Functional adaptation of bovine mesenteric lymphatic vessels to mesenteric venous hypertension

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1 Michael E. DeBakey Institute, Texas A&M University, College Station, Texas; 2 Department of Biomedical Engineering, Texas A&M University, College Station, Texas; 3 Department Biomedical Engineering, Dalhousie University, Halifax, Nova Scotia, Canada; 4 Large Animal Clinical Sciences, Texas A&M University, College Station, Texas; and 5 Systems Biology and Translational Medicine, Texas A&M Health Science Center, Temple, Texas

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Quick CM, Criscione JC, Kotiya A, Dongaonkar RM, Hardy J, Wilson E, Gashev AA, Laine GA, Stewart RH. Functional adaptation of bovine mesenteric lymphatic vessels to mesenteric venous hypertension. Am J Physiol Regul Integr Comp Physiol 306: R901–R907, 2014. First published March 26, 2014; doi:10.1152/ajpregu.00185.2013.—Lymph flow is the primary mechanism for returning interstitial fluid to the blood circulation. Currently, the adaptive response of lymphatic vessels to mesenteric venous hypertension is not known. This study sought to determine the functional responses of postnodal mesenteric lymphatic vessels. We surgically occluded bovine mesenteric veins to create mesenteric venous hypertension to elevate mesenteric lymph flow. Three days after surgery, postnodal mesenteric lymphatic vessels from mesenteric venous hypertension (MVH; n = 7) and sham surgery (Sham; n = 6) group animals were evaluated and compared. Contraction frequency (MVH: 2.98 ± 0.75 min⁻¹; Sham: 5.42 ± 0.81 min⁻¹) and fractional pump flow (MVH: 1.14 ± 0.30 min⁻¹; Sham: 2.39 ± 0.32 min⁻¹) were significantly lower in the venous occlusion group. These results indicate that postnodal mesenteric lymphatic vessels adapt to mesenteric venous hypertension by reducing intrinsic contractile activity.

Lymphangion; lymph flow; interstitial fluid; postnodal lymphatic vessels

THE LYMPHATIC SYSTEM drains fluid from the interstitial space and transports it as lymph to the venous system. On the one hand, lymphatic vessels have a very different structure than blood vessels. Unlike blood vessels with primarily circumferential arrangement of smooth muscle, lymphatic vessels also have smooth muscle layers that are oriented longitudinally (38). On the other hand, lymphatic vessels are similar to veins. Lymphatic vessels are relatively thin-walled and can collapse, have muscle allowing phasic and tonic contraction, contain unidirectional valves, and have a tone modulated by a number of vasoactive mediators (14, 23, 46). Gaining far more attention in the last decade, however, is the observation that lymphangions, the segments of lymphatic vessels bound by valves, form units that function like cardiac ventricles. Lymphangions can act as chambers that cyclically contract, and are capable of actively pumping lymph against an axial pressure gradient (35). Similar to ventricles (21), increases in transmural pressure can cause lymphangions to increase stroke volume and contraction frequency (22, 31). Not only does this property allow lymph to be propelled from the low-pressure interstitial space to the higher-pressure venous system, it also allows lymphatic vessels to respond to increases in interstitial fluid pressure by pumping more fluid out of the interstitium (3, 18, 43).

Mesenteric venous hypertension causes persistent intestinal edema. Intestinal microvascular hypertension and resulting interstitial edema may occur with portal venous thrombosis, hepatic fibrosis, or mesenteric venous thrombosis. Acute venous hypertension leads sequentially to increased intestinal microvascular pressure, increased microvascular filtration, increased interstitial fluid volume and pressure, and finally, a several-fold increase in mesenteric lymph flow (13, 20, 27). The increase in lymph flow is particularly pronounced when hypertension is induced regionally by increased resistance of regional veins (13, 36, 45), since, unlike central venous hypertension, regional venous hypertension does not raise the outlet pressure of the lymphatic system (10, 28). Gashev et al. (17) reported that increased luminal flow in vitro decreases the rate and magnitude of lymphangion contraction in mesenteric lymphangions. Like blood vessels, this response is due to increased endothelial shear stress and is mediated, in part, by the production of nitric oxide (17). Although regional venous hypertension has been used by numerous investigators to study edema formation and lymphatic function (12, 13, 45), all studies up to this point have been acute. Investigators have yet to report that persistent intestinal edema secondary to increased microvascular pressure involves adaptive responses by lymphangions. Recent studies have reported that postnodal bovine mesenteric lymphangions adapt to altered pressures in vivo in as little as 3 days (8). Therefore, we used a mesenteric venous hypertension model to test the hypothesis that postnodal bovine mesenteric lymphatic vessels adapt to become weaker pumps within 3 days.

METHODS

Experimental groups. Experimental groups and animal care were performed in compliance with protocols approved by the Texas A&M University Institutional Animal Care and Use Committee and were consistent with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Holstein cows used for this study were randomly divided into two experimental groups: mesenteric venous hypertension (MVH) and sham surgery (Sham). Functional and histological analyses of lymphatic vessels were performed to determine their response to MVH.

Surgical preparation. An intravenous catheter was placed in the jugular vein for drug administration. Oxytetracycline (20 mg/kg) was administered subcutaneously. Anesthesia was induced using xylazine (0.1 mg/kg iv), followed by diazepam (0.01 mg/kg iv) and ketamine.
(2 mg/kg). Animals were intubated, positioned in left lateral recumbency, and maintained using mechanical ventilation with 1–3% isoflurane in oxygen sufficient to maintain a surgical plane of anesthesia. Arterial oxygen saturation was monitored (Cardell Veterinary Monitor-model MAX-12 HD; CAS Medical Systems, Branford, CT) and maintained >95% throughout the procedure. The right flank was aseptically prepared and draped. A 25-cm incision was made midway between the tuber coxae and the last rib. The small intestine was exteriorized and kept moist using warm (37°C) 0.9% saline.

**MVH.** The veins draining the small intestine are arranged in arcades, such that complete obstruction of one mesenteric vein does not stop blood flow, but rather redirects flow, and increases local venous pressure (Fig. 1). The proximal jejunal venous arcade was identified. The vein draining one end of this arcade was carefully isolated by blunt dissection and ligated. The vein draining the other end of the arcade was isolated in a similar fashion and fitted with an appropriately sized vascular occluder (Kent Scientific, Torrington, CT). The occluder was fixed to the mesentery with a piece of suture (2-0 polygylactin 910) and filled with 50% dextrose solution. To measure pressure within the arcade, an upstream vein draining the proximal jejunal venous arcade was isolated by blunt dissection, and 1/8″, O.D. polyvinyl chloride tubing (VWR Scientific, Suwanee, GA) filled with heparinized saline was inserted into the vein and secured with 2-0 polygylactin 910 suture. The PVC tubing and the tail of the vascular occluder were exteriorized through a small skin incision dorsal to the abdominal incision. The jejunum was replaced in the abdomen, and the abdominal incision was closed in multiple layers. The animals then recovered from anesthesia.

**Sham surgery group.** The same surgical procedures were performed in the sham-treated animals. Sham-treated animals, however, were only instrumented with the intravenous tubing for measurement of intestinal venous pressure. The mesenteric vein was not ligated, and the vascular occluder was not applied.

**Measurement of venous pressure.** The day after surgery, mesenteric venous pressure was measured by placing the cow in a restraining chute. The venous catheter was flushed with heparinized saline to ensure patency. A water manometer was used to measure venous pressure in a fashion adapted from a common clinical technique for measuring central venous pressure (49). The manometer was attached to the venous catheter and fixed with the zero-point at the level of the point of the shoulder. The equilibrium column height was recorded as venous pressure. The system was zeroed, and pressures were recorded three times to ensure repeatability. If pressure in the MVH group was less than 30 cmH2O, dextrose solution was injected into the vascular occluder until that pressure was attained. This was done to ensure that the elevated mesenteric venous pressure obtained during surgery was maintained postoperatively. Venous pressure data were compared using the Mann-Whitney rank sum test.

**Sample collection.** Three days following surgery, animals were euthanized by captive bolt and exsanguination. Study samples were collected immediately following euthanasia. The small intestine was exposed for tissue sample collection. Postnodal mesenteric lymphatic vessels draining the affected segment were ligated at the downstream end. After carefully dissecting free from surrounding tissue, vessels used for functional and structural analysis were placed in a thermos containing warm (37°C) 1% albumin physiological saline solution (APSS, pH 7.4) containing (in mM) 145 NaCl, 4.70 KCl, 2.00 CaCl2, 1.17 MgSO4, 1.20 NaH2PO4, 5.00 dextrose, 2.00 sodium pyruvate, 0.020 EDTA, and 3.00 MOPS. All the tissue samples were transported a short distance to the laboratory.

**Determination of intestinal water content.** Intestinal water content was measured to characterize the effects of the preparation on interstitial fluid balance. A jejunal segment was rinsed with saline to remove the luminal contents, patted dry, and weighed. The segment was placed in a drying oven and dried at 60°C to a constant weight. Percent water content of the intestinal segment was calculated as (100 × (wet weight-dry weight)/wet weight. Data were compared using a Student’s t-test.

**Preparation of postnodal vessels.** Experimental procedures were similar to those reported previously (39, 48). Lymphatic vessels were carefully cleared of all adipose tissue. Vessel segments ~3 cm in length were isolated and instrumented in an isolated vessel bath in which inlet and outlet pressures could be independently manipulated and measured. Axial tension on the vessels was applied to a degree just sufficient to raise the center portion of the vessel segment from the bottom of the bath. Using vessel segments between valves, we could ensure that there was no potential for valve closure to isolate the pressure transducers from the lumen of the vessel. The vessels were perfused and bathed with APSS with pH of 7.4 and warmed to 37°C. Normal rectal temperature in beef cows is 36.7–39.1°C and in dairy cows is 38.0–39.3°C (1). Previous studies have set bath temperatures between 35°C and 38°C (15, 29, 32, 33). We chose 37°C for the bath temperature so that our results would be more comparable to those of prior studies of bovine mesenteric lymphatic vessels (26, 30, 42, 51).

External pressure was set to 1 cmH2O by setting the bath solution height 1 cm above the center line of the vessel. Transmural pressure was determined as the difference between inlet and external pressures. Flow through the vessel was minimized by maintaining inlet and outlet pressures at equal levels throughout the measurement process. Transmural pressure was adjusted by increasing or decreasing both inlet and outlet pressures in tandem. Instantaneous vessel outer diameter was measured using custom video caliper software (IMAQ, National Instruments, Austin, TX). Vessels were allowed to equilibrate for 15 min. Lymphatic vessel segments failing to exhibit stable spontaneous contractions were discarded.

**Functional analysis of postnodal lymphatic vessels.** Postnodal lymphatic vessels were exposed to five transmural pressures (3, 6, 9, 12, and 15 cmH2O). The instrumented vessel was allowed to equilibrate for 5 min at each transmural pressure setting followed by a 5-min recording period. Following analysis of the active vessel properties, the bath and lumen were perfused with Ca2+-free APSS for 30 min until the vessel was fully relaxed. Diameters of the passive vessel were recorded for 1 min at each transmural pressure level (i.e., 3, 6, 9, 12, and 15 cmH2O).

**Analysis of passive properties.** To detect changes in passive stiffness, passive pressure-diameter data were fit to an exponential function commonly used to characterize biomechanical properties (i.e., $D = c e^{\beta P}$) (34). The values of $\alpha$ and $\beta$ were compared using the Mann-Whitney rank sum test.
we estimated the area occupied by each constituent. were identified from H&E-stained images. Using the filtered images, from Trichrome-stained images, and fibrous structures of the media cle cells (stained pink) and collagen (stained blue) were identified with MATLAB (R2008a; MathWorks, Natick, MA). Lymphatic mus-

Changes in fluid balance parameters. Mesenteric venous pressure measured one day after surgery was significantly higher in MVH (31.4 ± 2.2 cmH₂O; n = 12) than Sham (11.6 ± 2.8 cmH₂O; n = 5). Although the intestinal water content determined post mortem 3 days after surgery in MVH (77.9 ± 1.6%) was elevated, it was not significantly different from the Sham group (73.3 ± 2.6%).

**Functional data analysis.** The functional data from postnodal vessels were analyzed using two-way repeated-measures ANOVA comparing the experimental groups (MVH vs. Sham) with transmural pressure as the repeated measure. The Holm-Sidak method was used for post hoc multiple-comparison tests. All volume measurements were expressed in terms of cross-sectional area (i.e., volume normalized by length assuming uniform cylindrical contraction). The functional parameters compared were diastolic tone (100 × [passive diameter – diastolic diameter]/passive diameter), maximum (diastolic) volume, minimum (systolic) volume, stroke volume, ejection fraction (stroke volume/diastolic volume), contraction frequency, and fractional pump flow (contraction frequency × stroke volume/dia-
stolic volume). A calculated P value of less than 0.05 was considered significant. In all cases, each cow contributed one vessel for analysis.

**Histological analysis of postnodal lymphatic vessels.** Changes in the structural components of the postnodal lymphatic vessels were determined using histological analysis. Each vessel was dehydrated using a series of graded alcohol (70%, 80%, 90%, and 95% EtOH) and then embedded with paraffin. Five-micrometer-thick cross sections of the vessels were then obtained using a rotary microtome (model no. RM2255, Leica Microsystems, Bannockburn, IL). The sections were mounted on plus-coated slides and stained with Masson’s Trichrome and hematoxylin and eosin (H&E). Images of each vessel cross section were obtained, and their contrast and saturation increased using image enhancement software (Picasa, Google, Mountain View, CA). Images were then analyzed with a custom application developed with MATLAB (R2008a; MathWorks, Natick, MA). Lymphatic muscle cells (stained pink) and collagen (stained blue) were identified from Trichrome-stained images, and fibrous structures of the media were identified from H&E-stained images. Using the filtered images, we estimated the area occupied by each constituent.

**Histological data analysis.** Cross-sectional area occupied by smooth muscle cells (SMCA), total cross-sectional area occupied by collagen (TCA), and cross-sectional area occupied by medial collagen were determined and normalized by total cross-sectional area (TA) and medial area (MA) and were reported as means ± SE. A calculated P value less than 0.05 was considered significant. Each cow contributed one lymphatic vessel for analysis.

**RESULTS**

**Fluid balance parameters.** Mesenteric venous pressure measured one day after surgery was significantly higher in MVH (31.4 ± 2.2 cmH₂O; n = 12) than Sham (11.6 ± 2.8 cmH₂O; n = 5). Although the intestinal water content determined post mortem 3 days after surgery in MVH (77.9 ± 1.6%) was elevated, it was not significantly different from the Sham group (73.3 ± 2.6%).

**Table 1. Comparison of passive, diastolic, and systolic diameters of bovine mesenteric postnodal lymphatic vessels in Sham and MVH groups**

<table>
<thead>
<tr>
<th>Transmural Pressure, cmH₂O</th>
<th>Passive Diameter, mm</th>
<th>Diastolic Diameter, mm</th>
<th>Systolic Diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham Group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.64 ± 0.37</td>
<td>3.38 ± 0.39</td>
<td>1.87 ± 0.27</td>
</tr>
<tr>
<td>6</td>
<td>3.74 ± 0.37</td>
<td>3.51 ± 0.39</td>
<td>2.15 ± 0.29</td>
</tr>
<tr>
<td>9</td>
<td>3.79 ± 0.38</td>
<td>3.59 ± 0.39</td>
<td>2.49 ± 0.29</td>
</tr>
<tr>
<td>12</td>
<td>3.80 ± 0.39</td>
<td>3.66 ± 0.38</td>
<td>2.89 ± 0.30</td>
</tr>
<tr>
<td>15</td>
<td>3.84 ± 0.40</td>
<td>3.71 ± 0.36</td>
<td>3.14 ± 0.30</td>
</tr>
<tr>
<td><strong>MVH Group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.23 ± 0.32</td>
<td>4.06 ± 0.31</td>
<td>2.15 ± 0.26</td>
</tr>
<tr>
<td>6</td>
<td>4.30 ± 0.32</td>
<td>4.21 ± 0.31</td>
<td>2.93 ± 0.30</td>
</tr>
<tr>
<td>9</td>
<td>4.34 ± 0.34</td>
<td>4.27 ± 0.33</td>
<td>3.40 ± 0.35</td>
</tr>
<tr>
<td>12</td>
<td>4.36 ± 0.35</td>
<td>4.30 ± 0.35</td>
<td>3.77 ± 0.35</td>
</tr>
<tr>
<td>15</td>
<td>4.39 ± 0.34</td>
<td>4.31 ± 0.35</td>
<td>4.00 ± 0.34</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. MVH, microvascular hypertension.
transmural pressure in bovine mesenteric lymphatic vessels by McHale and Roddie (31) used transmural pressures of 0 to 6 cmH₂O, while the current study used a pressure range of 3 to 15 cmH₂O. If we compare the transmural pressures common to both studies, the results for the Sham group appear similar to McHale and Roddie’s study. In this study, the increase in mean frequency from 3 to 6 cmH₂O was ~17%. In the current study, mean frequency in the Sham group at 6 cmH₂O was 9% greater than that at 3 cmH₂O. However, mean frequency in the MVH group in the present study was 5% lower at 6 cmH₂O than at 3 cmH₂O. This could be an aspect of the adaptive response to venous hypertension. Because the frequency change from 3 to 6 cmH₂O was not statistically significant, it is difficult to make any claim as to its importance. We chose a much greater pressure range than most in vitro studies, because the particular intervention that we used could potentially raise lymphatic transmural pressure in MVH vessels in vivo above normal values. Comparing Sham and MVH vessels in the same extended pressure range in vitro allowed us to detect any differences. Given the statistical difference between Sham and MVH vessels at 9 and 12 cmH₂O, this increased range is, in retrospect, justified. Because the passive properties of lymphatics have been reported to change significantly for the pressure range between 0 and 5 cmH₂O (41), it may be interesting in future biomechanical studies to explore changes in passive stiffness in lower pressures than those illustrated in Fig. 2, particularly in response to interventions that are less likely to raise lymphatic transmural pressures than MVH.

**Potential mechanical mechanisms for lymphatic pump inhibition.** The present work was primarily a functional study, and the transmural pressure in bovine mesenteric postnodal lymphatic vessels

### Table 2. Comparison of critical variables characterizing the response of bovine mesenteric postnodal lymphatic vessels pooled over all transmural pressure levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Diastolic Tone</th>
<th>Diastolic Volume, % of PV</th>
<th>Systolic Volume, % of PV</th>
<th>Stroke Volume, % of PV</th>
<th>Ejection Fraction</th>
<th>Frequency, min⁻¹</th>
<th>Fractional Pump Flow, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>5.57 ± 1.53</td>
<td>89.49 ± 2.83</td>
<td>47.61 ± 5.93</td>
<td>41.87 ± 6.88</td>
<td>0.47 ± 0.06</td>
<td>5.42 ± 0.81</td>
<td>2.39 ± 0.32</td>
</tr>
<tr>
<td>MVH</td>
<td>2.25 ± 1.42</td>
<td>95.64 ± 2.62</td>
<td>59.50 ± 5.49</td>
<td>36.14 ± 6.37</td>
<td>0.38 ± 0.06</td>
<td>2.98 ± 0.75*</td>
<td>1.14 ± 0.30*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Contraction frequency and fractional pump flow were significantly decreased in microvascular hypertension group (MVH; *n = 7*) compared to Sham (*n = 6*). *P < 0.05 vs. Sham. PV, passive volume.

Fig. 3. Functional parameters for bovine mesenteric postnodal lymphatic vessels. A: diastolic tone. B: systolic and diastolic volumes normalized by passive volume. C: ejection fraction. D: contraction frequency. E: fractional pump flow. Contraction frequency and fractional pump flow were significantly lower in MVH (*n = 7*) compared with Sham (*n = 6*). Data are presented as means ± SE. *P < 0.05 vs. Sham.
changes in function were not related to particular changes in biomechanical properties. In particular, the decrease in pump function with MVH may be related to changes in either circumferential or longitudinal smooth muscle. It is possible that changes in length, due to the longitudinal arrangement of smooth muscle, leads to significant shortening in vivo. Because our preparation set length, the difference in Sham and MVH vessels manifested only as a change in radius. Similarly, the mechanical stimuli in vivo that resulted in adaptation are difficult to identify. Adaptive responses occurred as a result of our hypertension model, but it is not clear whether this was due primarily to enhanced lymph flow, elevated lymphatic pressure, or even tissue oncotic pressure changes. Some or all of these mechanisms may be contributory, and their effects may be interrelated. Changes in contractility can affect transmural pressure and changes in transmural pressure can affect contractility (31). Elevated lymph flow increases shear stress, and it is known to acutely inhibit lymphangion pumping (17, 34). Since, in vivo, lymph flow is increased by increasing microvascular fluid filtration, it is not possible to increase lymph flow without altering interstitial protein concentration (44) and thus the content of lymph. Now that functional adaptations have been identified, further biomechanical study is warranted, including quantifying maximum tension generated (19, 52) or alterations of sensitivity to shear stress (17).

Potential inflammatory mechanisms. The alterations in lymphatic function observed in this study may be the result of an inflammatory response induced by the mesenteric venous hypertension. We did not seek to detect differences in immune cell populations or indicators of inflammation within the sections of the vessel walls or surrounding tissue. Inflammatory signaling molecules could originate in or near the intestinal interstitium and travel via lymph to affect the mesenteric lymphatic vessels. Using a model of bacterial tripeptide-induced ileitis in rats, Benoit and Zawieja (4) demonstrated that mesenteric lymphatic vessels examined in vivo increased stroke volume within the first hour. In that study, lymphatic contraction frequency was not significantly affected. However, Wu et al. (50) examined contractile behavior of mesenteric lymphatic vessels in vivo and in vitro 3 and 6 days after 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced ileitis in guinea pigs. They reported that intestinal inflammation was associated with decreased contraction frequency, decreased stroke volume, and increased diastolic diameter—results broadly consistent with the current study. The inflammation-related decrease in contraction frequency reported by Wu et al. (50) was moderated by application of a nonselective COX inhibitor (indomethacin), as well as by combined COX 1 and COX 2 inhibitors. Numerous inflammatory mediators have been demonstrated to affect lymphatic contractile behavior, including prostaglandins, bradykinin, histamine, and substance P (2, 6, 16, 23, 25). Recently, substance P has been shown to activate both inflammatory signaling pathways and contractile activity in lymphatic vessels (5). However, because substance P acutely stimulates lymphatic contraction, it may not be a good candidate for inducing the behavior observed in the present study. The finding by Wu et al. that COX inhibition partially reversed the effect of intestinal inflammation on lymphatic pumping (50) provides support for the possible role of prostacyclin.

Fig. 4. A: postnodal lymphatic vessel sections stained with Masson’s Trichrome and hematoxylin and eosin (H&E) to estimate the percent area of the constituents of interest: vessel total area (TA), medial area (MA), area occupied by collagen (TCA), and smooth muscle cells (SMCA). B: between the Sham (n = 8) and MVH (n = 8) groups, there were no significant differences in the smooth muscle cell area/total area ratio (SMCA/TA), total collagen area/total area ratio (TCA/TA), medial collagen area/total area ratio (MCA/TA), smooth muscle cell area/medial area ratio (SMCA/MA), and medial collagen area/medial area ratio (MCA/MA). C: Ratio of total cross-sectional area for SMC/TA, TCA/TA, and MCA/TA. Data are presented as means ± SE.
Novel bovine model for the study of lymphatic vessel adaptation. The present work is complementary to a recent study by Dongaonkar et al. (8), which reported changes in postnodal bovine mesenteric lymphatic pumping in response to altered transmural pressures. In that study, lymphatic vessels were partially constricted for 3 days, causing upstream pressure to be higher than downstream pressure in the same vessels. Although no change in contraction frequency was observed, systolic and diastolic diameters and the pump index (variation of fractional pump flow) of upstream vessel segments were higher than those of downstream segments. In effect, lymphangions adapted to higher pressures by becoming stronger pumps. In the present study, the primary stimulus was mesenteric venous hypertension, which is more clinically relevant. It is suspected that MVH results in higher flows, which could explain the fundamentally different result. Taken together, these two studies help establish the bovine model as a novel model for studying lymphatic vessel adaptation. Historically, the postnodal bovine mesenteric lymphatic vessel has been a well-established in vitro model of lymphatic function (30, 31, 37). The animal is large enough, not only to surgically create mesenteric venous hypertension, but also to measure and manipulate mesenteric venous pressure postoperatively. In developing this animal model to study lymphatic adaptation, we chose a conservative 3-day time frame for two reasons. First, this is the earliest time period that we could expect to document significant changes in function. Second, we wanted to initially avoid longer adaptation periods to minimize the potential confounding adaptive changes in the microvascular, interstitial (24), and serosal barriers. Dongaonkar et al. (7) identified no less than seven parameters affecting interstitial fluid volume. Changes in any of these seven could act to blunt the effects of mesenteric venous hypertension. The failure to demonstrate a significant difference in intestinal water content may be due, in part, to the effectiveness of these multiple adaptive processes. In particular, decreases in the microvascular filtration coefficient have been demonstrated with chronic increases in microvascular filtration pressure following venous hypertension (24, 47). Now that we have established adaptive changes in 3 days to a clinically relevant perturbation, we can suggest that there is value in repeating this complex, resource-intensive experiment for longer adaptive periods using this novel animal model.

Perspectives and Significance

The results of the present study are the first to suggest that postnodal lymphatic vessels can respond to edemagenic conditions by becoming weaker pumps. Given the great complexity of microvascular-interstitial-lymphatic interaction, mathematical models have been used to explore how changes in lymphatic pumping affects interstitial fluid balance. Quick et al. (40) developed a relatively simple model of a lymphangion that suggests that decreases in lymphangion pumping frequency raises lymphangion effective lymphatic resistance, first characterized by Drake et al. (11). Dongaonkar et al. (7) illustrated with a system-level model that increased effective lymphatic resistance will decrease lymph flow, increase interstitial volume, as well as “edemagenic gain” (i.e., the sensitivity of interstitial volume to increases in microvascular pressure) (9). These modeling insights suggest persistent adaptive responses manifesting as “lymphatic pump failure,” therefore, are either homeostatic, ameliorating elevated interstitial flow, or maladaptive, exacerbating interstitial edema.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


