Mesenteric lymphatic vessels adapt to mesenteric venous hypertension by becoming weaker pumps

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It has long been established that increasing the mesenteric venous pressure increases intestinal microvascular pressure and, thus, microvascular filtration into the intestinal interstitial space (14, 25). As a result, intestinal interstitial fluid volume and pressure become elevated, thus elevating lymphatic system inlet pressure. As expected, acute mesenteric venous hypertension also has been reported to significantly increase mesenteric lymph flow (14, 20). Mesenteric venous hypertension and resulting interstitial edema may also result from chronic conditions such as mesenteric venous thrombosis or portal hypertension. However, how lymphangion function is affected by increases in pressure and flow over the course of days has been investigated only recently (11, 38). Exposure to sustained increases in transmural pressure resulted in bovine mesenteric lymphangions adapting to become less effective pumps (11), whereas exposure to sustained mesenteric venous hypertension resulted in them adapting to become less effective pumps (38). These two studies characterized adaptation of lymphatic vessels in terms of standard indices of lymphatic function such as ejection fraction and pump index. Recapitulating the history of blood vessel research, lymphatic vessel research has only begun to transition from functional characterization to biomechanical characterization (19, 33, 57).

Transmural Pressure and Flow Affect Lymphatic Pump Function

Mesenteric lymphatic vessels play a critical role in intestinal interstitial fluid balance, nutrient absorption, and immune response by returning interstitial fluid (called lymph once in lymphatic vessels) to the central venous circulation (3). Historically, lymph transport has been viewed as a passive process governed by lymphatic vessel geometry (18). However, it is now recognized that active, spontaneous contractions of lymphatic muscle are essential to propel lymph from the lower-pressure interstitium to the higher-pressure central veins (13, 18, 21, 30, 32). Lymphangions, the segments of lymphatic vessels between two adjacent lymphatic valves, share several behaviors with cardiac ventricles, and thus lymphangions are typically characterized in terms of end-systolic and end-diastolic volumes and pressures (4, 27, 40). Like ventricles, acute elevations in lymphangion transmural pressure increase contraction frequency and stroke volume (4, 22, 31, 41). However, unlike ventricles, when lymphangion luminal flow increases acutely, the frequency of contraction and stroke volume decrease (16, 52). Edemagenic conditions thus tend to affect lymphatic pump function by altering transmural pressure and flow.

Mesenteric Venous Hypertension Alters Lymphatic Transmural Pressure and Flow

Although ejection fraction and pump index are two very useful indices of lymphatic function that affect lymph pressure and flow, these two indices are also affected by changes in lymph pressure and flow. Characterizing changes in intrinsic contractile properties of lymphatic muscle requires a more

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fundamental analysis of lymphatic muscle properties. The fact that lymphangions have the geometry of blood vessels and the function of ventricles, however, leads to new challenges. Although used extensively to characterize vascular muscle properties, wire myographs cannot be used to characterize the effects of endothelial shear stress on lymphatic muscle properties. Standard isobaric intact lymphangion preparations can have physiological shear stresses, but necessarily result in time-varying shear stress and have diminished developed tension from Hill effects (58). Consequently, investigators have begun to report the use of intact isovolumetric preparations to relate lymphatic function to muscle properties (33, 37). Such biomechanical studies have not yet characterized changes when lymphangions are exposed to prolonged increases in pressure and flow in vivo. Adaptive changes in developed tension in muscular arteries are affected by both intracellular Ca\(^{2+}\) concentration and sensitivity to intracellular Ca\(^{2+}\) (23, 24, 43). Ca\(^{2+}\) has also been identified to play a significant role in the acute lymphatic myogenic response (1, 2, 29, 51). Like changes in mechanical properties of lymphatic vessels, changes in Ca\(^{2+}\) homeostasis in response to prolonged changes in lymph pressure and flow have yet to be characterized. Therefore, the purpose of the present work was to use an isovolumetric intact lymphangion preparation to evaluate adaptive changes in mesenteric lymphatic muscle mechanical properties and intracellular Ca\(^{2+}\) in response to sustained mesenteric venous hypertension.

**METHODS**

**Surgical Preparation**

Experimental procedures and animal care were performed in compliance with protocols approved by the Texas A&M University Institutional Animal Care and Use Committee. Experimental procedures were similar to those published previously (38). Healthy cross-breed cows (n = 12) used for this study were randomly divided into two experimental groups: mesenteric venous hypertension (MVH, n = 6, weight = 417 ± 17 kg) and sham surgery (Sham, n = 6, weight = 423 ± 19 kg). Since all the cows used in the present study were purchased from commercial vendors, the precise age of each animal was not available, but they were relatively young (ranging from 2–8 yr out of an approximate life span of 14 yr). In both groups, cows were fasted for 48 h before surgery. Anesthesia was induced with xylazine (0.02 mg/kg), diazepam (0.01–0.03 mg/kg), and ketamine (2–6 mg/kg to effect), administered intravenously. Cows were ventilated mechanically and anesthesia was maintained using 1–3% isoflurane in oxygen by inhalation as needed to maintain a surgical plane of anesthesia. Through a right laparotomy incision, the small intestine and associated mesentery were exteriorized. Drying of the exposed mesentery was prevented by intermittent application of isotonic saline. The proximal jejunal venous arcade was identified. To induce mesenteric venous hypertension in cows from the MVH group, the vein draining this arcade at one end was isolated and ligated (38). The vein draining the other end of the arcade was isolated and fitted with a vascular occluder (Kent Scientific, Torrington, CT). The occluder, filled with 50% dextrose solution, was fixed to the mesentery with a piece of suture (2-0 polyglactin 910). The tail of the vascular occluder tube was exteriorized through a small skin incision dorsal to the abdominal incision. In cows from the Sham group, the mesenteric vein was not ligated, and the vascular occluder was not applied. To measure pressure within the arcade, an upstream vein from the venous arcade was isolated, and polyvinyl chloride tubing (1/8 in. O.D., Tygon R3603, Saint-Gobain Performance Plastic, Garden Grove, CA) filled with heparinized saline was inserted into the vein and secured with suture (2-0 polyglactin 910). The other end of the PVC tubing was exteriorized with the vascular occluder tubing through the skin incision. The small intestine was replaced in the abdomen, and the incision was closed in multiple layers. The animals were then recovered from anesthesia.

**Postoperative Measurements and Tissue Collection**

Cows were monitored during the 3-day period following surgery. Mesenteric venous pressure was measured once a day during this period using a water monometer as described previously (38). Briefly, after flushing the venous catheter with heparinized saline, the monometer was attached to the venous catheter and fixed with the zero-point at the level of the shoulder. The equilibrium column height was recorded as venous pressure. If the pressure in the MVH group was found to be <30 cmH\(_2\)O, dextrose solution was injected in the vascular occluder until that pressure was reached. On day 3 following surgery, cows were euthanized by captive bolt and exsanguination. After the small intestine was accessed, postnodal lymphatic vessels draining the affected intestinal segment were ligated at the downstream end. After carefully dissecting them free from surrounding tissue, lymphatic vessels were transported to the laboratory in chilled (4°C) albumin physiological salt solution (APSS, in mM: 145 NaCl, 4.7 KCl, 2 CaCl\(_2\), 1.17 MgSO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 5 dextrose, 2 sodium pyruvate, 0.02 EDTA, 3 MOPS; 10 g/l bovine serum albumin, and pH 7.4).

**Tissue Preparation**

Postnodal lymphatic vessels were cleared of surrounding fat and connective tissue. Two vessel segments were cut from each lymphatic vessel. Longer vessel segments (~3 cm in length (with two sets of valves) were prepared for characterizing radius-tension relationships. Shorter vessel segments (~1.5 cm in length (without valves) were prepared for measuring intracellular Ca\(^{2+}\). Lymphatic vessels were discarded if they failed to exhibit spontaneous contraction after initial equilibration.

**Radius-Tension Relationship Characterization**

**Experimental apparatus.** A modified isolated vessel bath (model Mayflower type 813/6, Harvard Apparatus, Holliston, MA) was used to control transmural pressure, luminal flow, and volume in isolated lymphatic vessels (Fig. 1). This setup allowed switching rapidly between isobaric and isovolumetric conditions while maintaining constant luminal flow. The vessel inlet was connected to the push side of a push-pull syringe pump (model KDS120, KD Scientific, Holliston, MA). The vessel outlet was connected by using a three-way stopcock to the pull side of the push-pull syringe pump and a horizontal glass tube open to the atmosphere. To establish isobaric conditions, the lymphatic vessel outlet was opened to the atmosphere by changing the stopcock to the open position. In this configuration, the syringe pump controlled luminal APSS flow, which passed out through the glass tube connected to the outlet. Luminal pressure of the lymphatic vessel was set by adjusting the height of the glass tube. To switch to isovolumetric conditions, the lymphatic vessel outlet was connected to pull side of the syringe pump by changing the stopcock position. Closing of the APSS flow loop between push and pull ends of the syringe pump thus established isovolumetric conditions while simultaneously allowing constant luminal flow. The segment of the lymphatic vessel between the two ties used to mount the vessel was isovolumetric. Unintended volume changes were minimized by using tubing of negligible compliance leading to and from the vessel. A microinjection pump (UltraMicroPump II, World Precision Instrument, Sarasota, FL) was connected at the inlet end to add or remove a controlled amount of volume from the circuit formed by the vessel, tubing, and syringe pump. The vessel chamber that bathed the vessel was open to the atmosphere.
Fig. 1. Schematic of the experimental apparatus to control transmural pressure, luminal flow, and volume in isolated lymphatic vessels. The vessel bath was open to the air. Changing the stopcock position allowed switching between isobaric (vessel lumen connected to glass tube open to atmosphere) and isovolumetric condition, the lymphanion and the connected flow loop had a constant volume. A microinjection pump connected at the inlet end allowed adding or removing a controlled amount of volume from the vessel lumen/flow loop. Instantaneous lymphatic vessel outer diameter was determined using video calipers from video captured by a camera. Lymphatic vessel luminal pressure was measured using a pressure transducer catheter inserted into the vessel lumen through a leak-proof port on the inlet end. A (at left and right) represents continuity.

**Data acquisition and measurements.** Lymphatic vessel wall movement was monitored using a CCD camera (model ST-XC50, Sony Electronics, Park Ridge, NJ). Instantaneous lymphatic vessel outer diameters at five locations along the vessel length were determined using video calipers (LabView 8.0, National Instruments, Austin, TX). The average of these five simultaneous measurements was taken to represent an instantaneous lymphatic vessel outer diameter. Lymphatic vessel luminal pressure was measured using a pressure transducer catheter (model SPR-1000 1F, Millar Instruments, Houston, TX) connected to a data acquisition system (PowerLab Pro, ADInstrument, Colorado Springs, CO). The catheter was inserted through a leak-proof port at the inlet end and was advanced into the vessel lumen until the catheter tip was positioned in the center of the vessel. Pressure and diameter data were acquired at 10 Hz.

**Experimental protocol.** Postnodal lymphatic vessel segments (~3 cm) were mounted in the vessel bath. Throughout the experiment these vessel segments were bathed (bath flow rate 2 ml/min) and infused with 1% APSS warmed to 37°C using thermoregulators (model Lauda E-200, Brinkman Instrument, Westbury, NY). Lymphatic vessel diameter and luminal pressure were continuously recorded during the entire experiment. With the vessel segment open to atmosphere (i.e., isobaric conditions), luminal flow was set to attain the diastolic shear stress of 0.0081 dyn/cm². This shear stress was chosen to be consistent with a lowest average diastolic shear stress calculated from the reported flow generated by spontaneously contracting lymphatic vessels (31). In addition, the flow allowed the luminal contents to be refreshed approximately once every minute.

The glass tube connected to the vessel bath outlet was then adjusted to set transmural pressure to 6 cmH₂O, estimated by subtracting the height of the bath APSS above vessel centerline (1 cm) from luminal pressure measurements (i.e., transmural pressure = luminal pressure - 1 cm). Each vessel segment was then allowed to equilibrate for 30–40 min until consistent contractions were observed.

**Simultaneous radius and pressure measurement.** Simultaneous radius and pressure data were recorded under three lymphatic contractile conditions in the following order: 1) spontaneous contractions, 2) stimulated constriction with varying concentrations of KCl, and 3) passive state. Following initial equilibration, lymphatic vessel segments exhibiting consistent spontaneous contractions were then switched to isovolumetric conditions. Lymphatic vessel diastolic diameter at transmural pressure of 6 cmH₂O was used to estimate the reference volume (100% volume) with the assumption that the vessel segment had a cylindrical shape. Consistent with approaches reported previously (33), five volume steps (50, 75, 100, 125, and 150% of reference volume) for each vessel segment were estimated. By either adding or removing APSS using the microinjection pump, luminal volume was randomly set to one of the five volume steps while lymphatic vessel segments contracted spontaneously. After adjusting the luminal flow to set endothelial shear stress, vessels were allowed to equilibrate for 5 min. Data were recorded for the next 2 min. Between two consecutive volume steps, luminal volume was restored to the reference level by either adding or removing APSS using the microinjection pump. Vessel segments were then allowed to equilibrate for 10 min. Next, lymphatic vessel segment diameters and luminal pressures were measured in response to stimulated constriction with varying concentrations of KCl. Following equilibration for 10 min, luminal flow was stopped by switching off the push-pull syringe pump. Under an isovolumetric state with transmural pressure set to 6 cmH₂O, the bath APSS was then replaced with APSS with KCl concentrations of 20, 40, 60, 80, or 100 mM in randomly selected order. The osmolarity of the APSS solution was maintained by reducing Na⁺ levels equal to additional K⁺ in the solution. Pressure and diameter data were recorded for 5 min immediately after the change of the bath solution. After 5 min, the vessel was rinsed twice by replacing the bath solution with regular APSS (with KCl concentration of 4.7 mM). The vessel was then allowed to equilibrate for 10 min before the next change in KCl concentration. After the active vessel diameter and luminal pressure were recorded, lymphatic vessel segments were switched to isobaric conditions. APSS in the bath and lumen was replaced with Ca²⁺-free APSS. Luminal flow was then set to attain an endothelial shear stress of 0.0081 dyn/cm², and transmural pressure was set to 6 cmH₂O. When lymphatic vessels relaxed completely, as evidenced by a lack of spontaneous contractions (change in diameter <1%), transmural pressure was lowered from the equilibrium pressure of 6 cmH₂O to 3 cmH₂O. Lymphatic vessel passive diameter and transmural pressure were recorded for 1 min at each transmural pressure step as transmural pressure was increased from 3 cmH₂O to 39 cmH₂O in steps of 3 cmH₂O.

**Data analysis.** Lymphatic vessels were assumed to be thin-walled and cylindrical in shape. Lymphatic vessel wall tension was calculated using recorded diameter and pressure data, based on Laplace’s law at each luminal volume step (tension = pressure × radius). Instantaneous wall tension for end diastole, end systole, and the passive state were used to derive active tensions for spontaneously contracting vessels: systolic active tension (end-systolic instantaneous tension – passive tension) and diastolic active tension (end-diastolic instantaneous tension – passive tension). Passive tensions corresponding to end-diastolic and end-systolic diameters were estimated by interpolation between measured passive tension-diameter values using nonlinear curve fitting in an exponential function. Instantaneous tension during KCl-stimulated constriction and passive tension at 6 cmH₂O transmural pressure were used to derive KCl-induced transient peak tension (KCl-stimulated peak tension – passive tension) and steady-state active tension (KCl-stimulated steady-state tension – passive tension). Steady state was assumed when the change in instantaneous tension between consecutive 10-s periods (averaged over 10 s) was <5%.

**Statistical analysis.** Active and passive tensions from MVH and Sham group vessels were analyzed and compared using the JMP 8.0 software package (SVS Institute, Cary, NC). Responses of MVH and Sham groups at different KCl concentrations were compared with repeated-measures ANOVA for a crossover design. All data are presented as means ± SE. A value of P < 0.05 was considered significant.
Intracellular Ca\(^{2+}\) Measurements

Experimental apparatus. A Lucite isolated vessel bath was used when measuring relative changes in intracellular \([Ca^{2+}]\). This setup allowed rapid switching between isobaric and isovolumetric conditions. Bath inlet to the vessel was connected to a syringe pump (model KDS120, KD Scientific, Holliston, MA). With the use of a two-way stopcock, outlet from the vessel was connected to a glass tube open to the atmosphere. Lymphatic luminal pressure was monitored using a pressure transducer (model MLT0699, ADInstruments, Colorado Springs, CO) connected to the outlet of the vessel bath. With outlet open to atmosphere, the inlet syringe pump controlled APSS flow through the lumen and out to the atmosphere. Luminal pressure was set by adjusting the height of a glass tube connected to the outlet. To switch to an isovolumetric state, the syringe pump was switched off, and the lymphatic vessel outlet was closed to the atmosphere using the stopcock. Uncontrolled luminal volume changes were minimized by using tubing of negligible compliance leading up to and from the vessel. Luminal pressure signals were acquired at 10 Hz using a data-acquisition system (PowerLab Pro, ADInstruments) connected to a Windows workstation.

Microscope system. An inverted microscope system (model Nikon Eclipse Ti-U, Nikon Instruments, Melville, NY) was used for fluorometry. Excitation light from a 175-W xenon arc lamp was passed through dual scanning galvanometers with 340- and 380-nm interference filters used for excitation wavelength selection (Sutter Instruments, Lambda DG-5). The fluorescence emission at 510 nm was synchronized with the appropriate excitation wavelength and reflected to an interline-transfer, progressive scan, cooled CCD video camera (CoolSNAP HQ, Photometrics) with a dichroic mirror. Fluorescence was analyzed with an analog fluorescence signal processor and was passed through analog-to-digital converter.

Experimental protocol. Each valveless lymphatic vessel segment, ~1.5 cm in length, was mounted in the vessel bath. The vessel segment was bathed and infused with 1% APSS solution warmed to 37°C using thermoregulators (model Lauda E-200, Brinkman Instrument, Westbury, NY). With outlet open to atmosphere, vessel luminal flow was set to 0.1 ml/min and luminal pressure was set to 6 cmH\(_2\)O. Following initial equilibration for 10 min, the bath solution was replaced with APSS containing 7.5 μM fura-2-AM (Sigma-Aldrich). After incubation for 90 min, the vessel segment was rinsed by replacing APSS in the bath three times. The vessel segment was then switched to an isovolumetric state, and the muscle layer was visualized. The microscope was switched to the fluorescence excitation mode and stimulated the visualized vessel wall with alternating 340 nm and 380 nm wavelengths. After recording the data at baseline for 10 min, bath APSS was replaced with APSS with KCl concentration of 20, 40, 60, 80, and 100 mM in the same order for radius-tension studies. Increased KCl in the APSS was balanced by reducing Na\(^{+}\) levels. After the fluorescence signal was recorded for 4 min, the vessel was rinsed three times by replacing bath APSS with regular APSS (KCl 4.7 mM). The vessel was then allowed to equilibrate for 5 min before it was stimulated with the next KCl concentration level.

Data analysis. Fluorescent images were acquired using dual-wavelength Ca\(^{2+}\) imaging software (NI-Elements AR 3.0). Final data for estimates of intracellular free Ca\(^{2+}\) are expressed as a fluorescence ratio (F340/F380) because of uncertainties in extrapolating in vitro calibrations to in situ measures as described previously (54). Instantaneous fura-2 (340/380) ratios were determined and used as a representative of relative changes in intracellular \([Ca^{2+}]\) in response to stimulation with KCl at different concentrations. Steady-state fura-2 340/380 ratios following stimulation with KCl from the MVH group vessels were compared with those from the Sham group vessels. Steady state was assumed when change in the fura ratio was <5%. The relationship between steady-state lymphatic active tension and steady-state fura-2 (340/380) ratio following stimulation with KCl was determined by correlating steady-state active tension and steady-state fura-2 (340/380) ratio data from two adjacent segments of the same vessel.

Statistical analysis. Responses of MVH and SHAM groups at different KCl concentrations were compared with repeated-measures ANOVA for a crossover design. All data are presented as means ± SE. A value of \(P < 0.05\) was considered significant.

RESULTS

A total of six MVH and six Sham experiments were performed. In MVH cows, mesenteric venous pressure was relatively stable over the 3 days following surgery (day 1, 28.4 ± 2.1; day 2, 26.6 ± 2.7; and day 3, 28.5 ± 2.3 cmH\(_2\)O). Therefore, the level of venous occlusion to raise venous pressure was not altered. Mesenteric venous pressure averaged 27.8 ± 2.2 cmH\(_2\)O over the 3 days after the surgery, which was significantly higher than pressure in Sham cows (13.7 ± 1.9 cmH\(_2\)O; \(P < 0.01\)). Following initial equilibration, transmural pressure and diameter data were recorded and analyzed in five spontaneously contracting vessels and six KCl-stimulated vessels from the MVH group, and six spontaneously contracting vessels and six KCl-stimulated vessels from the Sham group. An average flow of 0.12 ml/min was required to set endothelial shear stress to the desired level (0.0081 dyn/cm\(^2\)). Fura-2 (340/380) data were recorded and analyzed in six MVH group and six Sham group vessels.

Figure 2 illustrates changes in diameter and transmural pressure measurements from a representative lymphatic vessel segment from the Sham group. Passive, end-systolic, and end-diastolic diameters of MVH and Sham group vessels at 6-cmH\(_2\)O transmural pressure under isobaric condition are listed in Table 1. There were no significant differences in passive, end-systolic, and end-diastolic diameters between the two groups.

We next examined passive tension (Fig. 3A), end-systolic active tension (Fig. 3B), and end-diastolic active tension (Fig. 3C) from MVH and Sham group vessels as functions of normalized end-diastolic diameter. There was no significant difference in passive tension between MVH and Sham group vessels. Average end-systolic and end-diastolic active tensions developed by MVH group vessels (1.2 ± 0.1 and 0.5 ± 0.1 mN/mm, respectively) were significantly lower than those of Sham group vessels (1.9 ± 0.9 and 0.8 ± 0.1 mN/mm, respectively).

Lymphatic vessels from both groups when stimulated with KCl developed transient peak tension before reaching a steady state. The KCl-induced transient peak active tension in each vessel, however, was not significantly different from KCl-induced steady-state active tension in that vessel. KCl-induced steady-state active tension in the MVH group vessels was significantly lower than that of Sham group vessels at KCl concentrations above 40 mM (Fig. 4). The average KCl-induced transient peak active tension and KCl-induced steady-state active tension in MVH group vessels (1.5 ± 0.1 and 1.0 ± 0.1 mN/mm, respectively) were significantly lower than those of Sham group vessels (2.1 ± 0.1 and 1.3 ± 0.1 mN/mm, respectively).

The steady-state fura-2 (340/380) ratio in MVH group vessels in response to stimulation with KCl was significantly lower than that of Sham group vessels at each KCl concentration (Fig. 5), indicating lower intracellular Ca\(^{2+}\) concentration. We could not record changes in the fura ratio in the initial 30 s
following stimulation because of inherent limitations of our experimental setup. Figure 6 depicts lymphatic steady-state active tension as a function of steady-state fura-2 (340/380) ratio following stimulation with KCl. Sham and MVH steady-state active tension data at each KCl concentration were paired with Sham and MVH steady-state fura-2 (340/380) ratio data at corresponding KCl concentrations. The MVH steady-state active tension- fura-2 (340/380) ratio relationship exhibits a leftward shift, indicating elevated Ca$^{2+}$/H$^{100}$ sensitivity (Ca$^{2+}$ concentration per unit of tension generated).

**DISCUSSION**

The present work is the first to document adaptive changes in lymphatic vessel mechanical properties and calcium homeostasis within 3 days of surgically induced mesenteric venous hypertension. Specifically, spontaneously contracting lymphatic vessels from the mesenteric venous hypertension group generated end-systolic active tension and end-diastolic active tension lower than lymphatic vessels from the sham surgery group. Furthermore, steady-state active tension in response to KCl stimulation was also significantly lower in lymphatic vessels from the mesenteric venous hypertension group compared with the sham surgery group. However, there was no significant difference in passive tension in lymphatic vessels from the two groups. Analysis of Ca$^{2+}$ homeostasis revealed that the levels of cytosolic Ca$^{2+}$ following KCl stimulation were significantly lower in the lymphatic vessels from the mesenteric venous hypertension group compared with the sham surgery group. However, Ca$^{2+}$ sensitivity (Ca$^{2+}$ concentration per unit of tension generated) was elevated in the lymphatic vessels from the mesenteric venous hypertension group compared with the sham surgery group. Taken together, these results suggest that the postnodal mesenteric lymphatic vessels adapt to become weaker pumps following 3 days of mesenteric venous hypertension by reducing the cytosolic Ca$^{2+}$ concentration.

**Extending Understanding of Adaptive Responses of Lymphatic Vessels**

Acute functional responses of lymphatic vessels to mechanical and chemical stimuli have been investigated extensively. Characterizations of lymphatic muscle mechanical properties and Ca$^{2+}$ homeostasis are still in their infancy (12, 19, 37, 49, 56–58). Perhaps because of the requirement for chronic animal experiments, reports of adaptive responses to sustained stimuli in vivo are relatively rare, and have focused on changes in lymphatic vessel function. Wu et al. (55) reported that contractile activity of mesenteric lymphatic vessels was significantly impaired from 1 to 6 days following surgically induced inflammation. Gashev et al. (17) reported that mesenteric lymphatic vessel function adapts when rats were chronically exposed to simulated microgravity. Dongenkar et al. (11) used lymphatic coarctation in vivo to study the effect of a 3-day change in transmural pressure on mesenteric lymphatic vessel function. They found that vessel segments exposed to higher pressures adapted to become stronger pumps. Like the present study, Quick et al. (38) surgically produced sustained mesenteric venous hypertension, and found that lymphatic vessels adapted to become weaker pumps. 

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Table 1. Passive, end-systolic, and end-diastolic diameters of the Sham and MVH vessel segments at 6-cmH$_2$O transmural pressure

<table>
<thead>
<tr>
<th></th>
<th>Passive Diameter, mm</th>
<th>End-Systolic Diameter, mm</th>
<th>End-Diastolic Diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>3.21 ± 0.30</td>
<td>2.30 ± 0.21</td>
<td>2.69 ± 0.27</td>
</tr>
<tr>
<td>MVH</td>
<td>2.58 ± 0.24</td>
<td>2.04 ± 0.19</td>
<td>2.45 ± 0.21</td>
</tr>
</tbody>
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Value are means ± SE. MVH, mesenteric venous hypertension; Sham, sham surgery. No significant differences in diameters between the two vessel groups were detected.
**Hypertension-Induced Increases in Lymph Flow**

Mesenteric venous hypertension, like most clinically relevant edemagenic stimuli, leads to complex interactions involving the microvasculature, interstitium, and lymphatic system. A previous study in cats characterizing intestinal interstitial fluid balance reported that mesenteric venous hypertension acutely raises intestinal interstitial fluid pressure (from $-0.56$ to $7.3$ mmHg) (34). Such an increase in intestinal interstitial fluid pressure would increase mesenteric lymphatic inlet pressure and may provide two stimuli for adaptation of mesenteric lymphatic vessels. First, it may increase the axial pressure gradient (lymphatic inlet pressure - outlet pressure) and, therefore, increase passive lymph flow. It is unclear how increasing intestinal interstitial pressure affects the transmural pressure of terminal lymphatics within the intestine, because both luminal and external pressures can rise. However, an increase in inlet pressure may increase lymphatic transmural...
pressure in the mesenteric lymphatic vessels, because they are not imbedded in the intestine. The corresponding increase in endothelial shear stress and wall stress can provide the mechanical stimuli that cause lymphangions to adapt over the course of days. However, the results of the present study are consistent with results of acute studies illustrating that increased lymph flow and endothelial shear stress lead to diminished contractility and contraction frequency (16). Acute weakening of the lymphatic pump with elevated flow has been attributed to elevated production of endothelial nitric oxide (NO), which may also play a role in the adaptive responses observed in the present study.

Observed Changes May Be Due to Mesenteric Venous Hypertension-Induced Inflammation

Since the goal of the present study was to evaluate effects of mesenteric venous hypertension on mesenteric lymphatic muscle properties, we did not seek to characterize the inflammatory response. However, it is possible that the observed alterations in the lymphatic muscle properties were influenced by an inflammatory response induced by mesenteric venous hypertension. Inflammation signaling molecules could originate in the intestinal interstitium drained by mesenteric lymphatic vessels. In particular, prostaglandin (PGE2) and prostacyclin (PGI2) have been reported to relax lymphatic muscle and inhibit pumping of both bovine and guinea pig mesenteric lymphatic vessels in a concentration-dependent manner (8, 42, 55). PGE2 and PGI2 have been proposed as mediators of the compromised contractility of mesenteric lymphatic vessels observed in an animal model of intestinal inflammation, because COX inhibitors partially restore contractility (8, 42, 55). In addition to responses to exogenous prostanooids, lymphatic vessels have been reported to be capable of producing PGE2 and PGI2 (42). Taken together, PGE2 and PGI2, either exogenous or endogenous, may be the signaling molecules responsible for the observed alterations in lymphatic muscle properties in the present study.

Mesenteric Venous Hypertension-Induced Inflammation or Increase in Flow Could Decrease Cytosolic Ca2+

In blood vessels, K+ channels mediate vascular relaxation via hyperpolarization and reduction of [Ca2+]i (48). The decreased [Ca2+]i observed in the present study may be mediated by K+ channels stimulated by flow-induced release of endothelium-derived relaxing factors or inflammatory signaling molecules. NO has been reported to induce cGMP- and cAMP-dependent activation of KATP channels in lymphatic muscle cells and decrease lymphatic contraction frequency and amplitude (28, 53). KATP channel blockade diminished NO-induced decreased lymphatic contraction frequency and amplitude. Furthermore, recent studies suggest that K+ channels may also mediate effects of PGE2 and PGI2 in lymphatic vessels (42). PGE2 and iloprost (a prostacyclin analog) decreased the contractile activity of lymphatic vessels in a concentration-dependent manner. Furthermore, in the presence of the KATP channel blocker glibenclamide, the inhibitory actions of PGE2 and iloprost were strongly attenuated, indicating an involvement of KATP channels in diminishing pumping activity. Whether precipitated by prolonged increases in lymph flow or inflammatory mediators, the decrease in cytosolic Ca2+ with sustained mesenteric venous hypertension may be mediated by K+ channels.

Conventional Approaches to Study Fundamental Properties of Lymphangions Are Limited

The primary difficulty characterizing lymphangion properties arise from the complex interaction of biomechanics and mechanobiology. In terms of biomechanics, lymphangion pumping affects both transmural pressure and lymph flow and, therefore, wall tension and endothelial shear stress. In terms of mechanobiology, changes in wall tension and endothelial shear stress affect lymphangion pumping. In addition, because the lymphangion wall tension is not passive in diastole and can be regulated independently from systolic tension, two tensions must be characterized: the end-diastolic active tension and end-systolic active tension (4). Although it is not possible to control lymphatic wall tension or lymphatic endothelial shear stress in vivo, it is possible to control mechanical stimuli in vitro. However, the standard wire myograph, while allowing lymphatic muscles to spontaneously contract (56), does not allow a controlled physiological endothelial shear stress. While isobaric intact lymphangion preparations have physiological luminal flows, they yield variable endothelial shear stresses and result in diminished tension from Hill effects (58). The present work not only provides a novel technique to characterize lymphatic biomechanics, but it has yielded the first characterization of mechanical properties of lymphangions after prolonged changes in mechanical stimuli.

Intact Lymphangion Preparation Allows Characterization of Lymphangion Biomechanics

The intact lymphangion isovolumetric apparatus developed for the present work modified that of Meisner et al. (33) by including constant flow during an isovolumetric state. Unlike Meisner et al., we increased the length of the lymphangion segment to ensure the presence of valves. To minimize error due to nonuniform contraction of longer lymphangions, we measured radius at five different locations to derive an average circumference. We were limited in our ability to measure the inner radius. Although it is possible to illuminate vessels with brighter light source and visualize inner edge of the wall, visible light has been shown to induce nitric oxide production...
and relax vascular and lymphatic muscle (15). Our standard “thin-walled approximation” may have resulted in underestimation of endothelial shear stress. Although micropipettes inserted through the vessel wall have been used by other investigators (9, 45), the large size of the bovine vessels used in the present study allowed luminally placed transducer catheters (inserted through a port on the inlet end) to be used for pressure measurement. This avoided possible fluid loss through the puncture hole in the vessel wall. Fluid loss across lymphatic wall may have resulted in a decrease in volume. However, any volume lost would be small relative to the large volume of fluid circuit connecting the vessel lumen, the tubing, and the push-pull syringe pump. Notwithstanding these limitations, our isovolumetric methodology advances standard biomechanical characterizations as applied to spontaneously contracting lymphangions. While minimizing the effects of contraction-initiated time-varying shear stress, our apparatus allows characterization of the effects of an imposed shear stress on lymphatic muscle properties. Furthermore, the luminal flow also allows turnover of the luminal contents, and thus is helpful for keeping the vessel functioning during long experiments. To follow the historical development of classical blood vessel mechanics, the next logical step would be to alter our preparation for isotonic experiments (36).

Implications to Edema Resolution

Dongaonkar et al. (11) recently reported that lymphatic vessels adapt to changes in transmural pressure within a 3-day period. Blood vessels also have been reported to functionally adapt within 3 days of altered luminal flow (44). This time period is especially important for extended periods of intestinal edema leading to high lymph flow such as liver transplant (5), pancreatitis (10), and other abdominal surgeries (50). In these patients, increased microvascular pressure results in increased microvascular fluid filtration and severe intestinal interstitial edema (14, 25). Once formed, edema takes from days to weeks to resolve. Three days are not sufficient for development of new lymphatic vessels (i.e., lymphangiogenesis) (6, 47). Although it is possible that interstitial fluid pressure may rise so high that lymphatic pumping is no longer necessary to transport lymph (39, 41), weakening of lymphatic pumping (specifically decreased ability to develop active tension) may be responsible for delayed edema resolution following normalization of the edemagenic insult.

Perspectives and Significance

Although the impact of altered lymphatic function on interstitial fluid balance, lipid absorption, and immune response is just beginning to be understood, mechanisms of lymphatic adaptation have yet to be investigated. Findings from the studies characterizing blood vessel and ventricular adaptation may provide insight. Experimental studies have reported that when blood flow through an artery is increased chronically, blood vessel diameter increases as does the number of smooth muscle cells. These observations have been ascribed to increases in the expression of smooth muscle proteins and vascular remodeling, resulting in the ability to maintain vessel diameter at a higher pressure (26, 35). However, increased blood flow also increases endothelial shear stress-dependent nitric oxide production and leads to dilation. In the present study, we did not observe a significant difference in passive tension when lymphatic vessels were exposed to mesenteric venous hypertension. Decreased developed tension, however, is consistent with the acute effects of elevated levels of nitric oxide (7, 16, 52). Extensive studies focusing on molecular, cellular, and histological analysis are required to completely characterize the mechanisms of lymphatic adaptation. The present work characterizing changes in lymphatic muscle properties is the first step in that direction.

REFERENCES


LYMPHATIC BIOMECHANICAL ADAPTATION