Is nitric oxide important for the diastolic phase of the lymphatic contraction/relaxation cycle?

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Phasic contractions of collecting lymphatic vessels aid in the centripetal propulsion of lymph. The numerical model published in PNAS by Kunert et al. (1) describes how two "complementary feedback loops" involving Ca^{2+} and nitric oxide (NO) interact to drive the phasic contraction/relaxation cycle of lymphatic smooth muscle. The cycle is proposed to be initiated by a stretch-induced rise in lymphatic muscle Ca^{2+}, triggering a contraction (systole) that propels lymph through unidirectional valves. Subsequent elevation of shear stress in the narrow valve opening produces a burst of endothelium-derived NO that initiates and/or facilitates relaxation (diastole).

The model is based primarily on observations made in a single, in vivo study of mouse popliteal lymphatics, where chronic inhibition or ablation of endothelial NOS (eNOS) leads to an apparent loss of lymphatic tone, blunted contraction strength, and increase in contraction frequency (2). Unfortunately, the authors' interpretation of that data (1, 2) runs counter to the well-documented effects of NO in both blood and lymphatic vessels, whereby eNOS knockout/inhibition enhances basal tone (3) and enhances (4) or has no effect on (3) lymphatic contraction amplitude. Other explanations for the apparent effects of eNOS inhibition on the popliteal network in vivo are ignored, including changes in systemic arterial pressure, sympathetic tone, capillary filtration, and/or intraluminal pressure in both initial and collecting lymphatic vessels.

The model predicts that NO is critical for driving oscillations in lymphatic vessel diameter and that "without NO production, phasic contractions are inhibited" (1). However, when mouse popliteal lymphatics are studied ex vivo, where pressure is controlled and flow is determined solely by phasic contractions, genetic/pharmacologic blockade of eNOS does not impair diastolic relaxation, but instead leads to a slight increase in contraction amplitude as well as increases in ejection fraction and calculated pump flow (figures 3 *C–F*, 5 *C–F*, and S4 *C–F* in ref. 4).

The model also predicts that phasic NO production enhances the pressure range for effective lymphatic pumping (1). However, that idea has been tested and refuted: mouse popliteal vessels in which eNOS is inhibited pump with comparable effectiveness over the same range of controlled pressures (0.5–10 cmH_2O) as control vessels (4); similar results are reported for rat and bovine lymphatics (3, 5) studied ex vivo. Unsurprisingly, the model recapitulates primarily the in vivo behavior upon which it is based (e.g., figure 2 in ref. 2).

The model does offer an intriguing prediction: that NO alters the normal direction of contraction wave propagation—which can and should be tested experimentally under conditions of controlled pressure and flow.

1 Kunert C, Baish JW, Liao S, Padera TP, Munn LL (2015) Mechanobiological oscillators control lymph flow. *Proc Natl Acad Sci USA* 112(35):10938–10943.

2 Liao S, et al. (2011) Impaired lymphatic contraction associated with immunosuppression. *Proc Natl Acad Sci USA* 108(46): 18784–18789.

3 Nagai T, Bridenbaugh EA, Gashev AA (2011) Aging-associated alterations in contractility of rat mesenteric lymphatic vessels. *Microcirculation* 18(6):463–473.

4 Scallan JP, Davis MJ (2013) Genetic removal of basal nitric oxide enhances contractile activity in isolated murine collecting lymphatic vessels. *J Physiol* 591(Pt 8):2139–2156.

5 Hanley CA, Elias RM, Johnston MG (1992) Is endothelium necessary for transmural pressure-induced contractions of bovine truncal lymphatics? *Microvasc Res* 43(2):134–146.

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