Intrinsic increase in lymphangion muscle contractility in response to elevated afterload

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LYMPH PROPULSION is determined by the interplay of multiple factors, both passive and active, that influence lymphatic vessels to behave as pumps or conduits for lymph flow. Active lymph propulsion is achieved by the spontaneous, rhythmic contractions of collecting lymphatic vessels (5, 29, 37, 53), with one-way valves ensuring that net lymph flow proceeds centrally (6, 12, 56). Active pumping is critical for the maintenance of normal lymph flow (2, 21), with impairment contributing to lymphedema (39, 42, 43). Although the lymphatic vasculature is a low-pressure network, the lymphatic pump must adapt to the elevated output pressures (Pout) that are imposed by partial obstruction, increased central venous pressure, and/or gravitational shifts. In the dependent extremities of humans, Pout can be elevated to relatively high levels under both physiological and pathological conditions (42).

Several studies (16, 22, 23, 34, 36) of large animals have measured the maximal Pout against which lymphatic vessels can pump, which varies between 10 and 30 cmH2O, depending on pre- or postnodal location. However, only two studies have measured lymphatic vessel diameter and intraluminal pressure (PL) during similar loading conditions. Benoit et al. (4) measured pressures and diameters of rat mesenteric lymphatic vessels in situ before and after systemic volume expansion, which induced a modest increase in PL from ~1.5 to ~3 cmH2O over a time course of ~10 min. Indexes of pump function, including a rightward shift in the pressure-volume (P-V) relationship, suggested enhanced pumping ability of the vessels. Likewise, Li et al. (35) examined the effect of combined input pressure (Pin) + Pout elevation on the pump function of isolated chains of interconnected sheep mesenteric lymphatic vessels. However, in neither study was the effect of a selective increase in Pout assessed.

In recent experiments on collecting lymphatic vessels isolated from the rat mesentery, we noted several responses of the vessels to elevated PL that have not been previously detected in lymphatic vessels from large animals, including observations that lymphatic muscle exhibits rate-sensitive activation/inhibition of contraction frequency (FREQ) (10) and myogenic constriction (9). Because lymphatic vessels share functional and biochemical characteristics of both smooth and cardiac muscle (13, 40), we hypothesized that the lymphatic pump would behave similarly to the cardiac pump in its intrinsic adaptation to elevated afterload. To test this hypothesis, protocols were devised to determine the responses of single, isolated lymphangions to selective elevation in Pout (in effect, an increase in afterload), with particular emphasis on 1) the time course of diameter and PL changes, 2) how Pout influences the dynamics of the lymphatic P-V relationship, and 3) the maximal limit of the lymphatic pump to an imposed afterload. We adapted methods to measure intralymphangion pressure and diameter simultaneously with valve movements, thereby enabling a more complete analysis of the adaptation of a single lymphangion with each contraction cycle. Our results lead to the conclusion that lymphatic muscle undergoes an intrinsic increase in contractility when subjected to an elevated afterload. This response is also modulated by basal preload, resulting in unique interactions between these two determinants of...
lymphatic pump function, as described in a companion paper (50). Taken together, our results provide important new insights into the normal operation of the lymphatic pump and its intrinsic ability to adapt to the elevation in P_{out} that is known to occur in conjunction with edemagenic and/or gravitational loads.

**METHODS**

**Vessel isolation.** All animal protocols were approved by the Animal Care and Use Committee of the University of Missouri and conformed with National Institutes of Health guidelines (NIH Pub. No. 85-12, Revised 1996).

Male Sprague-Dawley rats (150–300 g) were anesthetized with pentobarbital sodium (60 mg/kg ip), and a loop of duodenum from each animal was exteriorized through a midline abdominal incision. Suitable lymphatic vessels [inner diameter: 80–220 μm] were identified, dissected from mesenteric arcades, and placed in physiological saline solution (PSS) containing albumin (APSS) at room temperature. The animal was subsequently euthanized with Nembutal (100 mg/kg ic). APSS contained (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, 1.2 NaH₂PO₄, 0.02 EDTA, 5.0 glucose, 2.0 sodium pyruvate, 3.0 MOPS, and 0.5% purified BSA (pH 7.4 at 37°C). PSS was identical to APSS except that albumin was not added. Near the end of most experiments, passive vessel properties were assessed in Ca²⁺-free APSS, which was identical to APSS except that 3.0 mM EDTA was substituted for CaCl₂. All chemicals were obtained from Sigma (St. Louis, MO) except albumin (no. 10856, U.S. Biochemicals, Cleveland, OH).

**Cannulation and pressurization methods.** After the removal of fat and loose adventitia, a segment of a lymphatic vessel, containing two valves, was transferred to a 3-ml chamber and cannulated at each end with a glass micropipette (outer tip diameter: ~80 μm and inner tip diameter: ~60 μm) filled with APSS and mounted in a Lucite holder on a Burg-style V-track system (15). The entire apparatus was attached to the stage of an inverted Zeiss ACM microscope. Pin and P_{out} were set to 3 cmH₂O from standing reservoirs for a 30- to 60-min equilibration period at 36–37°C until a stable pattern of spontaneous contractions developed. The length of the vessel was adjusted before the equilibration period at a pressure of 1 cmH₂O so that there was no axial slack in the vessel that would otherwise interfere with diameter tracking. Throughout the experiments, the bath solution (PSS, 3-ml total volume) was exchanged at a rate of 0.4 ml/min using a peristaltic pump to prevent potential changes in osmolarity associated with evaporation.

Pressure control at each end of the vessel was achieved using a custom-made, analog servo controller, as previously described (12). Pin and P_{out} were measured using low-pressure transducers (model 104, Cyber-Sense, Nicholasville, KY) connected to the pressure controller. Pressure steps between 0 and 20 cmH₂O could be achieved within 200 ms, and combinations of step or ramp pressure waveforms could be applied to either end of the isolated vessel segment.

**Diameter and valve tracking.** The vessel image was digitized using a monochrome, fire-wire charge-coupled device camera (model A641FM, Basler, Ahrensburg, Germany). The progressive scan settings were adjusted to acquire the video at 30 Hz in a custom format (1,624 × 510 pixels, 12 bits/pixel) aligned with the long axis of the vessel. Inner diameter was tracked continuously using a custom computer algorithm (7) and digitized in synchrony with the pressure signals using an analog-to-digital card (model PCI 6030e, National Instruments, Austin, TX). Video images were captured in an AVI file with embedded pressure and diameter data so that all analog parameters could be extracted while remaining synchronized during replay offline. Movements of the lymphatic valves were detected offline by measuring the densitometric change in image intensity at the base or midpoint of the valve leaflet (12). That signal was subsequently converted to a binary record of valve position by applying a threshold (where 1 = open and 0 = closed). In some cases, diameter tracking was also performed at additional sites along the vessel during video replay.

**Servo-null pressure recording.** Servo-null micropipettes were pulled from borosilicate glass (outer/inner diameter: 1.00/0.5 mm, Omega-dot, Frederick Haer, Bowdoin, ME) using a Sutter P-97 puller (Sutter Instrument, Novato, CA). The tips were either gently broken or beveled to an outer diameter of 2–3 μm. Pipettes were back filled with 2 M NaCl and connected to a servo-null micropressure system (model 4A, IPM, La Mesa, CA) to monitor pressure in the central lymphangion. Pipette positioning was achieved using a hydraulic micromanipulator (model MO-102, Narishige, East Meadow, NY). The servo-null system used another low-pressure transducer (model 104, Cyber-Sense), which was calibrated using a water manometer along with the other transducers before each experiment.

Diameter was typically monitored in the central lymphangion near the upstream side of the output valve, as shown in Fig. 1A. Servo-null pressure measurements were made immediately downstream from the input valve. Micropuncture was performed at room temperature before the development of spontaneous contractions. The servo-null system was zeroed with the micropipette positioned just outside the vessel wall, and successful micropuncture left the pipette tip free from obstruction in the vessel lumen. Initial penetration of the vessel was performed by making a pilot hole using an unfiled, unbeveled micropipette (tip diameter: <0.5 μm). The criteria used to assess valid servo-null pressure recordings were the same those previously described (33). Accurate calibration of each micropipette was confirmed periodically during the experiment at 36–37°C by simultaneously raising Pin and P_{out}, particularly if pipette plugging was suspected.

**Equilibration protocol.** After a lymphatic vessel developed a stable contraction pattern at 3 cmH₂O, Pin and P_{out} were lowered to a baseline pressure of 1 cmH₂O for ~5 min. Initial tests indicated that baseline levels of Pin and P_{out} = 1 cmH₂O were optimal for the assessment of the full range of responses to P_{out}; however, in some cases, the vessel did not spontaneously contract at 1 cmH₂O, so that baseline pressures of 2 or 3 cmH₂O were occasionally used. Only vessels in which the central lymphangion developed robust contractions [i.e., amplitude (AMP) was typically ~50% of the maximum passive diameter at a baseline pressure of 1 cmH₂O] were used for further experimentation. In all preparations, the contractions of the entire vessel segment (central lymphangion plus two adjacent, partial lymphangions) were essentially synchronized.

An estimate of the maximal pressure that the vessel could generate to open the output valve (P_{out,max}) was made in a few initial experiments before and after micropuncture by recording the movements of the output valve leaflets during a ramp increase in P_{out} (typically at 4 cmH₂O/min) with Pin held at 1 cmH₂O. This estimate permitted the assessment of whether P_{out} was altered by the presence of the servo-null pipette; P_{out,max} measurements in vessels used for subsequent data analysis before and after micropuncture were in very close agreement.

**Protocols to test the response to elevated P_{out} (afterload) at constant Pin.** For the left cardiac ventricle, afterload is closely related to aortic pressure (specifically to ventricular wall stress). Under the conditions of our experiments, afterload was determined by a combination of P_{out} and resistance of the output cannula and tubing as well as by lymphangion wall stress. Given these caveats, we use the terms “afterload” and “P_{out}” synonymously. We made every effort to minimize output cannulae resistance (34) and to use output pipettes of consistent dimensions.

After equilibration, the vessel was challenged with ramp- and/or step-wise changes in P_{out}. P_{out} ramps consisted of a graded rise in P_{out} from baseline to 8, 12, 16, or 20 cmH₂O at a rate of ~4 cmH₂O/min. Pin was held at the baseline pressure during a pressure ramp so that diastolic pressure in each contraction cycle fell to a level near Pin if the output valve was functioning properly (see Fig. 1). After the ramp was complete, P_{out} was returned to the baseline pressure until a stable...
**Fig. 1. Response of an isolated lymphangion to an output pressure (Pout) ramp.**

A: diagram of the isolated lymphatic vessel preparation showing relative positions of the input, output, and servo-null pipettes. The red and black rectangles show the approximate positions of the diameter tracking windows used in B. B: representative recording from a lymphatic vessel responding to a ramp-wise elevation in Pout. Initially, input pressure (Pin) and Pout were both set to 1 cmH2O, and, after several spontaneous contractions, a Pout ramp to −12 cmH2O (at 4 cmH2O/min) was imposed. The bottom trace shows the time course of the inner diameter changes in the central segment (black) and output segment (red). The pressure traces show Pin (blue), Pout (red), and intraluminal pressure (Pl; black; measured by the servo-null pipette) superimposed on a common scale. As the Pout ramp progressed, greater systolic Pl developed by the central segment during each contraction cycle, but diastolic pressure returned to 1 cmH2O during the diastolic phase of each cycle. The top and middle traces show the input (blue) and output (red) valve positions (see methods), where I = open and 0 = closed. Both valves opened and closed during each contraction cycle over the entire duration of the Pout ramp, indicating that alternate phases of ejection and refilling occurred at all Pout levels shown. The lower than control contraction frequency (FREQ) after completion of the ramp is consistent with rate-sensitive inhibition (10).

Contraction pattern redeveloped. This procedure was typically repeated at least once. For Pout steps, Pin was held at the baseline pressure while Pout was rapidly increased during diastole from 1 to 4, 8, 12, 16, or 20 cmH2O, depending on the value of Plimit. Pressure elevation was maintained for variable periods of time, but usually for a minimum of 10 contractions or until the response of the vessel appeared to stabilize.

Near the end of each experiment, the bath was exchanged for Ca2+-free APSS, and a simultaneous Pn and Pout pressure ramp was imposed, from 1 to 20 cmH2O, so that the passive diameter of the vessel at each pressure could be determined.

**Data analysis.** Custom analysis programs (in LabView) were used offline to detect 1) end-diastolic diameter (EDD) and end-systolic diameter (ESD) for each individual contraction from the inner diameter recording; 2) contraction FREQ, determined on a contraction-by-contraction basis and reported in contractions per minute; 3) dP/dt from Pin measured in the central chamber; and 4) the area under the P-V curve (stroke work) for each contraction cycle. Lymphatic vessel contraction parameters were calculated as follows:

\[
\text{Contraction AMP} = \text{EDD} - \text{ESD} \quad (1)
\]

\[
\text{Normalized EDD} = \frac{\text{EDD}}{\text{EDD}_{\text{control}}} \times 100 \quad (2)
\]

\[
\text{Normalized AMP} = \frac{\text{AMP}}{\text{PassD}} \quad (3)
\]

where EDDcontrol is EDD at the baseline pressure and PassD is the passive diameter in Ca2+-free APSS at the same pressure at which AMP was measured. Other contraction parameters were calculated as follows:

\[
\text{EF} = \frac{(\text{EDD} - \text{ESD})^2}{\text{EDD}^2} \quad (4)
\]

\[
\text{FPF} = \text{EF} \times \text{FREQ} \quad (5)
\]

\[
V = \pi \times r^2 \times L \quad (6)
\]

where EF is ejection fraction, FPF is fractional pump flow (a relative index of pump output), V is volume, r is the internal radius of the vessel in each video frame, and L is the distance between the bases of the two valves, as measured from a calibrated video montage of the vessel made at the beginning of the experiment. Stroke volume (SV) was computed as follows after converting ESD to end-systolic volume (ESV) and EDD to end-diastolic volume (EDV):

\[
SV = \text{EDV} - \text{ESV} \quad (7)
\]

An alternative (non-normalized) index of pump output, calculated pump flow (CPF), was calculated as

\[
\text{CPF} = \frac{\text{SV}}{\text{FREQ}} \quad (8)
\]

Normalized V, as used for P-V loop analyses, was calculated as

\[
\text{Normalized V} = \frac{V}{\text{MaxV}} \quad (9)
\]

where MaxV is the volume of the lymphatic segment at the maximal passive diameter (MaxD) determined in Ca2+-free APSS at 20 cmH2O. PassD was determined by continuously measuring the diameter in Ca2+-free APSS in response to a pressure ramp from 0 to 20 cmH2O for a subset (n = 10) of the vessels, binning the diameters in pressure increments of 0.5 cmH2O, and fitting the normalized passive pressure-diameter relationship with a second-order exponential curve. As this relationship was quite consistent between vessels (χ^2 = 0.00021), we measured the MaxD for each vessel in Ca2+-free APSS at 20 cmH2O and used that value in the best-fit equation for the passive pressure-diameter relationship to calculate PassD at any given pressure.

Stroke work was determined by the following calculation:

\[
\text{Stroke work} = \sum \left( \frac{[P_{L}(t) + P_{S}(t+1)]}{2} \times [V(t) - V(t-1)] \right) \quad (10)
\]

where PL is the servo-null pressure and V is the lymphangion volume at each time point (t) in the contraction cycle (47). The alternative method used by Guyton for stroke work calculation [the product of SV × (mean ejection pressure – diastolic filling pressure)] was not appropriate as it did not permit the detection of negative stroke work (27).

**Statistical analyses.** Typically, one vessel was used for data analysis from each animal. Data were compiled in Excel and plotted using Igor (Wavemetrics, Lake Oswego, OR) and/or Photoshop (Adobe, Mountain View, CA). For analyses of pressure ramps, data for individual vessels were combined by binning the calculated variables for each contraction according to the instantaneous Pout level into
1-cmH2O bins spanning the pressure range from 1 to 20 cmH2O. Statistical tests were performed using JMP 7.1 (SAS, Cary, NC). For the analysis of most data sets, one-way ANOVAs were performed using Pout as the independent variable. When appropriate, Dunnett’s or Tukey’s post hoc tests were used to test for significant within- or between-group variations, respectively. Significance was defined as $P < 0.05$.

RESULTS

Response to ramp-wise Pout elevation. Pout elevation tested the response of the isolated lymphangion to increases in pressure that would result from a gravitational load, outflow obstruction, or tissue compression. Pout ramps enabled the assessment of continuous adaptation of the lymphangion to a progressively increasing load, whereas Pout steps allowed the assessment of the time course of the response to a fixed, but elevated, afterload.

A representative example of the response of a single, isolated lymphangion to ramp-wise Pout elevation is shown in Fig. 1B. Pout elevation led to overt distention of the output segment (red diameter trace) concomitant with a slight constriction of the central lymphangion (black diameter trace). Despite these contrasting responses, the spontaneous contractions occurring in all sections of the vessel typically remained synchronized. A notable exception in this case was the sinus region near the input valve, which switched from being in phase with the rest of the vessel at baseline Pout levels to being out of phase, or even dilating, with each contraction when Pout exceeded ~2 cmH2O (see Supplemental Material, Supplemental Movie S1).1 FREQ increased with increasing Pout, with most of the increase occurring over the Pout range from 1 to 4 cmH2O. Contraction AMP in the central region decreased with increasing Pout level, due to a combination of a progressive decline in EDD and a progressive rise in ESD.

The changes in EDD, ESD, AMP, and other contraction parameters for 16 vessels subjected to Pout ramps are shown in Fig. 2. AMP, SV, and EF declined as Pout rose, whereas FREQ increased. FPF and CPF, indexes of pumping ability, remained

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1 Supplemental Material for this article is available at the American Journal of Physiology-Heart and Circulatory Physiology website.
relatively constant, due in large measure to the increase in FREQ. A decline in EDD as a function of $P_{out}$ (as shown in Fig. 1B) was not evident in the raw EDD values as a function of $P_{out}$ due to variability in the vessel size between experiments; however, decreases in normalized EDD were significant at multiple $P_{out}$ levels. After $P_{out}$ exceeded $\sim 10$ cmH$_2$O, contractions were not consistently maintained.

Ramp-wise $P_{out}$ elevation led to a progressive increase in peak systolic $P_l$, as recorded in the central lymphangion (black pressure trace), which equaled or exceeded the instantaneous value of $P_{out}$ at each respective point in time (Fig. 1B). Successful ejection (partial ejection) of the central lymphangion occurred with each contraction throughout the $P_{out}$ ramp, as denoted by opening of the output valve in systole (12). However, in about half the vessels subjected to a $P_{out}$ ramp to 12 cmH$_2$O, the lymphatic pump failed to eject at some point during the ramp, which we termed $P_{limit}$. In those cases, the output valve failed to open during systole and remained closed until $P_{out}$ reached its target level and was lowered subsequently (see Supplemental Movie S2). The mean value of $P_{limit}$ was $11.9 \pm 0.7$ cmH$_2$O ($n = 16$, range: 3–19 cmH$_2$O). Since $P_{limit}$ values were not normally distributed, the median value of 12.5 cmH$_2$O more accurately described the data. The reasons for the relatively large variation in $P_{limit}$ between lymphangions, or even lymphangions studied from the same vessel, were not consistently maintained.

The response of another lymphangion to ramp-wise $P_{out}$ elevation from 1 to 16 cmH$_2$O followed by a ramp-wise return to 1 cmH$_2$O is shown in Fig. 4A. In Fig. 4B, P-V loops are shown for three of the contractions in which the values of peak systolic $P_l$ were $\sim 2$, 4, and 8 cmH$_2$O, respectively. A few interesting features of these loops are worth noting. First, the $P_l$ waveform exhibited a “dip” below $P_{in}$ at the end of systole/beginning of diastole when $P_{in}$ and $P_{out}$ are equal and low; this resulted in the “boot heel”-shaped portion on the left edge of the P-V loop (arrowhead in Fig. 4B). Second, as $P_{out}$ increased, the P-V loops became more vertical and rectangular in shape.

To summarize P-V data from multiple vessels, we selected six different points that were consistently present in loops with positive stroke work (see Stroke work calculation from P-V loops for a full explanation). These points are indicated on the P-V loop in Fig. 4B for the contraction at $P_{out} = 8$ cmH$_2$O. Following the convention suggested by Li et al. (35), the points denote 1) EDV and end-diastolic pressure at the beginning of the quasiosovolumetric contraction (point $a$), 2) the end of the quasiosovolumetric contraction (point $b$), 3) peak systolic $P_l$, 4 cmH$_2$O (left) and after $P_{out}$ was elevated to $\sim 4$ cmH$_2$O (right). Vertical lines denote the beginning of systole and diastole. $P_{in}$ (blue) and $P_{out}$ (red) traces are overlaid on the $P_l$ trace (pressure recorded by the servo-null pipette in the central chamber) to facilitate comparisons of the different pressures at each point in time.

**Fig. 3.** Pressure-volume (P-V) loop analysis for the isolated lymphangion. A: time course of $P_l$ and diameter changes during two complete contraction cycles when $P_{in} = P_{out} = 2$ cmH$_2$O (left) and after $P_{out}$ was elevated to $\sim 4$ cmH$_2$O (right). Vertical lines denote the beginning of systole and diastole. $P_{in}$ (blue) and $P_{out}$ (red) traces are overlaid on the $P_l$ trace (pressure recorded by the servo-null pipette in the central chamber) to facilitate comparisons of the different pressures at each point in time. B: plot of $P_l$ versus calculated volume for the two contraction cycles shown in A. The color coding corresponds to the progression of time (relative to the start of contraction) from the beginning of the first contraction (dark brown) to the end (yellow) of each contraction (e.g., each color shade corresponds to the time point shown in the trace in A). The direction of both loops was counterclockwise, as indicated by arrows.
(point c), 4) the end of systole (point d), 5) the end of the quasisovolumetric relaxation (point e), and 6) the lowest pressure in diastole (point f). Based on measurements of these common points, summary data from 16 vessels subjected to P_out steps from 1 to 4, 8, 12, and 16 cmH2O are shown in Fig. 4C.

**Stroke work calculation from P-V loops.** Stroke work is a common index of cardiac function and can be calculated from the area under the P-V loop (47). We used a similar method to determine stroke work for single lymphangions challenged with elevated levels of P_out. Positive stroke work was defined as work performed by the vessel to expel its contents; negative stroke work implied work performed on the vessel (i.e., filling from one or both of the cannulation pipettes in our recording configuration).

The influence of afterload on lymphangion stroke work is not known. In Figs. 5 and 6, stroke work was calculated for two representative lymphatic vessels over the range of afterloads used in the P_out ramp protocols. Data for a vessel that was able to pump against P_out levels up to P_limit = 18 cmH2O are shown in Fig. 5A. The pressure and diameter traces for each spontaneous contraction are shown in response to a ramp elevation in P_out from 3 to 17 cmH2O, with six representative contractions labeled. The output valve opened at peak P_L during each contraction, indicating that the vessel partially ejected its contents. Figure 5B shows the pressure and diameter traces on an expanded timescale for the contraction 3 along with the time course of calculated stroke work (top trace). During systole, stroke work was positive as the volume declined, with a peak corresponding to the opening of the output valve. During diastole, there were two brief periods of negative stroke work, corresponding to the filling of the vessel from the input pipette. In Fig. 5C, P-V loops are shown for six labeled contractions, with red shading indicating net positive stroke work (only the areas inside the loops are shaded here for clarity). Stroke work rose with increasing afterload up to a maximum at P_out ~ 6 cmH2O (Fig. 5C) and then declined ~20% to a plateau level at P_out levels just below P_limit; this trend was evident on the graph of net stroke work as a function of P_out for these six contraction cycles (open circles in Fig. 5D; the closed circles are the stroke work for all other contraction cycles).

In contrast, a similar analysis of data from a vessel that failed to eject near the end of a P_out ramp from 1 to 14 cmH2O is shown in Fig. 6. Figure 6A shows the entire P_out ramp, with P_out and P_in traces overlaid on the P_L trace. Near the end of the P_out ramp, when afterload apparently was approaching P_limit (estimated earlier in the same experiment to be ~15 cmH2O), the output valve failed to open during systole for several consecutive contractions (beginning at the arrow). Contraction 5 is shown on an expanded timescale in Fig. 6B, where a period of negative stroke work is evident at the end of systole. Negative stroke work in this context indicated that net work was being performed on the vessel. Figure 6C shows P-V loops...
for the six labeled contractions, including contraction 6, which was the first of four successive contractions in which ejection failed. Interestingly, in this and similar vessels that failed to eject as P_{out} approached P_{limit}, the shapes of the P-V loops resembled “figure eights” in that the early phase of systole was characterized by positive stroke work (red shading), whereas the later phase of systole was characterized by negative stroke work (blue shading; only the areas inside the loops are shaded for clarity). As contractions continued at P_{out} = 15 cmH_2O, the proportion of negative stroke work increased. Figure 6D shows a graph of net stroke work for the all contraction cycles during this P_{out} ramp (with the contraction cycles in Fig. 6C marked by open circles), showing the progressive decline in net stroke work from positive to negative values.

**Contractility change in response to step-wise P_{out} elevation.**

A primary goal of our study was to test if the lymphatic adaptation to elevated P_{out} represented an increase in contractility, according to the strict definition of that term used in the cardiac literature (46). Step-wise changes in P_{out} were more useful than ramps for this purpose as they allowed analysis of the initial response and secondary adaptations, if any, over time at a constant afterload. The response of a lymphatic vessel to several stepwise increases in P_{out} is shown in Fig. 7A. P_{out} steps were initiated while the vessel was in diastole. On the first contraction after P_{out} elevation from 1 to 12 cmH_2O, there was a slight rise in EDD followed by a substantial increase in ESD, resulting in a fall in AMP and SV. However, over the subsequent eight to nine contractions, both EDD and ESD gradually declined to different degrees such that AMP increased slightly. The time-dependent decline in EDD represented an apparent myogenic constriction (decline in diameter with rise in mean PL), and, in most cases, the magnitude was roughly proportional to the size of the P_{out} step. However, the third P_{out} step shown in Fig. 7A shows that a secondary, gradual change in ESD could occur without a substantial change in EDD. Note the apparent rate-sensitive increase in FREQ (10), followed by a secondary decline, that was evident in response to most P_{out} steps shown in Fig. 7 and, to a lesser extent, the reverse response (rate-sensitive inhibition) that was evident when pressure was lowered (see also Fig. 1).

In Fig. 7B, data from portions of the actual recording were used to plot P_L as a function of volume (calculated from diameter and length) during the three P_{out} steps and one control period (where P_L = P_{out}). Time-dependent declines in EDV and ESV at a given level of P_{out} were apparent as leftward shifts in the sequential P-V loops at each afterload level. The common color coding used in Fig. 7, A and B, corresponds to the relative time from the start of the P_{out} step. Data for one to
two contractions at the control pressures preceding each $P_{\text{out}}$ step are included as the dark brown loops at $P_{\text{out}} < 2 \text{ cmH}_2\text{O}$. According to a convention commonly used in the cardiac literature, the slope of the line fitting the end-systolic $P$-$V$ relation (i.e., connecting the values of ESV at various levels of afterload) represents a given level of muscle contractility (46), such that:

$$P_{\text{ES}} = E_{\text{ES}} (V_{\text{ES}} - V_0)$$  \hspace{1cm} (11)

where $P_{\text{ES}}$ is end-systolic $P_L$, $V_{\text{ES}}$ is ESV, $E_{\text{ES}}$ is the slope of the line, and $V_0$ is the intercept on the volume axis. For the data shown in Fig. 7B, two lines were drawn to fit the P-V points at the end of systole immediately after $P_{\text{out}}$ elevation (dark brown line) and \( \sim 2 \text{ min} \) after the vessel was allowed to adapt (yellow line). The increase in the slope of the latter line suggests that an increase in contractility occurred with time after $P_{\text{out}}$ elevation. Notably, no substantial changes in diastolic $P_L$ (i.e., preload) occurred during this time.

To quantify the effect of elevated afterload on the end-systolic pressure-volume relationship (ESPVR), the responses of 16 vessels to step changes in $P_{\text{out}}$ from 1 to 4, 8, 12, and 16 cmH$_2$O were analyzed. Normalized volume was calculated to account for substantial differences (range: \( \sim 2\)-fold) in vessel-to-vessel diameter (see Table 1). Only counterclockwise P-V loops were used for this analysis. Data were analyzed on a contraction-by-contraction basis after each $P_{\text{out}}$ step, but only the mean data for the first, fourth, and eighth contractions are shown for clarity. Each of the three data sets was fit with a
first-order polynomial whose slope ($E_{ES}$), shifted from 34.8 (for contraction 1) to 41.5 (for contraction 4) to 46.7 (for contraction 8). ESPVR data points at $P_{out}/H11005 16$ deviated substantially from straight lines fitting the data points at lower values of $P_{out}$. However, linear fits of the mean data at $P_{out} = 4, 8, and 12 \text{ cmH}_2\text{O}$ gave a similar progression to higher $E_{ES}$ values (~30% increase) between the early and late contractions. Equations for both sets of analyses are shown in Table 2.

As an alternative analysis, time courses of changes in peak $dP/dt$ were measured for the same series of $P_{out}$ steps to determine if peak $dP/dt$ increased over time after $P_{out}$ elevation.

![Fig. 7. Secondary changes in EDV and ESV after a step elevation in $P_{out}$. A: time course of $P_t$ and diameter changes in response to three step changes in $P_{out}$ (from 1 to 12, 8, and 4, cmH$_2$O). Horizontal lines are reference points drawn to assess relative changes in ESD for the first $P_{out}$ step; the dotted line marks the ESD for the initial contraction, and solid line marks the ESD for contractions 8–12. $P_{in}$ (blue) and $P_{out}$ (red) traces are overlaid on the $P_t$ trace. The open circle denotes a spike artifact in the $P_t$ recording from table vibration or the pipette tip touching the vessel wall. B: P-V plots for the $P_{out}$ steps shown in A. The color coding corresponds to the progression of time from the beginning (dark brown) to the end (yellow) of each step. The initial one to two contractions before each pressure step are shown in dark brown (where $P_{out}/H11005 1 \text{ cmH}_2\text{O}$). The vertical lines indicate linear fits to P-V points at end systole for the first [early end-systolic pressure-volume relationship (ESPVR)] and last (late ESPVR) contractions at each $P_{out}$ level. C: summary plot of average values for PL and volume at the end of systole for the first contraction (c1), fourth contraction (c4), and eighth contraction (c8) after a $P_{out}$ step, with corresponding $P_{out}$ levels indicated on the right. Blue, red, and green lines are the respective best linear fits of the three sets of data points (c1, c4, and c8), and equation parameters are shown in Table 2.

### Table 1. Vessel dimensions

<table>
<thead>
<tr>
<th></th>
<th>Means ± SE</th>
<th>Range</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal passive diameter, μm</td>
<td>160.0 ± 3.5</td>
<td>100–202</td>
<td>34</td>
</tr>
<tr>
<td>Length (between base of valves), μm</td>
<td>1231.1 ± 76.6</td>
<td>344–3237</td>
<td>43</td>
</tr>
<tr>
<td>$P_{max}$, cmH$_2$O</td>
<td>11.9 ± 0.7</td>
<td>3–19</td>
<td>48</td>
</tr>
</tbody>
</table>

Maximal passive diameter determined at 20 cmH$_2$O in Ca$^{2+}$-free physiological saline solution with albumin. $P_{max}$, limiting pressure associated with ejection.

### Table 2. Parameter values for curve fits to equation 11

<table>
<thead>
<tr>
<th>$P_{out}$ Range of 4–16 cmH$_2$O</th>
<th>$P_{out}$ Range of 4–12 cmH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$</td>
<td>$E_{ES}$</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>Contraction 1</td>
<td>-9.5</td>
</tr>
<tr>
<td>Contraction 4</td>
<td>-11.8</td>
</tr>
<tr>
<td>Contraction 8</td>
<td>-12.4</td>
</tr>
</tbody>
</table>

$P_{max}$, output pressure; $V_0$, volume-axis intercept (in cmH$_2$O); $E_{ES}$, slope of the end-systolic pressure-volume relationship (in cmH$_2$O/normalized volume); r, internal radius of the vessel.
On average, however, the changes in peak dP/dt approximated the changes in \( E_{ES} \) over time (64% increase for \( P_{out} = 4 \) cmH\(_2\)O; 22%, 26%, and 24% increases for \( P_{out} = 8, 12, \) and 16 cmH\(_2\)O, respectively), even though the dP/dt calculation did not take into account compliance-mediated changes in volume.

**Time-dependent increase in contractility with sustained, step-wise \( P_{out} \) elevation.** \( P_{out} \) steps often elicited a variation in the response just described, as shown by the recording in Fig. 8. A rapid step in \( P_{out} \) from 1 to 8 cmH\(_2\)O, which was lower than the average value of \( P_{limit} \), led to an increase in systolic \( P_L \) (from 0.3 to 2.6 cmH\(_2\)O) on the first subsequent contraction; obviously, this change in \( P_L \) was insufficient to open the output valve, as evident from the trace shown in Fig. 8. However, as \( P_{out} \) remained elevated, peak systolic \( P_L \) increased with each subsequent contraction over the following 2-min period until peak \( P_L \) transiently exceeded \( P_{out} \) on contractions 43 and 44, as confirmed by opening of the output valve (arrow 1). Starting at contraction 51 (arrow 2), successful ejection occurred on all subsequent contractions over an additional 2-min period. The enhancement in peak systolic pressure was sustained for ≥10 min in some cases. Diastolic \( P_L \) always returned to near its control value (\( P_{in} \)) under these conditions, indicating that the response occurred independent of a change in preload. The adaptation of this vessel to step elevation in \( P_{out} \) is consistent with a time-dependent increase in the strength of contraction at constant preload.

To test if the time-dependent increase in the peak systolic \( P_L \) reflected an increase in contractility (as defined above), P-V loop analysis was performed. Figure 8B shows a plot of the P-V loops for the first half of the trace in Fig. 8A, with time color coded. Repetition of the analysis used in Fig. 7 was complicated by the nonrectangular P-V loops for this lymphangion, but loop height increased over time after rapid \( P_{out} \) elevation. Two lines approximating ESPVR are drawn from the ESVs of the first few contractions (black traces) to the last few contractions when successful ejection occurred. The ESPVR slope appeared to increase substantially between these two time points. In Fig. 8C, P-V loops were plotted for...

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**Fig. 8.** Time-dependent increase in the strength of contraction after a \( P_{out} \) step. A: diameter and pressure recordings along with d\( P_L/dt \) analysis from a vessel responding to a 5-min \( P_{out} \) step from 1 to 8 cmH\(_2\)O. On the first spontaneous contraction after the step, systolic \( P_L \) rose to only 2.5 cmH\(_2\)O, suggesting that this was a relatively weak pump; the output valve failed to open. However, over the ensuing 2-min period, peak systolic \( P_L \) progressively increased with \( P_{out} \) on contractions 43 and 44, as confirmed by opening of the output valve (arrow 1). Starting at contraction 51 (arrow 2), successful ejection occurred on all subsequent contractions over an additional 2-min period. The valve position trace reflects the position of the output valve leaflets. Arrow 1 indicates the first contraction associated with opening of the output valve at elevated \( P_{out} \); arrow 2 indicates the beginning of a continuous series of contractions that opened the output valve. The blue trace is the control period before the \( P_{out} \) step used for change of diameter over time (d\( D/dt \)) analysis, and the green trace is a recovery period used for d\( D/dt \) analysis. B: P-V analysis for the selected portion of the recording marked “B” in A. The color coding corresponds to the timescale in A. The blue loops are control loops. The dotted lines show estimations of the ESPVRs for the initial five P-V loops (black) and the final five P-V loops (yellow). C: P-V analysis for the selected portion of the recording marked “C” in A, showing that the loops shifted to the left as the lymphangion successfully ejected. The color coding corresponds to the timescale in A.

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the second half of the trace in Fig. 8A, and it was evident that a slight leftward shift in the loop (as in Fig. 7B) occurred over time as the lymphangion continued to eject successfully. Differentiation of the $P_t$ signal (second trace in Fig. 8A) revealed a progressive increase in $dP_t/dt$ from 5 to 16 cmH2O/s as peak systolic pressure increased from 3 to 8 cmH2O. When $P_{out}$ was returned rapidly back to control levels, ESV was substantially lower than for the control period, and SV was substantially larger than for the control period (green trace in Fig. 8A); an analysis of the change in diameter over time ($dD/dt$) revealed that peak $-dD/dt$ was $-70 \mu m/s$ for the first contraction after the pressure step and $-103 \mu m/s$ when adaptation was sufficient to restore ejection (arrow 2 in Fig. 8), indicating that higher velocity contractions persisted for a period of time after the $P_{out}$ step (i.e., at equivalent levels of preload and afterload). Vessels exhibiting this behavior were characterized by time-dependent increases in contraction AMP, with corresponding increases in peak systolic $P_t$, $-dD/dt$, and $dP/dt$. The time courses of the contractile characteristics were not averaged on a contraction-by-contraction basis because the adaptation to step-wise $P_{out}$ elevation sometimes occurred over only a few contractions and sometimes over many contractions (e.g., 51 contractions in Fig. 8A). However, in 15 vessels, the average peak systolic $P_t$ increased by $58 \pm 12\%$ and peak $dP_t/dt$ increased by $84 \pm 17\%$ (referred to the first contraction after the $P_{out}$ step). These changes, along with the changes in the slope of ESPVR, are consistent with an increase in lymphangion muscle contractility over time after a step elevation in $P_{out}$.

**DISCUSSION**

The present study is the first to independently control $P_{in}$ and $P_{out}$ of a single lymphangion while recording vessel dimensions, $P_t$, and the dynamics of ejection by tracking movements of the output valve. We used these methods to test the response and adaptation of the lymphangion to $P_{out}$ elevation and analyzed changes in the $P-V$ relationship to make comparisons with adaptation of the heart to elevated aortic pressure. In addition, we assessed the limit of the lymphangion to pump against an output load and analyzed the sequence of events leading to pump failure. Our results show that the isolated lymphangion has an intrinsic and robust capacity to adapt to $P_{out}$ elevation and, as a key part of the mechanism, exhibits a time-dependent increase in contractility, or positive inotropy, in the strict sense that those terms are used in the cardiac literature. The lymphangion response is compared and contrasted that of the cardiac ventricles below. Our findings have important implications regarding the intrinsic capacity of the lymphangion to adapt to edemagenic and/or gravitational loads and the progression of events leading up to pump failure when that capacity is exceeded.

**Response of the lymphatic pump to $P_{out}$ elevation and behavior near $P_{lim}$**

Collecting lymphatic vessels, particularly prenodal vessels, normally propel lymph against a hydrostatic pressure gradient (28, 57). Moreover, vessels in dependent extremities must pump against an additional gravitational load resulting from changes in posture and other factors. Our results, i.e., relatively constant values of FPF and CPF until $P_{out}$ exceeds $\sim 10$ cmH2O, are consistent with comparable indexes of pump function determined in other species (14, 16, 20, 22, 23, 34, 36), even though the other vessels were obtained from much larger animals (cows and sheep) and presumably experience much larger gravitational afterloads. As $P_{out}$ continuously rose while a lymphangion was contracting spontaneously, most lymphangions became unable to develop sufficient pressure to open the output valve and eject any of their contents. Laine et al. (34) noted that individual sheep lymphatic vessels exhibited a precipitous fall in pump output when $P_{out}$ reached a specific value, which is consistent with the sudden pump failure that we observed in single lymphangions as $P_{out}$ approached $P_{lim}$ (Fig. 6D). However, their aggregate data from multiple vessels suggested a more gradual decline in pump capacity than we observed as a function of $P_{out}$ (16, 34, 36), which might be expected if the individual lymphangions of a multilymphangion vessel had different values of $P_{lim}$ (17).

Stroke work describes the energy imparted to lymph per contraction of the chamber (47) and has been used in several studies of multilymphangion vessels to analyze lymphatic function in response to an imposed load. Power, a related index of pump function that represents the product of stroke work and FREQ (36), has been used in the same context. A general finding is a bell-shaped curve for stroke work or power as $P_{out}$ increases (16, 18, 35, 36), with a broader plateau than for the flow versus $P_{out}$ curve of the same vessel(s). The relationships between stroke work and $P_{out}$ for the two single lymphangions shown in Figs. 5 and 6 are consistent with the findings of previous studies (16, 18, 35, 36). In accord with the arguments advanced above, we would anticipate an even broader stroke work versus $P_{out}$ curve in an isolated chain of lymphangions from the rat mesentery.

The analyses shown in Figs. 5 and 6 are the first determinations of stroke work of single lymphangions subjected to $P_{out}$ elevation (derived from the $P-V$ relationship). Our methods allowed us to examine, for the first time, the sequence of events leading to pump failure in a lymphangion. We noted that stroke work for a single lymphangion shifted from a net positive to a net negative value at, or just before, $P_{lim}$ (Fig. 6C). Although the shift in net stroke work occurred within one contraction cycle, the presence of negative stroke work was evident well before that time, and the fraction of negative stroke work began increasing with each subsequent contraction as $P_{out}$ approached $P_{lim}$. Inspection of the time course of stroke work during the contraction cycle indicated that negative stroke work, i.e., retrograde filling from the output pipette, occurred before the completion of systole (Fig. 6B). In the case shown, negative stroke work was not associated with diastolic decompenation (17), because EDD (and EDV) progressively declined with rising $P_{out}$. It would be interesting to test whether agents that produce positive inotropy in lymphatic muscle, delivered at the first indication of declining positive stroke work, could prevent pump failure or at least extend the range of $P_{out}$ over which ejection could be maintained.

**Afterload-induced changes in lymphatic contractility**

The terms “contractility” and “positive inotropy” have been used broadly in the lymphatic literature to refer to various aspects of the lymphatic pump adaptation to a stimulus such as pressure. Most often, contractility is used to describe the enhancement of contraction AMP or FREQ in response to a pressure increase or agonist activation (4, 11, 24, 38, 41, 52). In contrast, these terms have much narrower definitions in the cardiac literature. Positive inotropic agents produce an increase in cardiac muscle...
contractility (meaning an increase in the strength and velocity of force development) that leads to a clearly defined fall in ESV at constant preload (EDV). Likewise, an increase in afterload (e.g., through an increase in aortic pressure) leads to an initial rise in ESV and fall in SV that are subsequently offset in the intact, beating heart by an immediate increase in EDV through the Frank-Starling mechanism (heterometric autoregulation). However, under some conditions, another mechanism (the Anrep effect, homeometric autoregulation) can be unmasked (49): after recovery of SV through a secondary compensation in preload, a gradual fall in left ventricular end-diastolic pressure can often be observed. This fall in end-diastolic pressure at constant SV in the face of elevated afterload reflects an underlying, intrinsic increase in contractility that is independent of, and more subtle than, the contractility changes induced by positive inotropic agents (49, 54). Recently, this intrinsic increase in contractility has been reproduced in strips of myocardium (1, 32, 44) and is termed the “slow force response” to distinguish it from the immediate increase in force after stretch (heterometric autoregulation) mediated by changes in myofilament overlap (19).

Our ability to control preload and afterload independently presented the opportunity to test whether the more conservative definition of contractility applies to lymphatic muscle. The most rigorous method to define and analyze contractility changes, devised by Sagawa and colleagues (46–48), is widely accepted in the cardiac literature (3, 30, 31), although changes in dP/dt are often used in clinical settings. ESPVR and end-diastolic pressure-volume relationship (EDPVR) reflect the mechanical properties of the ventricular chambers at peak contraction and relaxation, respectively. In the heart, ESPVR is insensitive to increases in transmural pressure or changes in diastolic filling (47). The slope of the ESPVR \([E_{ES}; \text{so-called end-systolic elastance (47)}]\) is a measure of contractility. \(E_{ES}\) rises after catecholamine stimulation while the volume-axis intercept \((V_0)\) is usually unchanged (47). Our analysis of the time-dependent shift in the ESPVR was based on the application of these same principles (Eq. 11) to the lymphatic pump. We confirmed, as did Li et al. (35) in sheep mesenteric lymphatics in situ, that ESPVR is linear with changes in \(P_{out}\) (over a limited range), whereas EDPVR is curvilinear.

In protocols in which \(P_{out}\) was selectively elevated while \(P_{in}\) was held constant, several aspects of the isolated lymphangion response to elevated afterload were consistent with an increase in contractility. First, after an initial increase in ESD (ESV), a secondary decline occurred at constant preload; this contributed to secondary increases in AMP, SV, and EF after an initial fall in each. Second, a time-dependent increase in the strength of contraction occurred at constant preload and afterload, as assessed by an increase in \(P_L\) pulse AMP and increase in dP/dt (Fig. 8). Third, after the increase in afterload, a temporal decline in EDD (and EDV) occurred as a function of \(P_{out}\) (Figs. 2 and 7), which may be analogous to the underlying increase in contractility associated with myogenic constriction in arterioles (8). The combination of these mechanisms resulted in a leftward shift in ESPVR and \(\sim 30\%\) increase in \(E_{ES}\) (Table 2 and Fig. 7C). Interestingly, the magnitude of the adaptation is comparable to the magnitude of the slow force response in the heart, which can account for 20–30% of the total force increase in response to stretch (19). However, our alternative analyses suggest that the average increases in peak systolic \(P_L\) (\(+58\%\)) and dP/dt (\(+84\%\)) can be substantially greater in the lymphangion.

**Conclusions and physiological relevance.** Our study provides important, new information about the ability of a single lymphangion to adapt to a \(P_{out}\) load. We specifically analyzed the effects of afterload elevation independent of changes in preload. Direct measurement of pressure in the lymphangion allowed us to examine the P-V relationship in the absence of interference from pressure waves and contractions of neighboring lymphangions. To that end, an analysis of the time course of the shift in the P-V relationship after step-wise \(P_{out}\) elevation led to the conclusion that afterload elevation triggers an intrinsic, time-dependent increase in contractility of the lymphatic muscle.

Lymphatic vessel afterload can increase in vivo as a result of a number of factors, including postural changes, respiratory movements, and increases in central venous pressure that elevate lymphatic \(P_{out}\). In addition, tissue compression or skeletal muscle contraction may lead to the partial occlusion of lymphatic vessel outflow tracts, which would increase the outflow resistance. Either way, the consequence is an elevated lymphatic vessel afterload that presumably must be overcome at the risk of compromising lymph pump output. Although some of these perturbations may be associated with rapid increases in \(P_{out}\), the changes in many cases are likely to be gradual, particularly in the lymphangions located furthest upstream. These gradual challenges to the lymph pump are probably best simulated by ramp-wise \(P_{out}\) protocols in the isolated lymphangion. One conclusion from comparison of the lymphangion responses to \(P_{out}\) ramps versus steps is that lymphatic muscle is continuously adapting to a gradual rise in \(P_{out}\) by increasing its contractility. This adaptation apparently occurs in between individual contractions during \(P_{out}\) ramps but can be unmasked after rapid, step-wise \(P_{out}\) elevation.

Our findings must ultimately be placed in the physiological context of a multilymphangion segment in vivo. For example, a median value of \(P_{limit} = 12 \text{ cmH}_2\text{O}\) for a single lymphangion may actually underestimate \(P_{limit}\) for the same lymphangion if other segments are interposed downstream between it and the \(P_{out}\) source. The most relevant comparison to our work in this regard is perhaps that of Eisenhoffer et al. (17), who measured pressure changes between interconnected segments of sheep lymphatic vessels, each containing four to nine lymphangions. In response to progressive \(P_{out}\) elevation, the upstream segments pumped better than the downstream segments, as measured by maintenance of lower diastolic pressures in between the upstream segments. Thus, the upstream segments were sequentially protected by the downstream segments from the imposed afterload. Because our single lymphangion preparation is analogous to the last downstream segment used in Eisenhoffer et al. (17), we predict that the average \(P_{limit}\) in the upstream lymphangion of a multivalve rat mesenteric lymphatic segment may be substantially greater than 12 cmH₂O. On the other hand, the behavior of the multisection preparation used by Eisenhoffer et al. (17) highlights a key advantage of studying a single lymphangion: the response to selective \(P_{out}\) elevation is difficult or even impossible to assess in a multiple segment vessel because once diastolic pressures begin to rise in one or more of the valve segments, preload is no longer controlled in each of the lymphangions even if pressure in the input cannulae is fixed.
Finally, evidence from human studies has suggested that $P_{\text{out}}$ in peripheral lymphatic trunks is substantially and chronically elevated in edematous limbs (26, 42, 43, 55). The conventional wisdom is that the lymphangions in these vessels undergo progressive diastolic decompensation as edema develops, with a concomitant decline in pump function (42). Many of the valves reportedly become insufficient, leading to a larger standing column of fluid in the lymphatic outflow trucks when the extremity is placed in a dependent position. A further elevation in $P_{\text{out}}$ would, in turn, exacerbate the decline in pump function. A study (39) in human breast cancer patients that develop lymphedema has indicated that the massive, effective increase in lymphatic resistance caused by surgical removal of the nodes and associated afferent and efferent lymphatics correlates with a decline in lymphatic pumping and with the degree of ensuing lymphedema. The authors of that study hypothesized that afterload-induced pump failure may help explain the well-known but puzzling temporal and spatial heterogeneity of the lymphedema associated with cancer therapy. Understanding the mechanisms by which lymphatics adapt to elevated afterload will provide insights into potential therapeutic approaches that might ameliorate or reverse the lymphatic pump failure associated with lymphedema, including the most prevalent forms currently diagnosed in industrialized countries: lymphedema secondary to cancer (45, 51).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.J.D., A.A.G., and D.C.Z. conception and design of research; M.J.D. performed experiments; M.J.D., J.P.S., J.H.W., and D.C.Z. interpreted results of research; M.J.D. drafted manuscript; M.J.D., J.P.S., J.H.W., and D.C.Z. reviewed and edited manuscript; M.J.D., J.P.S., J.H.W., M.M., A.A.G., and D.C.Z. approved final version of manuscript.

REFERENCES


