# A CRITICAL CONTRACTION FREQUENCY IN LYMPHATIC VESSELS: TRANSITION TO A STATE OF PARTIAL SUMMATION

A Thesis

by

JOSHUA KEITH MEISNER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Biomedical Engineering

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## ABSTRACT

A Critical Contraction Frequency in Lymphatic Vessels:

Transition to a State of Partial Summation. (August 2006)

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Although lymphatic vessel behavior is analogous to hearts (e.g. systole and diastole) and blood vessels (e.g. basal tone), hearts and blood vessels have fundamentally different contractile properties. While summation during contraction is minimized in the heart, summation is necessary for tonic contraction in blood vessels. Because lymphatic vessel behavior mimics cardiac and vascular behavior, we hypothesized that above a critical contraction frequency there is significant summation, evidenced by significantly increased diastolic active tension (i.e. basal tone). We used an isovolumic, controlled-flow preparation to examine the interaction of contraction cycle-time with contraction frequency. Using segments of isolated lymphatic vessels (~1 cm in length and 3-4 mm in diameter) from bovine mesentery, we measured transmural pressure and diameter for end-diastole and end-systole during spontaneous contractions for 10 volume steps. We found time between contractions (beat-to-beat period) decreases with increasing diameter, and total contraction time (vessel twitch length, 11.08 ± 1.54 s) slightly increases with increasing diameter. At the intersection of these relationships, there is a critical period, below which the vessel does not have time to fully relax. Above the diameter at the critical period, diastolic active tension (end-

diastolic minus passive vessel tension) significantly increases with increases in diameter (309 to 562% change in slope, $p<0.0001$), and, below the critical period, diastolic active tension increases with decreases in beat-to-beat period (712 to 2208% change in slope, $p<0.0014$). Because this transition occurs within a physiological range, it suggests summation may be crucial for lymphatic vessel function as a pump and a conduit.

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# INTRODUCTION

*Lymphatic function.* 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, 52, 53). Edemagenic conditions arise when lymphatic outflow is hindered (e.g., with venous hypertension or lymphatic occlusion), or when transcapillary flux increases (e.g., with increased permeability). Whereas lymphangions respond to higher lymph pressures by *increasing* frequency and strength of contraction (21, 22, 34, 47), they respond to increased flow by *decreasing* frequency and strength of contraction (16, 25).

*Analogies to ventricles and blood vessels.* Having been first observed in ventricles and blood vessels, the responses of lymphatic vessels to increased pressure and increased flow have influenced how investigators characterize lymphangions mechanics. Viewed as pumping chambers, lymphangions exhibit identifiable systolic and diastolic periods (6, 27, 34). Like the heart, the strength of lymphatic pumping increases with preload (14, 34, 47) (Frank-Starling effect), the rate of contraction increases with stretch (22, 34, 47) (Bainbridge effect (8)), and the force of lymphatic contraction is limited by the velocity of shortening (10) (Hill effect). However, viewed as tubular conduits, lymphangions can exhibit a basal tone in diastole (7, 39). Like blood

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vessels, lymphatic vessels release nitric oxide and dilate in response to flow (16, 25, 51) (shear dilation), and lymphatic vessels can regulate diastolic diameter (7, 43) (vascular basal tone). This behavioral dualism is reflected in structural dualism in that both cardiac and vascular muscle myosin heavy chain and α-actin isoforms have been found in lymphatic muscle (40).

*Between a heart and a blood vessel.* However, cardiac and vascular behaviors require fundamentally different contractile properties. Though traditionally associated with skeletal muscle tetany, summation is not specific to tetanus or skeletal muscle. In ventricles, which must pump, incomplete relaxation is associated with pathological behavior (e.g. diastolic dysfunction (20, 57)). Therefore, the relaxation rate of ventricles is high, and a calcium plateau creates an extended refractory period in the cardiac action potential to minimize summation. In contrast, blood vessels, which must regulate blood flow through tonic contraction, summation of electrical stimuli is a normal element of regulating vascular tone (e.g. myogenic response (9)). Therefore, the rate of relaxation of blood vessels is low (46), and vascular smooth muscle contraction is correlated to graded membrane depolarization subsequent to increases in intracellular calcium (9, 48).

The similarities in cardiac and vascular behaviors seen in lymphatic vessels, however, require a unique combination of these contractile properties, which could yield behaviors unique to lymphatic vessels. Although there has been no rigorous study of lymphatic muscle relaxation rates, the relaxation time period is longer than in the heart (~1.5 s in lymphatics (6) versus ~0.1 s in the heart (20, 24)). While lymphatic vessels possess a refractory period that prevents tetanus, the effective refractory period is less

than total contraction time, ending at ~50% relaxation (28). Taken together, no one has demonstrated a mechanism that prevents the summation of contractions in lymphatic vessels, as is seen in the heart. We, therefore, hypothesize that lymphatic vessels can exceed a critical contraction frequency, above which there is significant summation, and diastolic active tension increases with frequency.

## METHODS

*Tissue preparation.* Conducting lymphatic vessels from bovine mesentery were collected at an abattoir shortly after death. Post-nodal lymphatic vessels (3-4 mm outer diameter) were ligated at the downstream end, then dissected. They were transported in approximately 30°C, 1% albumin physiological saline solution (APSS; pH 7.4) containing (in mM) 145 NaCl, 4.70 KCl, 2.00 $CaCl_2$, 1.17 $MgSO_4$, 1.20 $NaH_2PO_4$, 5.00 dextrose, 2.00 sodium pyruvate, 0.020 EDTA, and 3.00 MOPS. Segments of lymphatic vessel that appeared valveless and devoid of fat and connective tissue were cut to ~1 cm lengths. The vessels were then mounted on an isolated, perfused vessel bath, which allowed for the control of transmural pressure, luminal flow, and volume. To form a continuous, incompressible volume of fluid between stopcocks S1 and S2 (Fig. 1, all figures are in appendix), air pockets were removed from the fluid lines, and a tight seal was ensured by securing vessels with two loops of suture.

*Setting baseline conditions.* Lymphatic vessels were perfused with APSS infused with room air at 37ºC. Baseline transmural pressure was set by height of an inlet pressure cannula (Fig. 1) at 4 mmHg to yield near maximal pumping (15, 34). Luminal flow was set by a syringe pump (SP120P, World Precision Instruments, Sarasota, FL) at 10 mL/hr, the minimal flow necessary for complete luminal turnover once every thirty seconds. After warming, vessels were allowed to equilibrate for 15 minutes. If the vessels did not exhibit spontaneous contractions, the transmural pressure was transiently elevated to induce contractions. Lymphatic vessels containing valves or failing to exhibit periodic contractions for 10 minutes were discarded.

*Recording lymphatic pump parameters.* Using a monochrome CCD camera (Sony ST-XC50), we monitored the external diameters of the lymphatic vessels. Transmural pressures were measured using a PX26-001GV pressure transducer (Omega, Stamford, CT) adjusted to the height of the solution bathing the vessel. The transducer was connected to a fluid-filled tube extending from the pressure port (P1) at the inlet to the vessel (Fig. 1). Under steady flow conditions, the inlet pressure approximately equaled the luminal pressure. Image and pressure readings were then digitized, at 30 samples per second. Instantaneous vessel diameter and pressure were continuously measured and recorded through a custom real-time diameter tracking virtual instrument using LabVIEW 7.0 (National Instruments, Austin, TX) (38).

*Experimental protocol.* The syringe pump set luminal flow during equilibration periods and baseline measurements, where S1 and S2 were open to the vessel only. To establish isovolumic conditions, S1 and S2 were opened to the bypass only (Fig. 1), thus sealing the lumen of the vessel, volume injection line, and the pressure sensor line as a continuous volume of fluid.

To create a baseline isovolumic dataset, we collected pressure recordings at 10 volumes. The vessel was always sealed to isovolumic conditions at end-diastole (maximum diameter just prior to contraction) from the 4 mmHg baseline isobaric conditions. We, then, added or subtracted a predetermined volume using a microsyringe attached to the injection port (P2, Fig. 1). For each vessel, a set of volumes to be added and subtracted was determined from the vessel volume under baseline isobaric conditions. An incremental volume, rounded to the nearest 0.001mL, was chosen that

equal 10% of baseline end-diastolic volume, assuming cylindrical shape. One volume change set was comprised of the following changes: -5, -4, -3, -2, -1, 0, +1, +2, +3, +4 increments. The vessel was allowed to pump for 4 beats or 2 minutes, whichever ended first, under the isovolumic conditions. Then, the stopcocks were opened to baseline isobaric conditions and luminal flow reestablished for 5 minutes before the next isovolumic step. The 10 volume steps were conducted in ascending order from -5 to +4 for each vessel, yielding ~40 isovolumic beats for a complete experiment.

Next, we obtained the passive pressure-volume curve of the vessel by perfusing the bath and lumen with $Ca^{2+}$-free APSS for thirty minutes. After that time, the vessel was assumed to be fully relaxed (16, 46). At this point, we pressurized the vessel from 0 to 50 mmHg, stepwise with ~30 steps.

*Data analysis.* The first 4 complete contraction cycles for each volume step were chosen for analysis. Figure 2 illustrates how the lymphatic contraction cycles were divided based on 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 where there was minimal change in pressure. It was identified by the rate of change of pressure with respect to time (first derivative, $dP/dt$) and the rate of change of the rate of change of pressure with respect to time (second derivative, $d^2P/dt^2$). After the maximum rate of relaxation, the vessel was considered as fully relaxed (*point C*) when $|dP/dt| \leq 0.1$ mmHg/s and $|d^2P/dt^2| \leq 0.01$ mmHg/s$^2$. Beat-to-beat period was the time between the current and preceding

contraction peak (*point B* to *B*). Frequency of contraction was the reciprocal of the beat-to-beat period. The time to peak was the time difference between end-diastole and end systole (*point A* to *B*). Time to full relaxation was the time difference between end-systole to the end of the relaxation phase (*point B* to *C*). Vessel twitch length was the time interval from end-diastole to the end of the relaxation phase (*point A* to *C*).

Assuming the vessel was a thin-walled cylindrical tube, end-systolic, end-diastolic, and passive tensions ($T$) were calculated by LaPlace's Law (*Eq. 1*) from pressure ($P$) and diameter ($D$) data.

$$T = P \cdot D/2 \qquad (1)$$

From these calculated tensions, we derived three "active tensions": systolic active tension (end-systolic tension minus passive tension), diastolic active tension (end-systolic tension minus passive tension), and differential tension (end-systolic tension minus end-diastolic tension). Because the calculated tensions were collected at different diameters, the passive tension had to be interpolated. First, we obtained a continuous approximation of the passive pressure ($P$) as a function of diameter ($D$) by fitting an exponential regression (*Eq. 2*) to passive pressure-diameter data over the same range of end-diastolic diameters as the calculated tensions.

$$P_{passive}(D) = \alpha \cdot e^{\beta \cdot D} \qquad (2)$$

From *Eqs. 1* and *2*, passive tension was then calculated.

We characterized the rate of isovolumic relaxation with a time constant ($\tau$) using a non-zero asymptote model, as occurs with non-zero diastolic active tension (11, 26, 50) (*Eq. 3*).

$$P(t) = (P_0 - P_\infty) \cdot \exp(-t/\tau) + P_\infty, \quad (3)$$

Where $P_0$ is pressure at the maximum rate of relaxation and $P_\infty$ is the pressure asymptote.

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 less than 0.5 mmHg above the end-diastolic pressure of the succeeding contraction.

To estimate when the vessel twitch length equaled the beat-to-beat period, we performed a least squares regression analysis on both the vessel twitch length and the beat-to-beat period. We then calculated the diameter and time interval when these two regression lines intersected (Fig. 6). The resulting period was termed the critical period.

The end-diastolic diameter at the critical period was used to divide 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, with significance taken at $p<0.05$. Similarly, the critical period was used to divide 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, with significance taken at $p<0.05$.

Given the variation between vessels, end-diastolic diameter was normalized to the end-diastolic diameter at the critical period, and tension was normalized to peak differential tension.

## RESULTS

*Isovolumic pressure-diameter relationships*. Figure 3 illustrates three (passive, end-systolic, and end-diastolic) distinct pressure-end-diastolic diameter relationships for 2 vessel segments (ranging from 3.4-4.2 mm in end-diastolic diameter and 3.4-5.6 mm in length at baseline). At small diameters, the passive and diastolic pressures are similar, but, at larger diameters, diastolic pressure diverges from passive pressure. Within the region of measured isovolumic end-diastolic diameters, *Eq. 1* fit the passive vessel relationship with at a range of $r^2$ values of 0.9510-0.8336. Both the systolic and diastolic pressure-diameter relationships increase with diameter.

*Active tension-diameter relationships*. Figure 4 illustrates A) systolic active tension, B) diastolic active tension, and C) 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 and the vessel twitch length both increased with diameter. Figure 5 illustrates that contraction frequency is positively correlated with end-diastolic diameter ($r^2$ values ranged from 0.8994 – 0.8606). The isovolumic relaxation time constant ($\tau$ in *Eq. 2*) is constant or increases with end-diastolic diameter with a value of 1.82 ± 0.30 (ranged from 1.16 to 2.43). *Equation 2* fit the isovolumic pressure decay curve with an $r^2$ value of 0.9988 ± 0.0097, with error of the estimate increasing with the decreased number of data points in the fit as end-diastolic diameter increased. The time to full relaxation was 9.86 ± 1.83 s,

and time to peak was 2.51 ± 0.50 s. The resulting vessel twitch length (time to full relaxation + time to peak) was 11.08 ± 1.54 s, and exhibited little or no increase with end-diastolic diameter (Fig. 6).

*Interaction of contraction cycle timing and active diastolic tension*. Figure 6 illustrates that twitch length increases with end-diastolic diameter and the beat-to-beat period (the inverse of contraction frequency) decreases with diameter. At the intersection of the two linear regression lines, the beat-to-beat period equals the vessel twitch length. Above this calculated diameter at this intersection, the slope of the active diastolic tension-diameter relationship significantly increases (309 to 562% increase, $p<0.0001$).

Figure 7 illustrates that when the beat-to-beat period is greater than the transitional period (dotted line), the diastolic active tension only changes slightly with beat-to-beat period. However, when the beat-to-beat period falls below the critical period, the slope of the diastolic active tension-period relationship significantly increases (712 to 2208% change, $p<0.0014$).

## DISCUSSION

Our data demonstrates that there is a significant element of summation in lymphatic vessel contraction beyond a critical diameter and beat-to-beat period. As the time between contractions shrinks below the time needed for the vessel to contract and fully relax, contractions begin to summate inducing an increase in diastolic active tension. Because diastolic active tension affects the pump filling and the passive conductance of a lymphatic vessel, the critical beat-to-beat period and diameter where this transition occurs is central 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 relationships: beat-to-beat period (time interval *B*-*B* in Fig. 2) and vessel twitch length (time interval *A*-*C* in Fig. 2). In agreement with previous reports (6, 42, 47), our data show that the contraction frequency—and, consequently, beat-to-beat period—is correlated with end-diastolic diameter (Fig. 5). The vessel twitch length, however, may only slightly increase 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 intercept of the beat-to-beat period-diameter and vessel twitch length-diameter relationships predicts the diameter and critical period after which temporal summation will occur (Fig. 6). The diameter where this intersection occurs divides the diastolic active tension-diameter relationship into two operating ranges: 1) below this diameter, diastolic active tension only slightly increases with diameter, and 2) above this diameter, summation results in a significant increase in diastolic active

tension with diameter (~4 fold increase in slope). Similarly, after the critical period, diastolic active tension has a significant, rapid increase with decreasing beat-to-beat period (14 fold change in slope) (Fig. 7). As opposed to the previously characterized relationship of increases in diastolic tension increased frequency (22, 42, 47), our results reveal that decreasing beat-to-beat period increases diastolic active tension.

*Influence of relaxation on critical frequency.* Relaxation is the key determinant of the vessel twitch length and, consequently, the transition to a state of summation. We, therefore, characterized the lusitropic (ability to relax) state of the vessel in two ways. First, we found that factors which influence the duration of the relaxation will have the largest impact, since the relaxation time comprises greater than two-thirds of twitch length, which is consistent with previous observations (5, 6). Second, we examined the time constant of relaxation, $\tau$, (*Eq. 2*),a standard method for characterization of the lusitropic state of the heart, first introduced by Wiess et al. (55). The observed value of $\tau$ (1.82 ± 0.30 s) is greater than that reported in left ventricular isovolumic relaxation (~0.03-0.11 s) (49, 54, 55, 59), which suggests the rate of lymphatic vessel relaxation is slower than cardiac relaxation. The slower relaxation rate creates a relatively longer relaxation phase, which allows for summation to potentially occur at lower frequencies.

*Uniqueness of lymphatic summation*. While summation is not unique to lymphatics, the apparent transition between no summation and summation appears to be different from skeletal, vascular, and cardiac contractile behavior. Summation in slow twitch skeletal muscle is the classic example of tetanic contraction summation, where repetitive stimulation of the muscle causes twitches to fuse, increasing the force of

contraction (i.e. systolic contraction) (17, 18). Summation in the heart, where summation is minimized by an extended refractory period and rapid rate of relaxation (18), takes the form of incomplete relaxation, with increases in diastolic tone (e.g. left ventricular diastolic dysfunction (20, 54, 57)). Summation in vascular smooth muscle, however, occurs in terms of electrical summation, where, as the muscle is repetitively stimulated, the membrane is depolarized in a graded fashion and contraction increases subsequent to increases in intracellular calcium (9, 48).

Summation in lymphatic vessels may be a combination of both incomplete relaxation and electrical summation, presenting a unique type of summation. A refractory period in the lymphatic action potential prevents tetany (28), but—like in cardiac summation—our results indicate there is summation in lymphatic vessels related to incomplete relaxation. Contraction summation is also evidenced in the reported results of a previous study (figure 4 from McHale et al (35)). This type of summation is related to the rate of relaxation and the frequency of contraction. Like vascular smooth muscle summation, however, electrical summation has also been described in lymphatics, where increased release of neurotransmitters results in a graded depolarization of the membrane (1, 2, 33). For example, the exogenous application of norepinephrine increases action potential frequency such that there is a marked membrane depolarization and an increase in diastolic active tension, similar to that seen in the portal vein (2). While our data cannot be used to separate the effects of electrical versus contraction summation, our work and previous studies demonstrate that lymphatic vessels exhibit a unique combination of electrical and contraction summation.

*Lymphatic summation occurs in a physiological range*. While our preparation minimizes the affects of muscle shortening and flow, high contraction frequencies and pressures have been have been reported in lymphatic vessels in vivo in pathological conditions with high afterload and low shear (12, 19). 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 (42), 22.65±1.58 s (42), 11.99±1.31 s (29), and 10.44±0.60 s (32). These values would predict summation starting at contraction frequencies as low as 3 beats per minute, a frequency commonly exceeded in numerous in vitro, in situ, and in vivo studies (6, 7, 15, 27, 31, 37). These frequencies place the potential for summation well within the range of reported lymphatic operating conditions.

*Complexity of chronotropic, inotropic, and lusitropic interactions.* With the potential for summation within a physiological range operation, changes in intropy, chronotropy, or lusitropy from an intervention (e.g. norepinephrine) may cause a transition between two fundamentally different contractile behaviors. Without consideration of summation, the well-documented positive chronotropic and inotropic effects of norepinephrine on lymphatic vessels (2, 5, 29, 35, 37) might be expected to increase lymph flow. However, several studies have reported conflicting effects of norepinephrine on lymphatic pump function (2, 5, 35, 36). Specifically, lymphatic pump flow decreased despite an increase in frequency (5, 35, 36), which was attributed to a decrease in stroke volume. The decreased stroke volume—and presumably diastolic

diameter—may have arisen from the increase in frequency, an idea first proposed by McHale et al (35) that has remained unanswered.

The present work suggests that an increase in strength and frequency by norepinephrine may have resulted in summation, whereby increases in frequency would, indeed, decrease diastolic diameter with increased diastolic active tension. The transition to a state of summation would be particularly pronounced in vessels that have a higher starting contraction frequency, as was noted in McHale et al (35). Other solutions for these conflicting reports have been proposed, but summation may help explain this complex interaction of chronotropic and inotropic effects.

*Functional impact on lymph flow*. By comparing the passive and end-diastolic pressure-diameter relationships (Fig. 3), it becomes clear that summation impacts the lymphatic vessel end-diastolic pressure-volume relationship. As the end-diastolic pressure-diameter curve diverges from the passive (fully relaxed) pressure-diameter curve during summation, the end-diastolic diameter is decreased for a given pressure. The decreased diameter can inhibit lymphatic vessel flow by both decreasing pump filling and increasing resistance to passive flow (7, 43, 45).

In the comparable situation in the heart during left ventricular diastolic dysfunction, lusitropic agents can shift the end-diastolic pressure-volume relationship and improve pump function (20, 58). Perhaps a similar approach could be used in lymphatic vessels to improve lymph pumping, having an additional benefit of decreasing resistance to flow. However, inherent in the analogy to left ventricular diastolic

dysfunction is the implicit assumption that summation in lymphatic vessels is pathological.

*Implications for physiological control*. With the fundamental question arising from this behavior being its functional significance in lymphatic vessels, it is important to consider that summation may be important for proper lymphatic vessel function. The two behaviors that govern the transition (contraction frequency and rate of relaxation) appear to be controlled by two separate mechanisms. Though the origins of rhythmicity in lymphatic vessel contraction remains unclear, a subpopulation of pacemaking cells has been hypothesized (30, 40). Similar to lymphatic pacemaker behavior, the specialized pacemakers of the sino-atrial node (8) and interstitial cells of Cajal (56) also increase frequency of contraction with increases in stretch, but may be modulated by other means (e.g. nervous stimulation, catecholamines, drug toxicity, and electrolyte imbalance). Additionally, the rate of relaxation and contraction is governed in part by the myofilament isoforms (4, 23). The unique combinations of vascular, cardiac, enteric, and skeletal muscle isoforms found in lymphatics may allow lymphatic vessels to optimize contractile behavior and efficiency under the variety of loading conditions seen throughout the lymphatic system (15, 40). In addition to optimization, the balance pacemaker activity and molecular composition the results in summation may preserve lymphatic vessel pump function under a wide range of conditions through lowering wall tension and maintaining synchronous contraction.

Summation may reduce the end-diastolic tensions seen by a lymphatic vessel. When compared to the passive state (fully relaxed), 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. Though 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).

The slow relaxation rate may also be a necessary feature in lymphatic vessels, which lack a specialized conduction system like that of the heart. A long relaxation time may ensure that the initial region in a lymphangion does not significantly relax before the contraction wave traverses the entire length of the lymphangion. When the heart is similarly limited to muscle conduction velocity (e.g. in ventricular pacing or left bundle branch block), the rapid rate of relaxation results in asynchronous contraction, which decreases pump effectiveness (44). Therefore, the slower relaxation rate in lymphatic muscle may represent a compromise between minimizing summation while ensuring synchronous contraction.

*Limitations of the isovolumic preparation*. There are four limitations to our current preparation that could impact our interpretation. First, we used a whole vessel segment instead of a single strip of muscle or muscle cell, which affected our vessel twitch length estimate by increasing the time in systole and diastole as the contraction wave propagates along the vessel. The estimated vessel twitch length is therefore a

*vessel* property, not an individual muscle fiber or cell property. Also, if given long enough segments and a slow enough wave propagation velocity, parts of the vessel may differentially contract out of phase, causing multiple pressure spikes during a single isovolumic contraction wave. Second, when we seal the vessel to create our isovolumic conditions, we establish no flow conditions. Removing flow can have several effects; the flow-induced release of nitric oxide (16, 25, 51) is reduced, and metabolites may accumulate within the lumen. Both phenomena could change the contractile state over the time-course of an isovolumic step. Third, the loading conditions could change over the time-course of isovolumic recording. For instance, viscoelasticity could diminish the loading force over time (41), and vessel permeability may allow a small amount of fluid flux across the vessel wall. Fourth, any non-uniform contraction or compliance in the system may cause small shifting of fluid during contraction, which would allow some muscle shortening.

*Advantages of the isovolumic preparation.* Our methods provide several key advantages, while minimizing the preparation's limitations. First, by using short segments of vessel, we minimize the time it takes to propagate the contraction wave, producing a more uniform contraction across the entire vessel. Second, our preparation allowed us to control flow through the vessel during baseline conditions—a technique traditionally used in vascular mechanics to minimize the effects of shear-dilation. Additionally, by shortening the time period of isovolumic conditions and choosing a comparatively low flow (13, 34, 35), we minimize the change in flow between baseline and isovolumic conditions but still provide steady luminal turnover to remove potential

metabolites. Third, the use of a short isovolumic period reduced the confounding effects of viscoelasticity and fluid flux across the vessel wall. Fourth, by minimizing muscle shortening (Fig. 2), we minimized the Hill effect—a technique traditionally used in cardiac mechanics preparations to obtain maximal force of contraction. Therefore, the use of a controlled flow, isovolumic approach draws upon the strengths of traditional techniques for vascular and cardiac mechanics to reconcile the heart-like and vessel-like behavior of lymphatic vessels.

# CONCLUSION

Using an isovolumic, controlled-flow preparation to reconcile the differences in vascular and cardiac muscle behavior seen in lymphatic vessels, we identified a transition in diastolic active that occurs when the beat-to-beat period decreases below the vessel twitch length, and contractions begin to summate. The slow rate of relaxation and periodic spontaneous contractions of lymphatic vessels create a unique physiological combination of rhythmic contraction and summation that occurs within a normal physiological range. Because the resulting diastolic active tension is significantly different than the passive tension, summation affects both the pump and conduit function of a lymphatic vessel. The present work therefore presents a novel mechanism that may play a fundamental role in lymphatic physiology and serve as a target for clinical intervention.

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# APPENDIX

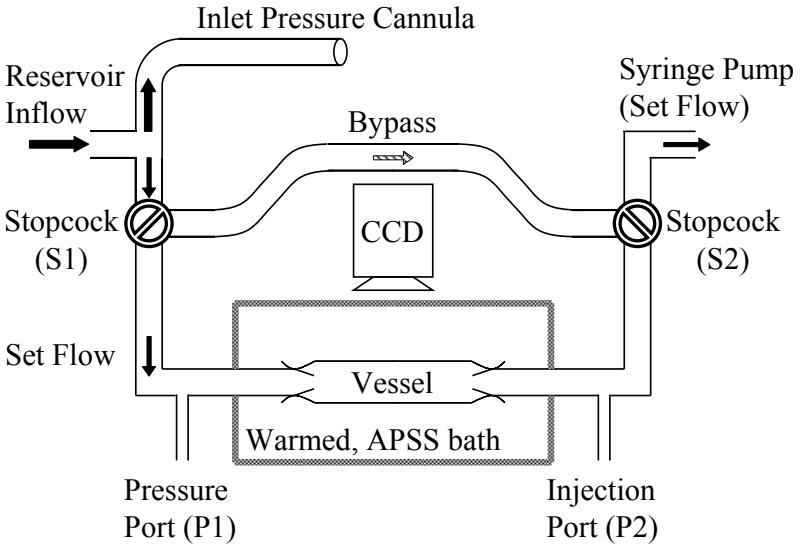


**Fig. 1.** Apparatus for controlling pressure, flow, and volume in isolated lymphatic vessel segments. The syringe pump draws physiologic saline (APSS) at a set flow across either the vessel lumen or the bypass, depending on the position of the stopcocks. Vessel diameter is measured from video captured by the CCD camera. The inlet pressure cannula sets the vessel transmural pressure during isobaric conditions. To set isovolumic conditions, stopcocks S1 and S2 are closed to vessel inflow and outflow. Transmural pressure was measured at port P1, and fluid was added or withdrawn from port P2.

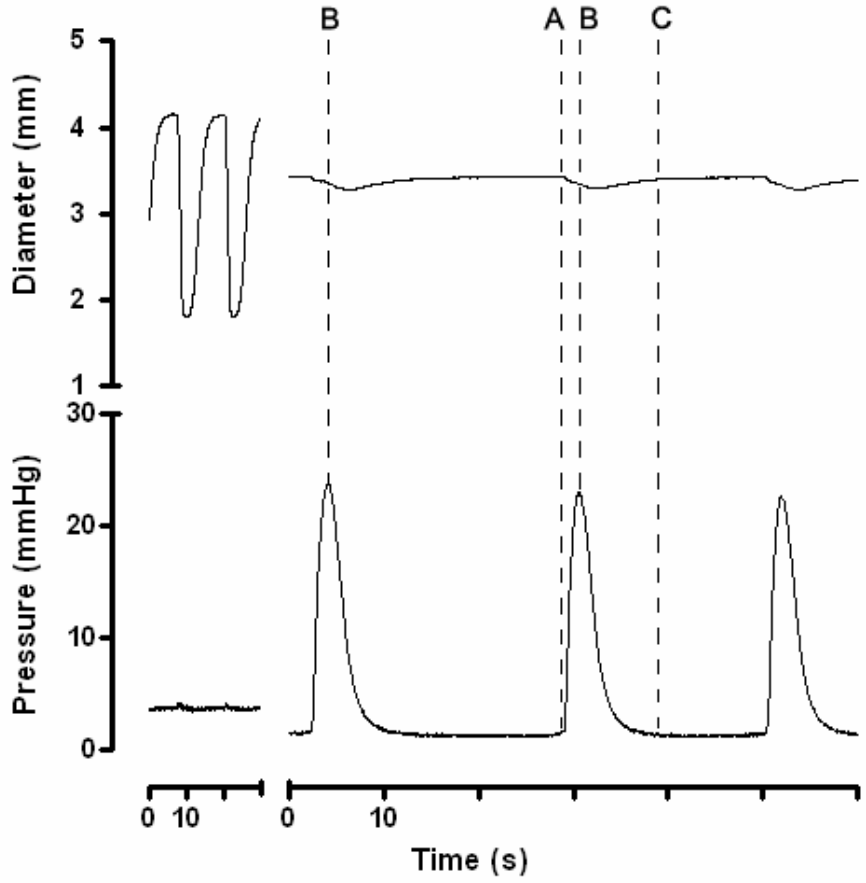


**Fig. 2.** Isovolumic contraction time periods. We defined end-diastole (*point A*) by the initial rise in pressure and end-systole (*point B*) by the peak in pressure. The vessel was considered to be fully relaxed at *point C*. Vessel twitch length constitutes the period from the start of systole to the fully relaxed state (*point A* to *C*). The beat-to-beat period is the time between the previous contraction peak and the current contraction peak (*point B* to *B*). The frequency of the contraction is the reciprocal of the beat-to-beat period.

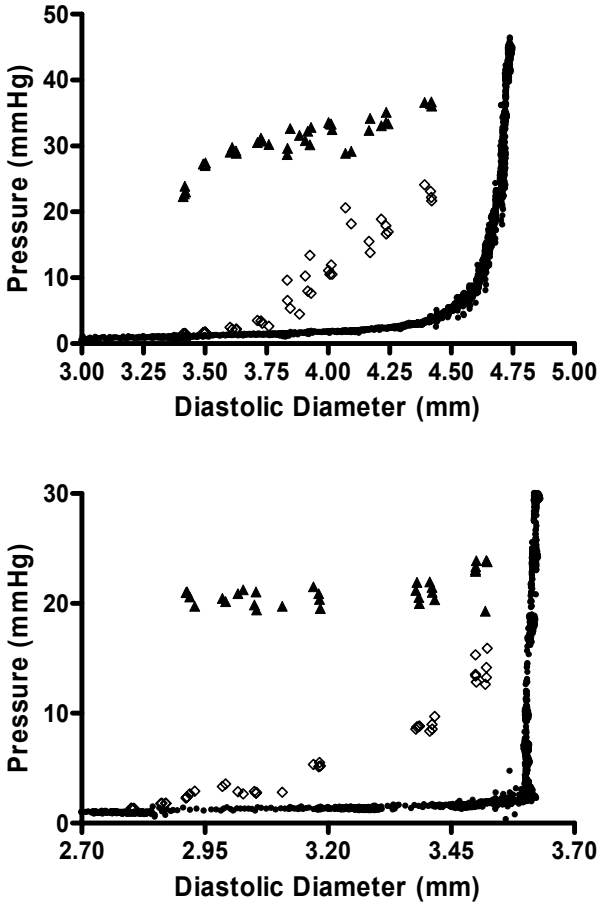


**Fig. 3.** Isovolumic end-systolic (▲), end-diastolic (◇), and passive (●) pressure-diameter relationships (data from 2 vessels). As end-diastolic diameter increases, the end-diastolic pressure-diameter relationship diverges from the passive pressure-diameter relationship.

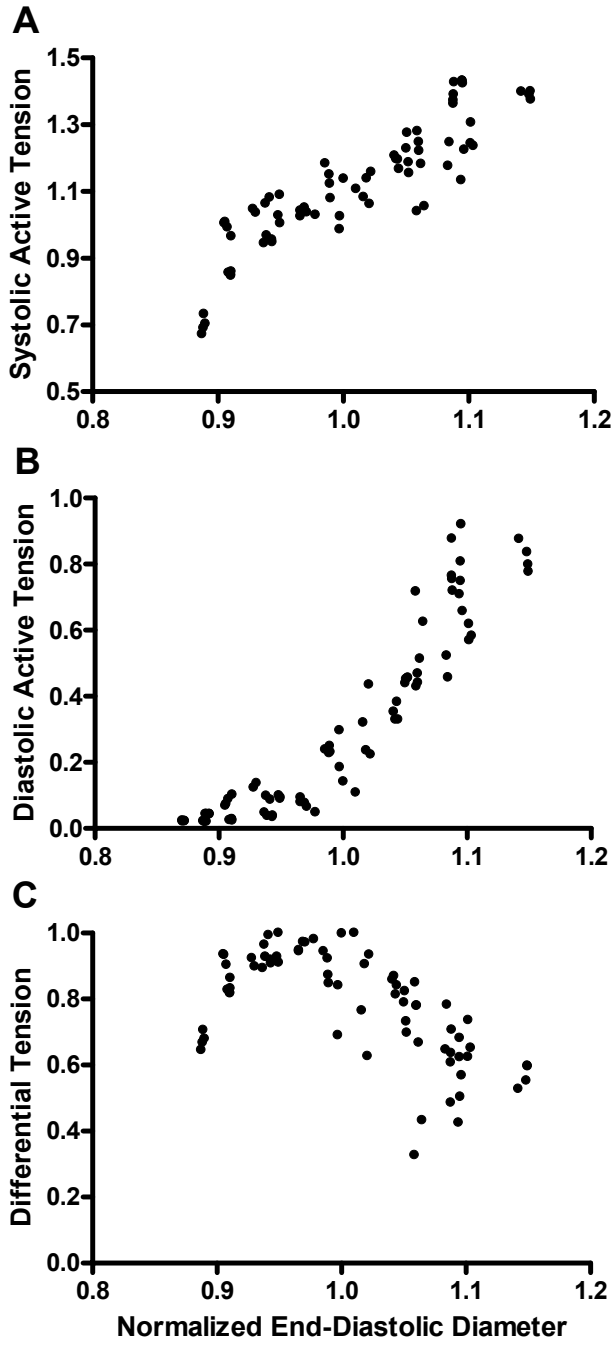


**Fig. 4.** Active tension-diameter relationships normalized to the end-diastolic diameter at the critical period (See Fig. 7) and peak differential tension (n = 2). Systolic active tension (end-systolic minus passive tension, A) increases with diameter. Diastolic active tension (end-diastolic minus passive tension, B) exhibits a transition near the diameter at the critical period. Differential tension (systolic minus diastolic tension, C) exhibits a peak near the diameter at the critical period.

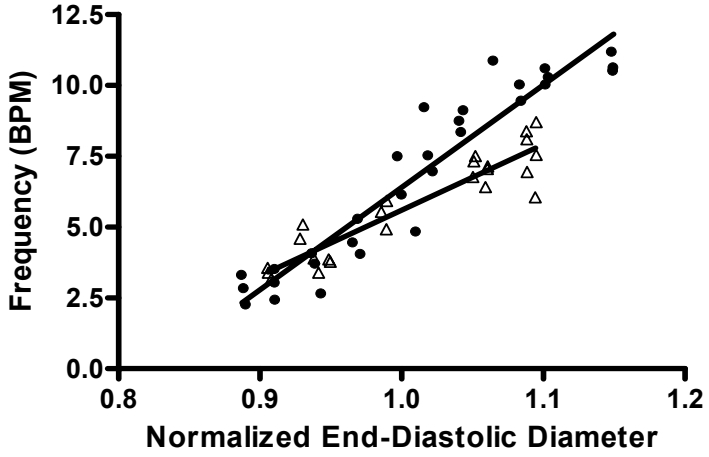


**Fig. 5.** Frequency-diameter relationship (data from two experiments). Frequency increased with increasing end-diastolic diameter (normalized to the end-diastolic diameter at the critical period) ($r^2$ ranges from 0.8994 to 0.8606).

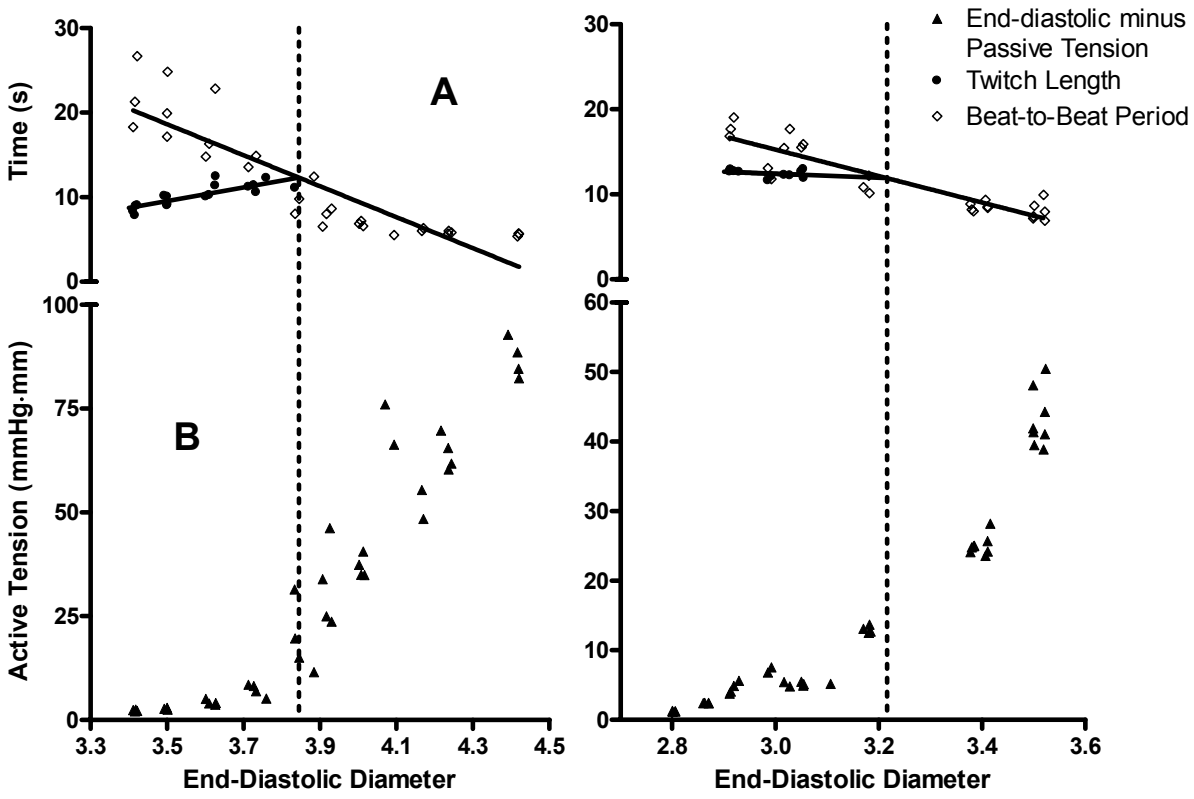


**Fig. 6.** The effect of contraction frequency and relaxation time (A) on diastolic active tension (▲) (B). As diastolic active diameter increases, beat-to-beat period (◇) decreases. The dotted line represents the intersection calculated from the linear regressions of beat-to-beat period and twitch length (●), which defined the critical period. Above this diameter, beat-to-beat period decreases below the vessel twitch length, and the slope of the diastolic active tension-diameter relationship significantly increases (309 to 562% change in slope, $p<0.0001$) (n = 2).

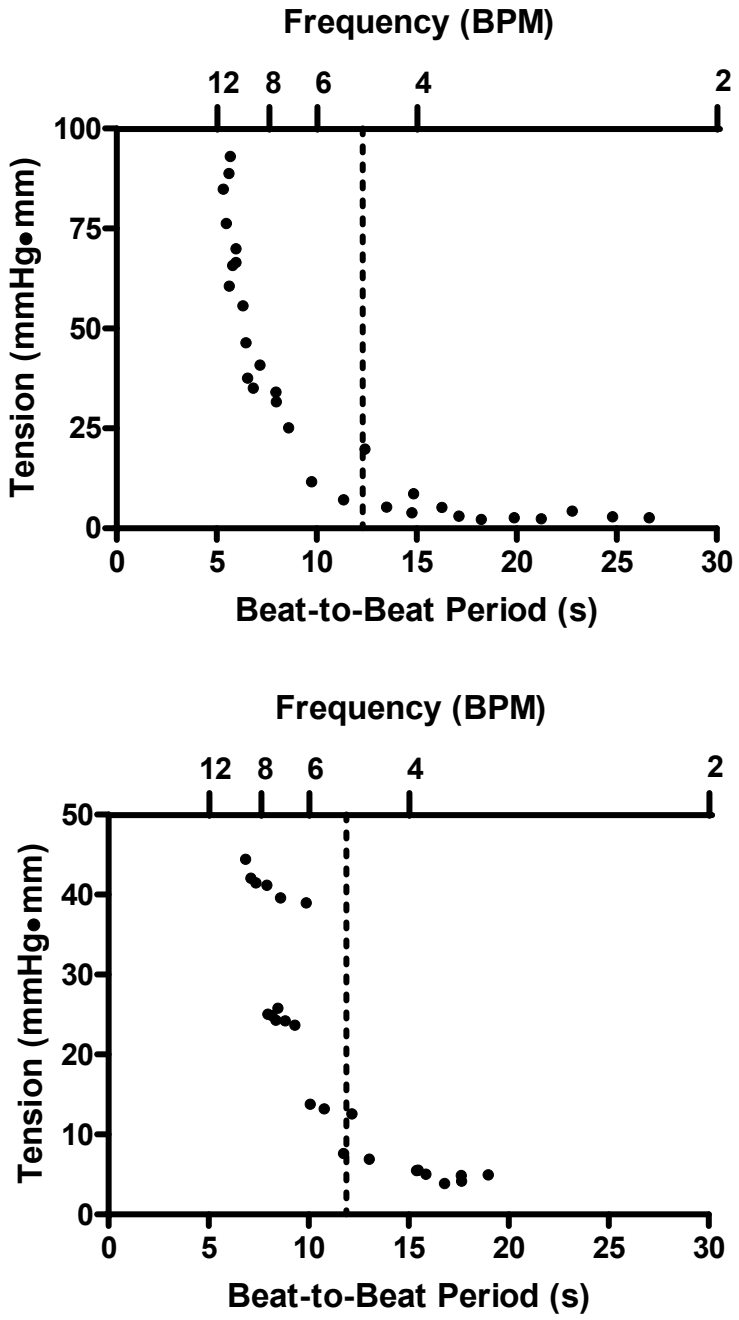


**Fig. 7.** Diastolic active tension versus beat-to-beat period (n = 2). As beat-to-beat period decreases below the calculated critical beat-to-beat period (dotted line), diastolic active tension builds rapidly due to incomplete relaxation (712 to 2208% change in slope, $p<0.0014$).

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