant shear-mediated NO release. The competing constraints provided by these four challenges may have limited the rigorous study of the biomechanics of isolated lymphatic vessels. We, therefore, created a unique controlled-flow isobaric isovolumetric apparatus and developed a rigorous experimental protocol to test the hypothesis that lymphatic vessels can transition to a state of summation when lymphatic vessel contraction frequency exceeds a critical value.

METHODS

Experimental apparatus. The experimental protocol required that the lymphatic bath setup provide rapid switching between two conditions: 1) constant transmural pressure and luminal flow and 2) constant luminal volume. To ensure rapid switching from one configuration to another, two pathways for fluid were established: 1) through the lumen of the vessel and 2) through a bypass (Fig. 1). In either configuration, lymphatic vessels were bathed and infused with 1% albumin-physiological saline solution (APSS, pH 7.4) containing (in mM) 145 NaCl, 4.70 KCl, 2.00 CaCl2, 1.17 MgSO4, 1.20 NaH2PO4, 5.00 dextrose, 2.00 sodium pyruvate, 0.020 EDTA, and 3.00 MOPS buffer. APSS of bath and lumen was infused with air via a membrane oxygenator (model OXR, Living Systems Instrumentation, Burlington, VT) and set to 37.0°C via thermoregulators (model Lauda E-200, Brinkman Instruments, Westbury, NY).

Establishing isobaric, steady-flow conditions. To establish isobaric, constant-flow conditions, stopcocks S1 and S2 (Fig. 1) were open to the vessel but closed to flow from the bypass. In this configuration, luminal flow was controlled by a syringe pump (model SP120P, World Precision Instruments, Sarasota, FL) set at 10 ml/h; the minimal flow was calculated to completely refresh the lumen approximately once every 30 s. Luminal pressure could be set by adjustment of the height of the inlet pressure cannula (Fig. 1). This isobaric, constant-flow condition was designed to ensure that luminal contents could be refreshed to eliminate the buildup of metabolites and 2) flows were low enough so as to minimize shear stress-induced release of NO.

Establishing isovolumetric conditions. To establish isovolumetric conditions, S2 was first closed to the vessel and opened to bypass. Then S1 was closed to the vessel and opened to the bypass. In this configuration, all flow was directed through the bypass, and no fluid flowed through the vessel. Therefore, the space between S1 and S2 was sealed to form a continuous volume of fluid containing the vessel. This isovolumic configuration was designed to ensure that 1) the effective afterload of the vessel was nearly infinite (since there was no outflow); 2) volume changes were minimized by assurance of negligible compliance in the tubing leading to and from the vessel; 3) a microinjection system at P2 could add or subtract a controlled amount of volume to the lumen; and 4) a pressure port allowed measurement of luminal pressure.

Recording critical variables. Using a monochrome charge-coupled device camera (model ST-XC50, Sony Electronics, Park Ridge, NJ), we monitored the external diameters of the lymphatic vessels. Transmural pressures were measured using a pressure transducer (model PX26-001GV, Omega, Stamford, CT) connected to a fluid-filled tube extending from the pressure port (P1) at the inlet to the vessel (Fig. 1) and adjusted to the height of the solution bathing the vessel. Image and pressure readings were then digitized at 25 samples per second (models NI PCI-6036E and NI PCI-1410, National Instruments, Austin, TX). Instantaneous vessel outer diameter and luminal pressure were continuously measured and recorded through a custom real-time diameter-measuring virtual instrument (LabVIEW 7.0, National Instruments) (33).

Validating measurement of vessel transmural pressure. The pressure drop from P1 to P2 (Fig. 1) was estimated to verify that the pressure measured at the pressure port (P1) approximated the vessel transmural pressure. A polyethylene (Tygon) tube approximating the vessel length and diameter (1.6 mm ID, 4 cm long) was inserted. At a high flow of 20 ml/h, the pressure drop from P1 to P2 was <0.05 mmHg. Therefore, P1 was presumed to serve as an adequate approximation for luminal pressure.

Tissue preparation. Conducting lymphatic vessels from bovine mesentery were collected at an abattoir immediately after the animals were euthanized. Postnodal lymphatic vessels (3–4 mm OD) were ligated at the downstream end and then dissected. They were transported in APSS at ~30°C. Segments of lymphatic vessels that appeared devoid of fat and connective tissue were cut to 1-cm lengths. The segments were then cleaned and mounted on the perfused, isolated vessel bath described above. Removal of air pockets from the fluid lines resulted in a continuous, incompressible volume of fluid between stopcocks S1 and S2 (Fig. 1), and the vessels were connected with two loops of suture to ensure a tight seal.

Establishing baseline isobaric conditions. Baseline transmural pressure was set by the height of an inlet pressure cannula (Fig. 1) at 4 mmHg to yield near-maximal pumping (16, 30). Luminal flow was set by a syringe pump at 10 ml/h. After they were warmed, vessels

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**Figure 1.** Apparatus for controlling pressure, flow, and volume in isolated lymphatic vessel segments. Syringe pump draws a set flow across vessel lumen or bypass, depending on position of the stopcocks. Vessel diameter is measured from video captured by a charged-coupled device (CCD) camera. Inlet pressure cannula sets vessel transmural pressure during isobaric conditions. To set isovolumic conditions, stopcocks S1 and S2 were closed to vessel lumen flow/outflow and opened to flow across the bypass. Transmural pressure was measured at port P1, and fluid was added to or withdrawn from port P2. Under isovolumic conditions, S1 and S2 positions created a sealed volume between S1 and S2 containing the vessel, whereby any volume of fluid subtracted/added by the microsyringe attached to P2 almost exclusively subtracted/added to the vessel luminal volume. APSS, albumin-physiological salt solution.
were allowed to equilibrate for 15 min. If the vessels did not exhibit spontaneous contractions, the transmural pressure was transiently elevated to induce contractions.

Establishing isovolumetric pressure-diameter relationships. To create a baseline isovolumic data set, we collected pressure recordings at 10 volumes. Baseline volume was estimated from end-diastolic diameter under isobaric conditions with the assumption that the vessel segment had a cylindrical shape. We used the microsyringe attached to the injection port (P2 in Fig. 1) to randomly vary volume from baseline −50% to +40% in 10 increments (rounded to the nearest 0.001 ml). The vessel was switched from the 4-mmHg isobaric condition to the isovolumetric condition during the relaxed phase of the contraction cycle (i.e., end diastole) to standardize baseline volumes. After each change in volume, the vessel was allowed to pump under isovolumic conditions for four beats or 2 min (whichever ended first). The system was then switched to baseline isobaric, constant-flow conditions for 5 min before each subsequent step in volume. This protocol resulted in ~40 complete isovolumic contractions per vessel for analysis.

Establishing passive pressure-diameter relationships. After analyzing the active vessel properties, we recorded data to establish the passive pressure-diameter relationship. First, we perfused the bath and vessel lumen with Ca2+-free APSS for 30 min, after which the vessel was assumed to be fully relaxed (17, 42). Then we adjusted the vessel luminal pressure from 0 to 50 mmHg in ~30 steps by incrementally raising the height of the inlet tubing. The passive vessel pressure and volume data obtained during these steps were used to construct passive pressure-diameter relationships.

Vessel exclusion criteria. Analysis required that valveless vessels spontaneously contract cyclically, baseline frequencies be constant throughout the experiment, and sufficient data be collected to establish tension-diameter relationships. Therefore, the following exclusion criteria were employed. 1) On cannulation, vessels found to contain valves or leaks were discarded. 2) Vessels that did not exhibit spontaneous contraction within 15 min of equilibration or did not maintain spontaneous contraction throughout isovolumetric protocols were removed from study. 3) If the frequency of contraction was not reestablished after the vessel was subjected to baseline conditions (between each step in volume), that vessel was removed from analysis. Specifically, if the frequency after each of the 5-min baseline conditions varied by a factor of 2, the vessel was removed from analysis. 4) If 4 of the 10 steps in volume yielded fewer than two complete contraction cycles, the vessel was removed from analysis.

Definition of contraction cycles. The first four complete contraction cycles for each volume step were chosen for analysis. Figure 2 illustrates how the lymphatic contraction cycles were divided on the basis of identifiable points in the recorded pressure. Point A (end diastole) was defined as the local minimum in pressure before a sharp increase in the rate of pressure development. Point B (end systole) was defined as the maximum pressure. Point C (end of the relaxation phase) was defined as the point of minimal pressure change. It was identified by the rate of change of pressure ([dP/dt]) and the rate of change of the rate of change of pressure ([d^2P/dt^2]) calculated from differentiation algorithms [similar to those of Benoit et al. (6)]. After the maximum rate of relaxation, the vessel was considered fully relaxed (point C) when [dP/dt] ≤ 0.1 mmHg/s and [d^2P/dt^2] ≤ 0.01 mmHg/s². Beat-to-beat period was defined as the time between the present and the preceding contraction peak (point B to point B) and was, thus, the reciprocal of contraction frequency. Time to peak was defined as the maximum pressure (point B to point B). Time to full relaxation was the difference between end systole and end systole (point A to point C). Vessel twitch length was the time interval from end diastole to the end of the relaxation phase (point A to point C), constituting the contraction cycle of the vessel.

Calculation of wall tension. With the assumption that the vessel was a thin-walled cylindrical tube, wall tension (T) was calculated by Laplace’s law (Eq. 1) from measured pressure (P) and diameter (D)

\[ T = \frac{PD}{2} \]  

Since bovine mesenteric lymphatic vessels are exceedingly thin walled, D was approximated by the outer, rather than the inner or the...
midline, diameter (6). From the calculated wall tension for end systole, end diastole, and the passive vessel state, we derived three “active tensions”: systolic active tension (end-systolic tension – passive tension), diastolic active tension (end-diastolic tension – passive tension), and differential tension (end-systolic tension – end-diastolic tension). Because the calculated tensions were collected at slightly different diameters, the passive tension had to be interpolated. First, we obtained a continuous approximation of the passive pressure \( P_{\text{passive}} \) as a function of diameter by fitting an exponential curve (Eq. 2) to passive pressure-diameter data over the same range of end-diastolic diameters as the calculated tensions

\[
P_{\text{passive}}(D) = \alpha e^{\beta D}
\]

where \( \alpha \) and \( \beta \) are mathematically determined constants used to fit the equation to the data. Passive tension was then calculated by substituting \( P_{\text{passive}}(D) \) into Eq. 1.

Characterization of relaxation rate. We used a nonzero asymptote model that mimics a nonzero diastolic active tension (10, 23, 44) to characterize the rate of isovolumic relaxation with a time constant (\( \tau \))

\[
P(t) = (P_0 - P_\infty)\exp(-t/\tau) + P_\infty \tag{3}
\]

where \( P_0 \) is pressure at the maximum rate of relaxation and \( P_\infty \) is the pressure asymptote. The value of \( \tau \) was determined using the Levenberg-Marquardt technique to fit Eq. 3 to the pressure decay curve of each measured beat. To avoid the confounding effects of the succeeding contraction, Eq. 3 was fit starting from the time of maximum rate of relaxation to the time when pressure had fallen to just <0.5 mmHg above the end-diastolic pressure of the succeeding contraction.

Characterization of twitch length and beat-to-beat period. To estimate when the vessel twitch length (point A to point C, Fig. 2) equaled the beat-to-beat period (point B to point B, Fig. 2), we performed a least-squares regression analysis on the vessel twitch length- and the beat-to-beat period-end-diastolic diameter relationships. We then calculated the diameter and time interval when these two regression lines intersected. The resulting measure of time was termed the “critical period.”

Data analysis. The end-diastolic diameter at the critical period was used to separate the diastolic active tension-diameter data into two groups. A linear regression was calculated for each group. The slopes were then compared with a paired \( t \)-test. Similarly, the critical period was used to separate the diastolic active tension period data into two groups. A linear regression was calculated for each group. The slopes were then compared with a paired \( t \)-test. \( P < 0.05 \) was considered significant.

Normalization of diameters. For purposes of graphing pressure-diameter and tension-radius relationships of vessels with disparate diameters, end-diastolic diameter was normalized to end-diastolic diameter at the critical period, and tension was normalized to peak differential tension. Values are means ± SE.

RESULTS

Data collection. Over a 5-mo period, >40 vessels were collected for study. More than 35 cannulated vessel segments failed to maintain spontaneous contraction throughout the experimental isobaric and isovolumetric protocols, developed leaks, or were found to contain valves (exclusion criterion 1 and 2). One of the five remaining vessels did not maintain consistent baseline frequencies (exclusion criterion 3). Another of the five remaining vessels yielded too few complete contraction cycles to establish valid tension-radius relationships (exclusion criterion 4). Therefore, of the >40 total vessels collected and cannulated, only 3 met all inclusion criteria to provide valid analysis, yielding a 90% failure rate. Analysis was performed on data from all three vessel segments.

Isovolumic pressure-diameter relationships. Figure 3 illustrates passive pressure-, end-systolic pressure-, and end-diastolic pressure-end-diastolic diameter relationships for each of the three vessel segments (3.4- to 4.2-mm end-diastolic baseline diameter and 3.4–5.6 mm long). At small diameters, the passive and diastolic pressures are similar, but, at larger diameters, diastolic pressures diverge from passive pressures. Within the range of measured isovolumic end-diastolic diameters, Eq. 2 fit the passive vessel relationships \( r^2 = 0.83, 0.90, \) and 0.95). The systolic and diastolic pressures increase with diameter.

Fig. 3. Isovolumic end-systolic (A), end-diastolic (C), and passive (o) pressure-diameter relations (data from 3 vessels). As end-diastolic diameter increases, end-diastolic pressure-diameter relationship diverges from the passive pressure-diameter relationship. Dashed line, baseline diameter. A, B, and C: vessels 1, 2, and 3, respectively.
Active tension-diameter relationships. Figure 4 illustrates systolic active tension, diastolic active tension, and differential tension. Systolic active tension increases monotonically with end-diastolic diameter. Diastolic active tension exhibits an apparent transition from low slope to high slope, with an increase in end-diastolic diameter. This apparent transition coincides with peak differential tension.

Characterizing contraction cycle timing. Contraction frequency increased with diameter, although vessel twitch length exhibited little or no increase with diameter. Figure 5 illustrates that contraction frequency is positively correlated with end-diastolic diameter ($n = 3$, $r^2 = 0.64, 0.86$, and 0.90). The isovolumic relaxation time constant ($\tau$ in Eq. 3) had a value of $1.78 \pm 0.06$ s (range $0.27–3.57$ for 112 cycles from 3 vessels), and $\tau$ did not significantly change or increased with increasing end-diastolic diameter. Equation 3 fit the isovolumic pressure decay curve ($r^2 = 0.99–0.93$ for 112 cycles from 3 vessels). The error of the estimate increased when contraction time decreased, an artifact of the smaller number of data points available for fitting. The time to full relaxation was $9.91 \pm 0.33$ s (for 37 cycles reaching full relaxation from 3 vessels), and time to peak was $2.29 \pm 0.05$ s (for 112 cycles from 3 vessels). The resulting vessel twitch length (time to full relaxation + time to peak) was $11.01 \pm 0.28$ s (for 37 cycles reaching full relaxation from 3 vessels) and exhibited little or no increase with end-diastolic diameter (Fig. 6).

Interaction of contraction cycle timing and active diastolic tension. As shown in Fig. 6, the vessel twitch length-diameter relationship intersects the beat-to-beat period-diameter relation. At the intersection of the two linear regression lines, the beat-to-beat period equals the vessel twitch length. The slope of the active diastolic tension-end-diastolic diameter relationship was significantly greater at diameters above this calculated intersection than at diameters below the intersection ($P < 0.0001$, $n = 3$). Although all three vessels were analyzed, Fig. 6 displays only one case for the sake of clarity; the change in the diastolic active tension-length relationship after the critical diameter for all vessels is illustrated in Fig. 4B.

Increase in diastolic active tension. When the contraction period is greater than the critical beat-to-beat period, the diastolic active tension changes only slightly with beat-to-beat period ($P = 0.0014$, $n = 3$). When the contraction period is less than the critical beat-to-beat period, the slope of the diastolic active tension-beat-to-beat period relationship significantly increases ($P < 0.0014$, $n = 3$).

DISCUSSION

Data collected from our unique in vitro preparation demonstrate that lymphatic vessels transition to a state of mechanical summation at high contraction frequencies. As frequency increases, the time between contractions decreases below the time needed for the vessel to contract and fully relax (i.e., the vessel twitch length). At this transition point, contractions begin to mechanically summate, inducing an increase in diastolic active tension. Because diastolic active tension affects pump filling and lymphatic vessel conductance, the transition
astolic tension increased frequency (21, 37, 43), our results reveal that decreasing beat-to-beat period increases diastolic active tension. Limitations of experimental success rate. The results of the present study have been made possible by developing the first system capable of producing isovolumetric, isobaric, and constant flow for spontaneously contracting lymphatic vessels. The methodological rigor required for combining classical biomechanics studies on the heart with classical biomechanical studies of blood vessels not only is a particular strength of the present work, it also led to its most obvious limitation. Of the >40 samples obtained for study, 37 failed to meet inclusion criteria. Although initial viability may have been hampered by our established procedure of transporting vessels in a warm solution (29, 37), it is likely that the cumulative effect of the constraints inherent in the preparation limited the experimental success rate. In particular, the protocol required short vessel segments to maintain very stable, repeatable spontaneous contractions for a long time. By relaxing our rigorous requirements, it would have been possible to analyze more samples and, thus, make more general statements about the isometric tension-radius relationship of isolated postnodal bovine mesenteric vessels. However, we erred on the side of methodological rigor to ensure that we could provide the solid, statistically significant evidence that mechanical summation can occur in such vessels. Limitations of the isovolumic preparation. We have identified four limitations to our present preparation that may impact our interpretation of our data. 1) We used a whole vessel segment, instead of a single strip of muscle or muscle cell. The estimated vessel twitch length is therefore a vessel property, rather than an individual muscle fiber or cell property. 2) When we sealed the vessel to create our isovolumic conditions, we established a condition with no flow. Stopping flow reduces flow-induced release of NO (17, 22, 45) and allows for accumulation of metabolites within the lumen. Both phenomena could have changed the contractile state over the time course of an isovolumic step. 3) Loading conditions could have changed to a state of summation could be fundamental to the physiological operation of lymphatic vessels. Contraction cycle timing and summation. We tested the hypothesis that summation significantly increases diastolic active tension by examining two competing relations: the beat-to-beat period (point B to point B in Fig. 2) and vessel twitch length (point A to point C in Fig. 2). In agreement with previous reports (6, 37, 43), our data indicate that contraction frequency and, consequently, beat-to-beat period are correlated with end-diastolic diameter (Fig. 5). The vessel twitch length may increase slightly with diameter (Fig. 6). When beat-to-beat frequency equals vessel twitch length, the vessel does not have enough time to fully relax before the next contraction. Therefore, the intersection of the beat-to-beat period-diameter and vessel twitch length-diameter relationships predicts the diameter and critical period after which mechanical summation will occur (Fig. 6). The diameter at which this intersection occurs divides the diastolic active tension-diameter relationship into two operating ranges: 1) a diameter below which diastolic active tension only slightly increases with diameter, and 2) a diameter above which summation results in a significant increase in diastolic active tension with diameter. Similarly, after the critical period, diastolic active tension increases markedly with decreasing beat-to-beat period (Fig. 7). In contrast to the previously characterized relationship that noted increasing diastolic tension builds rapidly because of incomplete relaxation (P < 0.0014 for each of 3 experiments). Data from 1 experiment are shown for visual clarity.
over the time course of an isovolumic step recording. For instance, viscoelastic creep (36) could diminish transmural pressure and, thus, preload, or vessel permeability may allow a small portion of luminal volume to exude across the vessel wall. 4) Any nonuniform contraction or stray compliance in the system may have caused small fluid shifts within the vessel segment during contraction, which would allow some incidental muscle shortening.

Endothelial shear stress and limits to interpretation of lymphatic function in vivo. One of the most critical aspects of our experimental design was the minimization of shear stress-mediated release of NO. Shear stress was negligible during isovolumetric contractions (which prevented flow). Since each step in volume was preceded by only a very low level of controlled flow under isobaric conditions to wash out luminal metabolites (Fig. 2), it is conceivable that NO was produced. However, the flow was relatively small (14, 30, 31), and thus shear was estimated to be well below levels that would significantly impact function. It is, therefore, unlikely that our conclusion that summation increases diastolic active tension was an artifact of changes in shear stress. However, a more important issue is that, in vivo, shear stress generated by contraction is significant enough to affect function (18). With physiological levels of shear stress in vivo, it is entirely possible that the resulting release of NO may decrease diastolic active tension, thus minimizing the effect of summation. Shear stress-mediated NO release in vivo may also decrease the frequency of contraction or increase the rate of relaxation (18), both of which could limit the propensity of a vessel for summation.

Lymphatic summation occurs in a physiological range. Although our experiments were conducted in vitro, the isovolumic and low-flow conditions are similar to certain pathological states with high afterload and low flow (e.g., venous hypertension and lymphatic obstruction). Venous hypertension and lymphatic obstruction produce high contraction frequencies and pressures (11, 19), similar to the frequencies and pressures reported in the present study (Figs. 3 and 5). More importantly, the vessel twitch length of 11.08 ± 1.54 s observed in our study is similar to those we obtained from analysis of data digitized from previous isovolumic and isometric reports on bovine mesenteric lymphatic vessels: 15.75 ± 1.33 s (37), 22.65 ± 1.58 s (37), 11.99 ± 1.31 s (26), and 10.44 ± 0.60 s (27). These values would predict summation starting at contraction frequencies as low as three beats per minute, a frequency exceeded in numerous in vitro, in situ, and in vivo studies (6, 7, 24, 32).

Influence of relaxation on critical frequency. Additionally, we characterized the lusitropic state (ability to relax) of the vessel in two ways. 1) We evaluated contraction and relaxation time periods and found that the duration of relaxation (9.91 ± 0.33 s, time to full relaxation) is greater than two-thirds of twitch length (11.01 ± 0.28 s), which is consistent with previous observations (5, 6). 2) We examined τ (Eq. 2), a standard method for characterization of the lusitropic state of the heart. The observed value of τ (1.776 ± 0.056 s, n = 112) is greater than that reported in left ventricular isovolumic relaxation (0.03–0.11 s) (47, 51). This long relaxation phase in lymphatic vessels allows summation to occur at lower relative frequencies, which is demonstrated when τ is expressed as a percentage of the beat-to-beat period. A lymphatic vessel with the observed τ (1.776 ± 0.056 s) would constitute >25% of the beat-to-beat period when the contraction frequency is >8.4 beats/min, which is commonly observed (6, 7). In comparison, for the relaxation time constant to constitute the same percentage of the beat-to-beat period in a normal human heart [τ = 0.039 s (38)], the contraction frequency would need to be >380 beats/min, which far exceeds the maximum heart rate. Since the rate of relaxation is the key determinant of the vessel twitch length, it is fundamental to the transition to a state of summation.

Uniqueness of lymphatic summation. Summation in lymphatic vessels may be a combination of mechanical and electrical summation. Although a refractory period in the lymphatic action potential prevents fused tetany (25), our results indicate there can be incomplete relaxation in lymphatic vessels. Mechanical summation is also evidenced in the reported results of a previous study (Fig. 4 in Ref. 31). Using electrical stimulation of lymphatic vessels, McHale and Roddie (31) demonstrated that end-diastolic tension increased at high frequencies. Similar to vascular smooth muscle summation, however, graded depolarization due to rapid repetitive stimulation has also been observed in lymphatic vessels, where increased release of neurotransmitters results in a graded depolarization of the membrane (1, 2, 28). Although our data cannot be used to discriminate between the effects of electrical and mechanical summation, our work demonstrates that lymphatic vessels exhibit significant mechanical summation.

Possible role in bell-shaped pressure-flow relationship. Lymphatic mechanical summation may play a role in the previously reported “bell-shaped” relationship between transmural pressure and lymph flow (12, 13). At lower transmural pressures, lymphangions exhibit behavior consistent with the Frank-Starling effect, where increases in preload increase stroke volume. However, as transmural pressure is increased above a critical level in lymphatic vessels, further increases in pressure cause lymph flow to decrease, presumably from decreased stroke volume. The resulting “pump failure” can be attributed to decreased systolic diameter resulting from stretching smooth muscle beyond its optimal length or decreased diastolic radius resulting from summation. However, it has recently been shown that optimal force is generated at pressures well into the pump failure region (49). Mechanical summation may, therefore, help explain the recent finding that “mesenteric lymphatics produce maximal force under pressures higher than normal pressures even though the amplitudes of the phasic contractions are known to be decreased at those pressures” (49).

Summation may allow lymphatic pumping at high pressures. Additionally, summation may preserve lymphatic vessel pump function under a wide range of conditions by lowering wall tension. Compared with the passive (fully relaxed) state, summation results in a decreased end-diastolic diameter for a given pressure. As such, we predict that, for a given pressure, the end-diastolic tension during summation would be less than that of the fully relaxed vessel (Eq. 1). Consequently, the decrease in wall tension as a result of summation may preserve the ability of the lymphatic vessel to contract at high pressures. However, a decrease in diastolic diameter could negatively impact pump filling and resistance to passive flow (7, 35, 38). Although fused tetany is prevented, summation may also increase the force of systolic contraction at the expense of a
smaller stroke volume, which would additionally preserve lymphatic operation under conditions with high pressure and afterload (e.g., venous hypertension and lymphatic obstruction)

**Summation may preserve synchronous contraction of lymphangions.** Furthermore, the slow relaxation rate, which allows for summation, may be a necessary feature in lymphatic vessels, which lack a specialized conduction system similar to that of the heart. A long relaxation time may ensure that the region of initial contraction in a lymphangion does not significantly relax before the contraction wave traverses the entire length of the lymphangion. The relatively slow relaxation rate in lymphatic muscle may represent a compromise between minimizing summation and ensuring synchronous contraction.

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**REFERENCES**


