Maximum shortening velocity of lymphatic muscle approaches that of striated muscle

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Zhang R, Taucer AI, Gashev AA, Muthuchamy M, Zawieja DC, Davis MJ. Maximum shortening velocity of lymphatic muscle approaches that of striated muscle. Am J Physiol Heart Circ Physiol 305: H1494–H1507, 2013. First published August 30, 2013; doi:10.1152/ajpheart.00898.2012.—Lymphatic muscle (LM) is widely considered to be a type of vascular smooth muscle, even though LM cells uniquely express contractile proteins from both smooth muscle and cardiac muscle. We tested the hypothesis that LM exhibits an unloaded maximum shortening velocity (Vmax) intermediate between that of smooth muscle and cardiac muscle. Single lymphatic vessels were dissected from the rat mesentery, mounted in a servo-controlled wire myograph, and subjected to isotonic quick release protocols during spontaneous or agonist-evoked contractions. After maximal activation, isotonic quick releases were performed at both the peak and plateau phases of contraction. Vmax was 0.48 ± 0.04 lengths (L)/s at the peak: 2.3 times higher than that of mesenteric arteries and 11.4 times higher than mesenteric veins. In cannulated, pressurized lymphatic vessels, shortening velocity was determined from the maximal rate of constriction [rate of change in internal diameter (−dD/dr)] during spontaneous contractions at optimal preload and minimal afterload; peak −dD/dr exceeded that obtained during any of the isotonic quick release protocols (2.14 ± 0.30 L/s). Peak −dD/dr declined with pressure elevation or activation using substance P. Thus, isotonic methods yielded Vmax values for LM in the mid to high end (0.48 L/s) of those the recorded for phasic smooth muscle (0.05–0.5 L/s), whereas isobaric measurements yielded values (>2.0 L/s) that overlapped the midrange of values for cardiac muscle (0.6–3.3 L/s). Our results challenge the dogma that LM is classical vascular smooth muscle, and its unusually high Vmax is consistent with the expression of cardiac muscle contractile proteins in the lymphatic vessel wall.

isometric; isotonic quick release; maximum shortening velocity; force-velocity; slack test; spontaneous contraction

THE LYMPHATIC VASCULATURE is vital to cardiovascular homeostasis by virtue of its role in collecting and returning to the great veins more than 2 liters of fluid and protein per day (in humans) that otherwise would accumulate in extravascular compartments. An extensive network of lymphatic vessels runs in parallel to the blood vascular system, with initial lymphatic vessels serving an absorptive role and collecting lymphatic vessels possessing intrinsic pump behavior to move lymph against a hydrostatic pressure gradient. The robust, synchronized contractions of lymphatic muscle (LM) cells are an essential component of active lymph propulsion (34, 63, 81), with contraction facilitating central movement of the luminal contents through one-way valves (20). The weakening of LM is an essential contributor to the lymphatic pump dysfunction underlying many forms of lymphedema (55). Unfortunately, current therapies to ameliorate lymphedema are limited to promoting passive lymph transfer due to our lack of understanding of the molecular and mechanical properties of LM. As a consequence, strategies for improving lymphatic pump function have not yet been developed.

Collecting lymphatic vessels share common functional characteristics with both arterial blood vessels and the heart. Like small arteries and arterioles, muscular lymph vessels develop and sustain spontaneous tone that can be modulated by vasoconstrictors and vasodilators (16, 18, 30, 47), 2 dilate to intrinsic and imposed flow (8, 9, 30, 31), and 3 show myogenic constriction to pressure elevation that is comparable or greater in magnitude (per unit change in pressure) to that of arterioles (16, 61, 66). LM exhibits spontaneous contractions following Ca2+–dependent action potentials (72, 74) that may be initiated by Ca2+-activated Cl− current (67, 71, 74) and/or a hyperpolarization-activated cation current (45), resembling the cardiac “funny” current (1). In addition, LM expresses contractile proteins common to both vascular smooth muscle (VSM) and cardiac muscle, including smooth muscle actin isoforms and myosin heavy chains (MHC) I and II, along with cardiac actin isoforms and β-MHC (51). Despite these substantial functional and molecular differences between LM and VSM, LM is widely considered to be equivalent in function to the VSM found in arteries/arterioles (3, 27, 43, 45).

The length-tension and force-velocity relationships reflect two fundamental mechanical properties of muscle. The passive length-tension relationship describes the force contributed primarily by elastic elements in parallel with the contractile apparatus. Lymphatic vessels are highly distensible, as evident from comparisons of their passive length-tension and stress-strain curves to those of arteries and veins (53, 54, 79). The active length-tension relationship describes how force developed by the contractile apparatus depends on the initial muscle length (50). LM has an optimal (and relatively broad) initial length over which substantial force development can be supported (46, 54, 79). Length-tension relationships of rat mesenteric lymphatic vessels reveal that, when maximally activated with substance P (SP) or norepinephrine (NE), the muscle layer has the potential to develop 5- to 10-fold higher active force than that exhibited during a spontaneous contraction (79).
The force-velocity relationship has been called the "mechanical measurement most directly reflecting the molecular and chemo-mechanical transduction process" (50), e.g., the actomyosin cross-bridge cycling rate (65). Estimates of shortening velocity for LM in vivo (5), albeit measured without precise control of preload or afterload, suggest that it may be much higher than that for VSM (37, 41, 49). However, despite the fundamental importance of this relationship to understanding its contractile properties, the maximum unloaded shortening velocity ($V_{\text{max}}$) for LM has not been measured, with the exception of a single curve for circularly oriented strips excised from bovine mesenteric lymphangions in a Russian journal (44). Knowing the $V_{\text{max}}$ for LM is of particular importance if LM shares biochemical (22, 51) and functional properties common to both VSM and cardiac muscle, as this would open up potential avenues for selective therapeutic targeting of lymphatic vessels. The present study aimed to determine the force-velocity relationship for rat mesenteric LM and to compare it with that of arterial and venous VSM in the same vascular bed. We tested the hypothesis that LM exhibits an unloaded shortening velocity that is intermediate between that of smooth muscle and cardiac muscle.

**METHODS**

**Vessel isolation.** All animal protocols were approved by the Texas A&M University Laboratory Animal Care Committee or the University of Missouri Animal Care and Use Committee and conformed with the Public Health Service Policy for the Humane Care and Use of Laboratory Animals (PHS Policy, 1996).

Male rats (150–300 g) were anesthetized with pentobarbital sodium (60 mg/kg ip), and a loop of the duodenum from each animal was exteriorized through a midline abdominal incision. After identification, suitable lymphatic vessels (inner diameter: 100–220 μm), small arteries (inner diameter: 180–280 μm), or small veins (inner diameter: 210–390 μm) were dissected from mesenteric arcades and placed in physiological saline solution with albumin (APSS) at room temperature. The animal was then euthanized with pentobarbital. APSS contained (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl$_2$, 1.2 MgSO$_4$, 1.2 NaH$_2$PO$_4$, 0.02 EDTA, 5.0 glucose, 2.0 sodium pyruvate, 3.0 MOPS, and 0.5 g/100 ml purified BSA (pH 7.4 at 37°C). K-PSS was made by adding 2.0 mM CaCl$_2$ to K-PSS to make K-PSS + Ca and 2.0 μM 5,8-dIDEO-ECAM (5,8-dideoxy-5,8-diido-ECD acetylcholine), a nicotinic agonist, to APSS to make APSS – Ca. The animal was then euthanized with pentobarbital.

**Wire myograph methods.** After careful removal of loose fat and adventitia, a vessel was cannulated on both 40-μm diameter stainless steel wires, trimmed axially to a length of 2 mm, and transferred to a small vessel wire myograph (model 310A with a direct output option, Danish Myo Technology, Aarhus, Denmark). The wires were secured to the opposing jowls of the myograph. The bath temperature was raised to 36–37°C over the course of 30 min with force set to a minimum preload to allow for wire expansion. When temperature stabilized, preload [passive force ($F_{\text{pass}}$)] was set at a predetermined optimal value for the respective vessel type (79), and the vessel was allowed an additional 15- to 30-min equilibration period in APSS.

For servo control of force, the wire myograph was equipped with an Inchworm piezo drive (EXFO-Burleigh, Victor, NY) in series with the micrometer position (i.e., diameter) control, as previously described (19). The system was mounted onto the stage of a Leica DMIL inverted microscope, and the vessel image was viewed using a Sony XC-55 camera through a ×10 objective. The force signal was amplified and filtered (300 Hz) using modules (models PA-1S and LPBF-01G, respectively) from NPI Electronic (Tamm, Germany). A Pentium-based computer (Dell, Austin, TX) acquired the data at 800 Hz through a PCI-6030a analog-to-digital/digital-to-analog card and BNC-2090 interface. The acquisition and servo-control algorithms were written using LabView (National Instruments, Austin, TX). The position control signal for the Inchworm drive was sent through a serial port to the EXFO 8200 controller. Analyses were performed using LabView and IGOR (Wavemetrics, Oswego, OR). In some protocols, the diameter of the vessel was simultaneously tracked using edge detection software (14). All diameters represent inner diameters.

**Isotonic release protocols.** In APSS, passive tension was set to 0.3 mN for lymphatic vessels and small veins or to 3.0 mN for small arteries, values corresponding to the respective optimal preloads for maximal force production, as determined in a previous study (8). Small arteries and small veins were activated with K-PSS + 1 μM NE. Lymphatic vessels were activated with K-PSS + SP because SP produced higher maximum force than NE (18, 80). After maximal force development, where $F_{\text{max}}$ is maximal active force (i.e., peak force – $F_{\text{pass}}$), quick release protocols were initiated. During each release, force rapidly dropped to a designated fractional afterload level [force/maximal force ($F/F_{\text{max}}$)] between 0 and 1, where 0 $F/F_{\text{max}}$ and 1 $F/F_{\text{max}}$ correspond to any given release, and the correction factor was adjusted, assuming exponential decay, was added to the otherwise “square” release step, using the “supercharging” principle similar to that for series resistance compensation in patch-clamp amplifiers (64). To empirically determine the appropriate correction factor, releases of different magnitudes were performed on several vessels, and the difference between the actual force and the target force 100 ms after the release (the fractional error) was plotted as a function of the normalized afterload (see inset in Fig. 1C). Estimates for the amplitude of the correction factor as a function of afterload were then made from a double-exponential fit of that data (line in inset in Fig. 1C). The value at any given afterload was used to correct the force target at 100 ms after the release, and the correction factor was adjusted, assuming exponential decay, to correct the force target at subsequent time points. The middle trace in Fig. 1C shows a well-compensated force trace using this method, whereas the bottom trace shows a trace that was intentionally overcompensated with the force target for the first 0.5 s after the release: an additional amount of force, with exponential decay, was added to the otherwise “square” release step, using the “supercharging” principle similar to that for series resistance compensation in patch-clamp amplifiers (64). To empirically determine the appropriate correction factor, releases of different magnitudes were performed on several vessels, and the difference between the actual force and the target force 100 ms after the release (the fractional error) was plotted as a function of the normalized afterload (see inset in Fig. 1C). Estimates for the amplitude of the correction factor as a function of afterload were then made from a double-exponential fit of that data (line in inset in Fig. 1C). The value at any given afterload was used to correct the force target at 100 ms after the release, and the correction factor was adjusted, assuming exponential decay, to correct the force target at subsequent time points. The middle trace in Fig. 1C shows a well-compensated force trace using this method, whereas the bottom trace shows a trace that was intentionally overcompensated with an exaggerated correction factor of −2X. Compensation was subsequently used for all quick release protocols, and different compensation parameters were determined empirically for each type of vessel.

Shortening velocity, the rate of change in diameter as reflected by the change in relative piezo position, was measured between 50 and 150 ms after the release, with care taken to avoid piezo ringing that occurred occasionally immediately after the release (Fig. 2A). The position data were fit to a first-order polynomial whose slope approximated the initial shortening velocity (65). The data were plotted against the fractional afterload and fit to the following Hill equation:
where \( a/F_{\text{max}} \) and \( b \) are constants related to the curvature of a rectangular hyperbola, \( V \) is shortening velocity, and \( V_{\text{max}} \) is represented by the \( y \)-intercept (65).

Slack test protocol. The slack test described by Edman (25) was used as an alternative method to estimate \( V_{\text{max}} \) in maximally activated vessels. When a vessel was subjected to a large, rapid release to a value below its slack length (i.e., slack diameter), force dropped to zero. The time required for the vessel to take up the slack was equal to the time required to redevelop force; that time was roughly proportional to the magnitude of the change in vessel diameter. This procedure has been used as an alternative method to estimate unloaded shortening velocity (25, 77). The fractional diameter changes (normalized to the passive vessel diameter) can be plotted as a function of the time required to take up slack, with the slope of the line being equal to the shortening velocity and the intercept being an index of vessel compliance (25, 77). We adapted our isotonic release algorithm to perform slack tests on lymphatic vessels and small arteries as an alternative method to measure \( V_{\text{max}} \). After an equilibration period with force set to \( F_{\text{pass}} \), the diameter at which the vessel would generate zero force (\( D_i \)) was estimated by slowly ramping the force down to zero. The difference between \( D_i \) and the diameter at \( F_{\text{pass}} \) (\( D_o \)) served as an estimate of the diameter change required to produce slack, i.e., to drop force to zero, when the vessel was later stimulated. Force was then returned to \( F_{\text{pass}} \) (and diameter to \( D_o \)) for several minutes before maximal activation. At the peak of maximal
force development, force was dropped to zero by rapidly stepping diameter to \( D_o \) (or a value slightly less than \( D_o \)). Force would subsequently redevelop over a time course of 0.1–2.0 s. Diameter was then returned to \( D_o \), the bath was exchanged for APSS, and the vessel was allowed to reequilibrate for several minutes before the procedure was repeated. Typically, progressively larger diameter steps were used on subsequent trials, in increments of 5 or 10 \( \mu \)m. The procedure was repeated three to eight times for each vessel. Data from trials in which an unusual amount of piezo ringing, transducer noise, or transducer drift occurred were not used for analysis.

**Spontaneous contraction protocols.** The quick release protocol was modified to estimate shortening velocity during the systolic phase of the spontaneous contraction cycle in wire-mounted lymphatic vessels. Preload was set to the optimal level (0.3 mN), and a steady pattern of spontaneous contractions was allowed to develop in APSS (in the absence of agonist-induced stimulation). Several control contractions were recorded for the determination of amplitude (termed \( F_{\text{max}} \) for this protocol), and a quick release was performed on the subsequent contraction. Servo gain was kept sufficiently high to override spurious \( F_{\text{max}} \), calculated as described above. The contraction then switched from isometric to isotonic and was held at the lower force for \(~5\) s. Shortening velocity was determined from the diameter change (i.e., piezo position) required to maintain the isotonic contraction. As before, the initial shortening velocity was determined from the data recorded during the first 50–150 ms after the release, excluding piezo ringing. The control system was then switched back to isometric mode, and several spontaneous contractions were recorded before another isotonic quick release at a different afterload. This procedure was repeated 5–12 times on each vessel; the initial shortening velocities were subsequently fit to \( \text{Eq. 1} \) in the manner described above.

**Isobaric methods and protocols.** In pressurized lymphatic vessels, shortening velocity was estimated from the rate of the lymphatic vessel diameter change during isobaric contractions at a fixed pressure level. After dissection and cleaning, a two-valve lymphatic vessel segment was transferred to a 3-ml chamber, cannulated at each end with a glass micropipette on a Burg-style V-track system (24), and mounted on the stage of an inverted microscope. Input pressure (\( P_{\text{in}} \), which determines the preload) and output pressure (\( P_{\text{out}} \); which determines the afterload) were set to an optimal level (typically 3 cmH\(_2\)O (62)), and a steady pattern of spontaneous contractions was allowed to develop for 30–60 min with the vessel bathed in APSS. The image was digitized with a progressive scan, firewire camera (model A641FM, Basler, Ahrensburg, Germany) at 30 or 60 Hz, and internal diameter was measured using an edge-detection algorithm (14). Pressures were measured using low-pressure transducers (model 104, CyberSense, Nicholsville, KY) and digitized at 30 or 60 Hz in synchrony with diameter using a PCI 6030e analog-to-digital card (National Instruments). Pressures were controlled in the input and output cannulae using a custom-made analog servo control system (Cardiovascular Research Institute, Texas A&M University), as previously described (80). LabVIEW analysis programs were used offline to calculate the rate of change in internal diameter (\(-dD/dt\)) from the internal diameter recording. Before differentiation, diameter data were filtered to remove glitches associated with occasional tracking artifacts. Three pressure protocols were used: 1) simultaneous \( P_{\text{in}} \) + \( P_{\text{out}} \) steps in the range of 0.5–13 cmH\(_2\)O; 2) \( P_{\text{out}} \) steps over the same pressure range, with \( P_{\text{in}} \) held at 1 cmH\(_2\)O; and 3) \( P_{\text{out}} \) ramps over the same pressure range, with \( P_{\text{in}} \) held at 1 cmH\(_2\)O. In some experiments, SP was added to the bath (\( 3 \times 10^{-6} \) M) to test the effect of muscle activation on shortening velocity. In the pressure ramp protocol, contractions did not always occur at exactly the same pressure, so the data collected from individual vessels were combined by binning the \(-dD/dt\) values according to the pressure level, across the entire pressure range, to perform statistical analyses (80).

**Confocal microscopy.** To determine the average length and orientation of LM cells in the wall of mesenteric lymphatic vessels, vessel segments were observed at \( \times 40–50 \) magnification using a Leica AOBBS SP2 confocal multiphoton microscope system. Cells were loaded with CellTracker green (5 \( \mu \)M in PSS, Molecular Probes). Images were acquired at 0.3-\( \mu \)m intervals using 488-nm excitation and 508-nm emission wavelengths. The image stacks were reconstructed using Leica confocal software to produce various three-dimensional projections. The average length of the muscle cells was 79.9 ± 5.6 \( \mu \)m (\( n = 72 \), range: 66–107 \( \mu \)m). The average pitch of the cells was 6.0 ± 0.9° off the circumferential axis of the vessel (i.e., 84° to the normal direction of flow).

**Statistics.** Data were analyzed using IGOR, Excel, and JMP (SAS, Cary, NC). For analysis of isobaric data, one-way ANOVAs were performed, with pressure designated as the independent variable. Dunnett’s post hoc tests were used to test for differences between the group (pressure) with the highest shortening velocity and the other
groups. To compare control and SP responses in the same vessels, an ANOVA was used. Significance was defined as $P < 0.05$.

**RESULTS**

Isotonic quick releases after maximal activation. An example of the time course of activation for a mesenteric lymphatic vessel is shown in Fig. 1A. Immediately upon the addition of activating solution to the wire myograph bath, the lymphatic vessel developed a maximal force of 2.6 mN, which peaked at 30–60 s. Over the next 3–4 min, force declined to a plateau level of ~2.0 mN and remained at that level for ~15 min. The average value of $F_{\text{max}}$ for 12 lymphatic vessels was 2.1 ± 0.1 mN at the peak and 1.3 ± 0.1 mN at the plateau. During the plateau, a series of isotonic quick releases was performed, as indicated by the downward spikes in the force trace. The protocol was repeated using small arteries. In contrast to lymphatic vessels, small arteries developed approximately eight times more force ($F_{\text{max}} = 17.6 ± 6.8$ mN, $n = 12$), over a significantly longer time course, in which peak force was maintained for 15–30 min without a secondary plateau (79). These results are shown in Table 1, along with the absolute values of diameter for the different vessel types.

Examples of two isotonic quick releases are shown on an expanded timescale for a lymphatic vessel in Fig. 1B. The top traces reflect the relative positions of the piezo drive during each release and directly correspond to the movements of the myograph jaw that controlled internal diameter. The bottom traces represent the force changes associated with the two quick releases. In both cases, the contractions were isometric in the first second and isotonic from time $= 1$–$9$ s. At time $= 9$ s, force was slowly returned to trough control. This vessel was maximally activated before the first release, washed in APSS, and then reactivated to obtain the second release. $F_{\text{max}}$ (peak force) was nearly identical for the two trials ($1.20$–$1.22$ mN).

Isotonic quick release data were collected from 12 lymphatic vessels, 12 small arteries, and 3 small veins. The procedure for measuring shortening velocity for each isotonic release is shown in Fig. 2A (the example shows a lymphatic vessel). After piezo ringing ceased, the diameter data over the subsequent 100 ms were fit with a first-order polynomial to estimate the initial shortening velocity. This method yielded an estimate of initial shortening velocity at one particular afterload. Shortening velocities determined from 5–12 isotonic releases/vessel were then plotted as a function of fractional afterload and fit with a hyperbolic function according to $\text{Eq. 1}$, for which the $y$-intercept was an estimate of $V_{\text{max}}$ (65). A data set obtained for one lymphatic vessel using this method is shown in Fig. 2B (the same vessel as in Fig. 2A). Because this method of determining $V_{\text{max}}$ was biased by low values of fractional afterload (<0.2 $F_{\text{max}}$), we typically attempted to perform multiple releases to afterloads in this range to ensure the repeatability of the measurements.

Lymphatic vessels exhibited biphasic contractions in response to activation with an exogenous agonist (Fig. 1A); therefore, we measured $V_{\text{max}}$ both at the peak and plateau of maximal force production. Figure 3 shows two families of isotonic quick release recordings obtained from the same lymphatic vessel. The vessel was activated to obtain one quick release from the peak and one from the plateau, washed with APSS, and then reactivated to obtain another set of two quick release recordings. This procedure was repeated until the full sets of traces shown here were obtained. Up to 30% variation in $F_{\text{max}}$ occurred with repeated activation, as is evident from the force values during the isometric phases (time $= 0$–1 s) in both graphs. The time course of the diameter changes (Fig. 3, top) indicates that shortening velocity slowed considerably during the plateau phase of contraction. The values of $V_{\text{max}}$ obtained after initial shortening velocity measurements and the curve fitting procedure for the same vessel were 81.4 and 56.0 $\mu$m/s at the peak and plateau, respectively. This procedure was repeated on 12 vessels (in most cases, either peak or plateau measurements, but not both, were obtained), and the results are shown in Table 1. The average values of $V_{\text{max}}$ were $103.9 ± 7.1$ $\mu$m/s measured at peak force and $57.1 ± 5.7$ $\mu$m/s measured at plateau force.

To compare $V_{\text{max}}$ for LM with $V_{\text{max}}$ for arterial smooth muscle, the isotonic quick release protocol was repeated using mesenteric small arteries, which were located adjacent to the main collecting lymphatic vessels in each mesenteric arcade. In most cases, the small artery and lymphatic vessel were taken from the same arcade in the same animal. Small arteries were slightly larger in diameter and developed substantially more active force than lymphatic vessels, 17.6 vs. 2.1 mN for lymphatic vessels (Table 1). Representative sets of quick release recordings from a lymphatic vessel (A) and a small artery (B) are shown in Fig. 4. At the peak of force production (30–60 s for lymphatic vessels vs. 3–5 min for small arteries), quick releases were performed. $V_{\text{max}}$ was consistently and significantly higher for lymphatic vessels than for small arteries. The values of $V_{\text{max}}$ for these two individual vessels were 73.3 and 16.4 $\mu$m/s, respectively, whereas the average values for 12 lymphatic

| Table 1. Comparison of $V_{\text{max}}$ for rat mesenteric lymphatic vessels, small arteries, and small veins |
|---------------------------------|-----------------|-----------------|-----------------|
| **Lymphatic Vessel**            | **Plateau**     | **Small Artery** | **Small Vein**  |
| Number of vessels               | 12              | 12              | 3               |
| Diameter at $F_{\text{pass, mN}}$ | $218 ± 8^*$     | $216 ± 7^*$     | $243 ± 8^*$     |
| $F_{\text{max, mN}}$            | 0.3             | 0.3             | 0.3             |
| $V_{\text{max, mN}}$            | $2.1 ± 0.1^*$   | $1.3 ± 0.1^+$   | $17.6 ± 6.8$    |
| In $\mu$m/s                     | $103.9 ± 7.1^*$ | $57.1 ± 5.7$    | $45.4 ± 2.5$    |
| In $L/s$                        | $0.48 ± 0.04^*$ | $0.26 ± 0.03^+$ | $0.19 ± 0.01$   |

Data are means ± SE. $V_{\text{max}}$, maximum unloaded shortening velocity; $F_{\text{pass, mN}}$, passive force; $F_{\text{max, mN}}$, maximal force; $L/s$, lengths per second. $^a$Diameter as measured in a wire myograph; all vessels were 2 mm in axial length. $^b$Vein measurements were performed before compensation methods were used and thus may be slightly overestimated. $^*P < 0.05$ compared with the small artery; $^+P < 0.05$ compared with the lymphatic vessel at peak.
vessels and 12 small arteries were 103.9 and 45.4 μm/s, respectively. In addition, the shapes of the curves were subtly different for the two vessels. Shortening velocity slowed exponentially over time in both cases, but the first 3 s of isotonic shortening were very well fit by a single-exponential function; a similar observation was made by Uvelius and Hellstrand (70) for fitting force-velocity data from the portal vein. To account for differences in diameter between the two types of vessels and to facilitate comparison with other studies, \( V_{\text{max}} \) values (in μm/s) were normalized in two ways: 1) to the passive vessel diameter at the optimal preload and 2) to actual cell length as derived from confocal imaging. The normalized data were then expressed in units of lengths per second (L/s) using both methods; however, in the text descriptions and comparisons to follow, we use normalization to passive diameter unless specifically stated. \( V_{\text{max}} \) for lymphatic vessels was 0.48 L/s, and \( V_{\text{max}} \) for small arteries was 0.19 L/s. \( V_{\text{max}} \) in the plateau phase of lymphatic vessel contraction was 0.26 L/s, a value closer to, but still significantly higher than, \( V_{\text{max}} \) for small arteries (Table 1).

**Slack test protocol.** Figure 5 shows examples of slack tests performed on a mesenteric lymphatic vessel (A) and a mesenteric small artery (B). In both Fig. 5, A and B, the time required to redevelop force (as denoted by arrows) was plotted as a function of the normalized diameter change (inset graphs). \( V_{\text{max}} \) was 0.36 L/s for the lymphatic vessel and 0.16 L/s for the small artery. As described in Methods, we used data only from vessels in which force measurements were not substantially contaminated by transducer noise or artifacts. For reasons that are not entirely clear, this method worked better for lymphatic vessels, where only small force changes (<2 mN) were required to produce vessel slack, as opposed to small arteries, where larger force changes (~12–18 mN) were required. Thus, reliable data were obtained from four of six lymphatic vessels but from only one of eight small arteries. The average value for LM shortening velocity was 0.37 ± 0.03 L/s using the slack test method versus 0.48 ± 0.04 L/s using the isotonic release method (Table 2). The value for small artery smooth muscle shortening velocity was 0.16 L/s using the slack test method compared with an average value of 0.19 ± 0.01 L/s using the isotonic release method (Table 2).

**Releases triggered during spontaneous contraction.** Collecting lymphatic vessels spontaneously contract under basal conditions, even in the absence of an exogenously applied agonist. To estimate initial shortening velocity during a spontaneous contraction, a quick release was initiated during systole of the spontaneous contraction cycle. The example shown in Fig. 6 illustrates the protocol. Preload was set to the optimal level (0.3 mN), and a steady pattern of spontaneous contractions was allowed to develop in APSS. This particular vessel consistently developed ~1.0 mN peak force with each contraction. At a predetermined fraction of peak total force (0.82 mN for this vessel), an isotonic quick release was triggered; the trigger threshold was based on that determined for the portal vein (35).
At that time, force was rapidly lowered to a predetermined value of the normalized afterload and was held at the lower force for 5 s. Shortening velocity was determined from the diameter change (i.e., piezo position) required to maintain the isotonic contraction. The initial shortening velocity was estimated from the data during the first 50–150 ms after the release. The vessel then entered diastole within 1–2 s, as indicated by an upward deflection in the diameter trace ("spontaneous relaxation" in Fig. 6A). Several spontaneous contractions were then recorded before another isotonic quick release at a different afterload. Three isotonic releases (not consecutive), along with the respective preceding isometric contraction, are shown in Fig. 6A. The traces are aligned in time relative to the point of release (at \( t = 19 \) s), so that the time shift between the peaks of the isometric contractions reflects the variability in spontaneous contraction frequency that occurred over the course of these measurements (additional variability in frequency was often introduced by the release protocol). As before, we attempted to perform 5–12 quick releases in this manner on each vessel, after which the values of shortening velocity were plotted as a function of normalized afterload and fit with Eq. 1. Figure 6B shows the best fit of the three data points from Fig. 6A along with three more data points obtained at other afterloads; \( V_{\text{max}} \) was estimated to be \( \approx 57 \) \( \mu \text{m/s} \) for this vessel. These measurements were performed on nine lymphatic vessels, but the results from two vessels were not included in the analysis due to the fact that they were very poorly fit by a hyperbolic function (the \( V_{\text{max}} \) values for the two vessels were 26 and 140 \( \mu \text{m/s} \)). The average value of \( V_{\text{max}} \) for seven spontaneously contracting lymphatic vessels was 63.9 \( \pm \) 4.6 \( \mu \text{m/s} \) (range: 45–80 \( \mu \text{m/s} \)), corresponding to 0.32 \( \pm \) 0.02 \( L/s \).

Isobaric protocols. The rate of change in diameter during systole in cannulated, pressurized lymphatic vessels potentially provides an estimate of LM shortening velocity under more physiological conditions. After an initial equilibration period at a pressure associated with optimal pumping (3 cmH2O), pressure was lowered to 0.5 or 1 cmH2O. These were the lowest pressures that allowed consistent contractions to occur in most vessels. \( -dD/dt \) was computed on a point-by-point basis from the acquired diameter tracking data (60 Hz). For each contraction cycle, the peak \( -dD/dt \) in systole was determined. To determine how \( -dD/dt \) might vary with afterload, \( P_{\text{out}} \) was slowly raised from 0.5 to 10 cmH2O over the course of \( \approx 7 \) min. This procedure typically produced a gradual reduction in amplitude and an increase in frequency (80). Figure 7A shows an example of data collected using this protocol. Contraction amplitude was initially \( \approx 115 \mu \text{m} \) at 1 cmH2O and progressively declined to \( \approx 20 \mu \text{m} \) at 12 cmH2O. \( -dD/dt \) was at its most negative value at 0.5 cmH2O (\( \approx 162 \mu \text{m/s} \), average of the first 3 contractions shown) and gradually became less negative as pressure increased to 12 cmH2O (\( \approx 34 \mu \text{m/s} \), average of 17 contractions). The peak \( -dD/dt \) typically occurred 30–50% of the way into systole, as indicated by the open symbols in the first three contraction cycles in Fig. 7A. The average values of peak \( -dD/dt \) were plotted as a function of pressure (\( P_{\text{in}} + P_{\text{out}} \) raised simultaneously) in Fig. 7B after normalization to the passive diameter at the respective preload (in units of \( L/s \)) and followed the same trend as illustrated in the example. The summary data were fit to the Hill equation to determine unloaded \( V_{\text{max}} \). These data, along with the other lymphatic vessel shortening velocity measurements, are shown in Table 2.
For comparison with data obtained in isotonic release protocols in which LM was activated with an exogenous agonist, we also tested the effect of partially activating the vessel with 3/1000 M SP on peak \( \frac{dD}{dt} \). This dose has been previously shown to enhance the frequency and amplitude of spontaneous contractions (18); higher doses depressed amplitude even to the point of producing sustained tonic contractions.

Progressive \( P_{\text{out}} \) elevation also produced a progressive decline in peak \( -\frac{dD}{dt} \), as illustrated by the ramp-wise \( P_{\text{out}} \) elevation protocol shown in Fig. 8A. These protocols were performed with diameter tracking at 30-Hz video rates, which largely accounts for lower peak \( -\frac{dD}{dt} \) values than the control data set than in Fig. 7. SP produced a reduction in \( \frac{dD}{dt} \) that was significantly lower than control at almost all \( P_{\text{out}} \) levels (Fig. 8B).

![Fig. 5. Slack test protocol. A: a mesenteric lymphatic vessel \([162 \, \mu m \text{ at passive force } (F_{\text{pass}})]\) was subjected to large, rapid releases to the slack diameter so that force dropped to zero. \( F_{\text{max}} \) ranged from 1.0 to 1.3 mN. The corresponding pairs of diameter and force traces are identified by 1–4; the diameter steps were identical for traces 1 and 2. Force traces are corrected for slight transducer drift. The time required to redevelop force (denoted by arrows) was plotted as a function of the normalized diameter change (inset). The slope of the line = \( V_{\text{max}} \) \([0.36 \text{ lengths } (L)/s]\). B: a mesenteric small artery \((340 \, \mu m \text{ at } F_{\text{pass}})\) was subjected to a similar protocol. Force traces are corrected for slight transducer drift. \( F_{\text{max}} \) ranged from 15.7 to 17.7 mN. The corresponding pairs of diameter and force traces are identified by 1–3; \( V_{\text{max}} \) = 0.16 \( L/s \). For both A and B, data from additional releases are included in the data analysis but the corresponding force recordings are omitted for clarity.](http://ajpheart.physiology.org/)

**Table 2. Comparison of \( V_{\text{max}} \) for rat mesenteric lymphatic vessels using different methods**

<table>
<thead>
<tr>
<th></th>
<th>Lymphatic Vessels</th>
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<tbody>
<tr>
<td></td>
<td>Peak</td>
</tr>
<tr>
<td>Number of vessels</td>
<td>12</td>
</tr>
<tr>
<td>Diameter at ( F_{\text{pass}} ), ( \mu m )</td>
<td>218 ± 8*</td>
</tr>
<tr>
<td>( F_{\text{pass}} ), mN</td>
<td>0.3</td>
</tr>
<tr>
<td>( F_{\text{max}} ), mN</td>
<td>2.1 ± 0.1*</td>
</tr>
<tr>
<td>( V_{\text{max}} ) ( \text{In } \mu m/s )</td>
<td>103.9 ± 7.1</td>
</tr>
<tr>
<td>( V_{\text{max}} ) ( \text{In } L/s )</td>
<td>0.48 ± 0.04*</td>
</tr>
<tr>
<td>( V_{\text{max}} ) ( \text{In } L/s )</td>
<td>1.31 ± 0.09</td>
</tr>
</tbody>
</table>

Data are means ± SE. Peak, plateau, and spontaneous values are isotonic quick release measurements. \(^{*}\)Diameter as measured in wire myograph; all vessels were 2 mm in axial length. \(^{1}\)\( V_{\text{max}} \) estimated from the rate of change in inner diameter change in systole with pressure = 0.5 cmH\(_2\)O. \(^{2}\)\( F_{\text{max}} \) expressed as the peak amplitude of spontaneous contraction. \(^{3}\)Determined at 3 cmH\(_2\)O. \(^{4}\)Normalized to passive diameter, except for the slack test. \(^{5}\)Normalized to average muscle cell length (79.9 ± 6.5 \( \mu m \), range: 66–107 \( \mu m \), \( n = 72 \)). \(^{*}\)\( P < 0.05 \) compared with the small artery; \(^{†}\)\( P < 0.05 \) compared with the lymphatic vessel at peak.
DISCUSSION

Our results represent the first comprehensive measurements of the force-velocity relationship for LM; the unusually high values of shortening velocity for LM suggest a unique phenotype that is intermediate between that of VSM and cardiac muscle. For the purpose of comparison with published values of $V_{\text{max}}$ in other muscle types, measurements were performed using four different experimental approaches, including after-loaded contractions (Figs. 2–4) and slack tests (Fig. 5) of LM after activation with an exogenous agonist, isotonic quick releases of spontaneously contracting isometric segments (Fig. 6), as well as estimates of shortening velocity in isobaric lymphatic vessels (Figs. 7 and 8). The first three methods yielded $V_{\text{max}}$ values for LM in the mid to high end (0.48 L/s) of those recorded for phasic smooth muscle, whereas the latter measurements yielded $V_{\text{max}}$ values (2.03–2.14 L/s) that overlap with the midrange of values obtained from cardiac and skeletal muscle. Similar to VSM, the $V_{\text{max}}$ of LM slowed with progressive activation of the muscle; much higher values were recorded under the most physiological conditions. The highest values for LM shortening velocity were obtained using isobaric preparations with basal levels of contractile machinery activation (i.e., in the absence of agonist stimulation). Shortening velocities for LM that are intermediate between those for smooth muscle and cardiac muscle are consistent with the functional properties that lymphatic vessels share with blood vessels and cardiac tissue as well as with our previous findings showing that LM expresses both smooth and cardiac muscle contractile protein isoforms (51). Importantly, rapid shortening of LM would permit robust, synchronized contractions of the lymphangion that would permit efficient pumping and a high ejection fraction (~80%). Below, we discuss the implications of our results, critique the methods, and frame our findings in a physiological context.

Comparison of $V_{\text{max}}$ for LM with $V_{\text{max}}$ for other muscle types. Given the relative paucity of data on LM mechanical properties, it is important to compare measurements of shortening velocity from LM with similar measurements from other types of muscle. The force-velocity relationship reflects the actomyosin cross-bridge cycling rate and is perhaps the best functional indicator of the underlying molecular and chemomechanical transduction processes of the contractile machinery (50). To facilitate comparisons, a graph of representative $V_{\text{max}}$ data from the literature is shown in Fig. 9. $V_{\text{max}}$ values for the five major muscle types range from 0.1–0.3 L/s for tonic smooth muscle (e.g., small arteries), 0.1–0.8 L/s for phasic smooth muscle, 0.6–4.1 L/s for skeletal muscle, to 0.7–3.4 L/s for cardiac muscle. The scatter in the data within the groups shown in Fig. 9 reflects a number of differences, including inevitable variations related to species and tissues (e.g., blood vessels from different organs and different subtypes of skeletal muscle fibers) as well as differences related to the methods of $V_{\text{max}}$ determination. Within each type of muscle, the values of $V_{\text{max}}$ for whole tissue (e.g., vessels), isolated fibers, and single cells are stratified, with single cells consistently representing the higher values within each group; this effect has been noted previously and attributed to a greater content of connective tissue elements in whole tissue (50, 77).
Given these caveats, $V_{\text{max}}$ values for LM span the upper range of $V_{\text{max}}$ values recorded for phasic smooth muscle to the upper range of slow twitch skeletal muscle and midrange of cardiac muscle. The difference between LM and other smooth muscle types is even more pronounced (~2-fold higher average) if the portal vein is excluded from the list of “representative” phasic vascular smooth muscles (see explanation below). Although LM is traditionally considered to be smooth muscle (3, 27, 43, 45), our measurements suggest that the $V_{\text{max}}$ of activated LM is significantly higher (~2.2-fold) than that of adjacent small arteries, recorded using the same method, even though the maximal active force developed by the small arteries is approximately eightfold greater. The $V_{\text{max}}$ value that we determined for small mesenteric arteries (0.19 L/s) is intermediate between the value of 0.26 L/s reported by Wang and Stephens (76) and the value of 0.13 L/s reported by Mulvany (49) for the same species and vascular bed (rat mesentery). Our measurement of $V_{\text{max}}$ for mesenteric veins (0.03 L/s) is consistent with previous data (0.017 L/s) recorded in tonic venous smooth muscle (36).

$V_{\text{max}}$ declined with time after activation of LM. When measured at the peak of lymphatic vessel isometric contraction, ~60 s after activation with SP, $V_{\text{max}}$ was nearly twofold higher than the value determined at the force plateau, 3–5 min after activation (0.48 vs. 0.26 L/s, respectively), suggesting that it fell as LM became fully activated. Isobaric protocols confirmed that even partial activation with a relatively low dose of SP led to substantial reduction in contraction velocity at any given level of afterload (Fig. 8). A similar pattern was observed by Brozovich and Morgan (11) for single smooth muscle cells isolated from the aorta, where $V_{\text{max}}$ was 0.064 L/s at the peak of force development induced by phenylephrine but subsequently declined by 60% (to 0.02 L/s) during the force plateau.

Thus, the decline in $V_{\text{max}}$ with time after activation of LM is similar to that recorded in VSM (76).

Our measurements of $V_{\text{max}}$ for LM range from 0.26 (plateau) to 0.48 (peak) L/s using isotonic quick release protocols during activation of the muscle with an exogenous agonist (Table 2). The only other report of $V_{\text{max}}$ of LM is a single measurement of 0.6 L/s from agonist-activated circular strips of bovine mesenteric vessels recorded by Lobov (Fig. 5 in Ref. 44), which is well within the range of velocities that we report (Table 2). We used the slack test protocol as an alternative method of determining $V_{\text{max}}$. Slack test protocols gave an estimate of $V_{\text{max}}$ at the peak of isometric contraction that was slightly lower than the average $V_{\text{max}}$ from isotonic shortening protocols (0.37 vs. 0.48 L/s; Table 2). However, the group of lymphatic vessels used for slack tests developed significantly less $F_{\text{max}}$ than the group of vessels used for isotonic shortening protocols (1.2 vs. 2.1 mN, respectively; Table 2).

Although the unloaded isotonic shortening velocity of a maximally activated vessel provides a good reference point for the comparison of LM with other types of muscle studied under similar conditions, this condition is far from physiological because it is difficult to imagine if, or when, LM becomes activated to this degree in vivo. Previously, we estimated that the (basal) phasic activity of rat mesenteric lymphatic vessels is associated with ~15% activation of the LM (79). Thus, we also attempted to measure unloaded shortening velocity during the systolic phase of a spontaneous contraction. We expected to record higher values, based on the precedent that $V_{\text{max}}$ for the rat portal vein (considered phasic smooth muscle) was 0.74 L/s during spontaneous contractions compared with 0.33 L/s after activation with an exogenous agonist (35). Surprisingly, our measurement of $V_{\text{max}}$ during spontaneous lymphatic vessel contractions was lower than during activated
afterloaded contractions (0.32 vs. 0.48 L/s). However, in previous portal vein protocols, spontaneous activity was induced using solutions containing 25 mM K⁺ and 20 mM Ca²⁺ (35), which are known to increase both Fmax and Vmax (41).

Importantly, our measurements of shortening velocity in lymphatic vessels under isobaric conditions yield Vmax estimates that are much higher than those obtained under isometric/isotonic conditions. The significance of this measurement is that it more closely reflects the actual shortening velocity of LM under actual working conditions. Benoit et al. (5) reported an average peak −dD/dt equivalent to 2.0 L/s for lymphatic vessels in the rat mesentery in vivo. That value is similar to our measurement in isolated lymphatic vessels taken from the same tissue (2.14 L/s), although our highest values were recorded at 0.5 cmH2O and declined substantially at the equivalent in vivo pressure, ~5 cmH2O, reported by Benoit et al. (5). Our measurements of −dD/dt at multiple pressures reveal that the normalized peak −dD/dt versus intraluminal pressure relationship (Figs. 7 and 8B) closely resembles the velocity versus afterload relationship obtained with the isotonic quick release protocol (Figs. 2 and 6). Furthermore, at each pressure, partial activation of the lymphatic muscle by SP produced a significantly slowing of shortening velocity (Fig. 8). Collectively, these results suggest that the requirement for agonist activation in the isometric/isotonic LM protocols substantially slows contraction velocity and leads to substantial underestimation of the shortening velocity that normally can be achieved under in vivo conditions.

Is LM a unique muscle phenotype? The finding that the Vmax for LM is intermediate between that of VSM and cardiac muscle is consistent with a number of the unique functional properties of lymphatic vessels. As previously mentioned, LM shares several common electrophysiological characteristics with cardiac myocytes. Like ventricular muscle, lymphatic vessels exhibit synchronized spontaneous contractions (within a lymphangion) that are initiated by a pacemaker (2, 45) and produce rapid activation of LM (75) during each contraction cycle. The contractile unit of a lymphatic vessel, the lymphangion, is surrounded at each end by a one-way valve, thereby imparting properties of filling and ejection that are in many ways similar to those of the cardiac chambers. These functional similarities to cardiac muscle presumably depend on a correspondingly high shortening velocity or else the ejection fraction of the lymphangion would be extremely low at high pressures, which are known to increase both Fmax and Vmax (41).
contraction rates, thus limiting the range of intrinsic regulation by changes in preload and afterload.

Among the various types of smooth muscle, the $V_{\text{max}}$ of LM is perhaps closest to that of the portal vein (Fig. 9). There are additional similarities, mechanical, physiological and electrophysiological, between the portal vein and LM as well. For example, both types of vessels exhibit spontaneous contractions composed of relatively short periods of systole interspersed with long diastolic intervals (40, 80), in contrast to the periodic, sinusoidal vasomotion, composed of multiple frequency components (6, 13, 48), that characterizes some arteriolar smooth muscle. With perhaps a single exception among VSM [bat wing venules (15, 21)], only the portal vein exhibits large-amplitude, spontaneous contractions [a few other veins show spontaneous contractions (15, 38, 78) but those are small in amplitude]. Both portal vein muscle and LM also exhibit rate-sensitive responses to rapid changes in pressure/stretch (17, 40). With respect to their electrophysiology, both the portal vein and LM express ion channels that are more characteristic of cardiac myocytes than typical VSM, including a TTX-sensitive fast Na$^+$ current (39, 60) and a hyperpolarization-activated funny current (32, 45); these channels are thought to underlie or contribute to spontaneous pacemaking activity. Mesenteric lymphatics must normally move lymph against a small pressure gradient to transport it back to the vena cava. While the pressure gradient for blood flow between the portal vein and the inferior vena cava is small (3–4 mmHg), the ways in which these atypical mechanical and electrophysiological characteristics of the portal vein contribute to the regulation of the portal circulation are unknown: do the high-velocity contractions of the longitudinal layer actually result in propulsive movement of portal flow or even a substantial change in portal vascular pressure or resistance? For these reasons, we contend that portal vein is a unique, rather than representative, example of VSM. Thus, the observation that the $V_{\text{max}}$ values of the portal vein and LM are somewhat similar does not negate our conclusion that the shortening velocity of LM is intermediate between that of cardiac muscle and (typical) VSM. $V_{\text{max}}$ values measured for LM are in the midrange of those reported for cardiac muscle, which is consistent with the molecular phenotype of the contractile proteins previously described for LM (51). LM preferentially expresses the smooth muscle MHC B isoform, which confers a higher ATPase activity that may explain in part a relatively high shortening velocity (58). Can the relatively high shortening velocity of LM be explained completely by the expression of a faster MHC isoform? This question cannot be answered at the present time, but it seems unlikely since veins expressing smooth muscle MHC B have a much lower $V_{\text{max}}$ than either LM or striated muscle (58). Like cardiac muscle, but unlike smooth muscle, LM expresses cardiac isoforms of actin, $\beta$-MHC, and troponin C, along with striated $\alpha$-tropomyosin (51, 52, 73); nevertheless, the contractile filaments of LM, while highly organized, are not arranged in a distinct striated pattern (51). Although the functional significance of these cardiac protein isoforms in LM remains to be defined, we propose that they are critical for conferring a relatively high shortening velocity. The resolution of these issues likely will require manipulation of the expression of the cardiac-like contractile protein isoforms in LM (29) in combination with the approaches to quantify LM function developed in the present study.

Conclusions. Our results represent the first comprehensive measurements of shortening velocity for LM. We find that $V_{\text{max}}$ for LM is higher than that of phasic smooth muscle and close to that of cardiac muscle, which is consistent with both the molecular expression profile of contractile protein isoforms in LM and the functional characteristics that lymphatic vessels share with both blood vessels and the heart. Our findings challenge the assumption that LM is classical VSM (3, 27, 43, 45). Because current therapies are aimed solely at improving passive drainage in the lymphatic vasculature using extravascular compression in the millions of annual cases of primary or secondary lymphedema that occur in the United States (57), a more extensive understanding of the molecular properties of LM will potentially allow selective therapeutic targeting of LM to improve lymphatic pump function in many of these cases.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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