SUPPLEMENTAL METHODS

Acoustic Field Testing

Acoustic pressures generated by the X5-1 transducer were measured in a degassed water tank using a calibrated a capsule hydrophone (HGL-0200/AH-2010, ONDA Corp., Sunnyvale, CA). The 3-dimensional pressure fields produced from the custom pulsing sequence were mapped by translating the hydrophone using a 3-axis motorized positioning system. Hydrophone signals were captured in MATLAB at each position within the volume of interest and analyzed offline. Volumes of interest were sampled in step sizes of 2 mm axially, 0.5 mm laterally, and 0.5 mm elevationally. In order to accurately represent the spatial pressure distribution with the high azimuthal and elevational transmit density, the 3-dimensional pressure distributions measured with limited spatial sampling above for sparse transmit designs were combined with simulated X5-1 transmit beam profiles at high spatial sampling and high line density using the Field-II Simulation Program (Jensen JA; Med Biol Eng Coput;1996;34;1:351-352 [Suppl. 1]). This approach was previously validated for the X5-1 transducer (Mason O, et al, JACC CVI 2020;13:641).

In vitro Erythrocyte ATP and NO Release

Release of the vasoactive mediators ATP and NO from RBCs during cavitation was assessed. Dialysis cassettes (Thermo Fisher) were filled with 3 mL of heparinized blood from normal human volunteers (within 1 hour of collection), to which luciferase (10 μg) (Thermo Fisher, Waltham, MA), D-luciferin (1.0 mg) (Thermo Fisher), and lipid-stabilized decafluorobutane MBs (5×10^8) were added. The transducer was positioned over the cassette membrane using gel standoff. Ultrasound settings were similar to those generated in mice except the mechanical index was set to 0.9 to compensate for muscle attenuation, which was estimated at 1 db/cm/MHz. Ten US frames in 2-D mode were generated over 10 seconds while scanning across

the membrane. Cassettes (n=7 experiments) were immediately placed in an optical scanner (IVIS Spectrum, Caliper Life Sciences) together with a paired, non-US-exposed control cassette; and ATP-dependent light generation was measured and expressed as photons/s/cm^2.

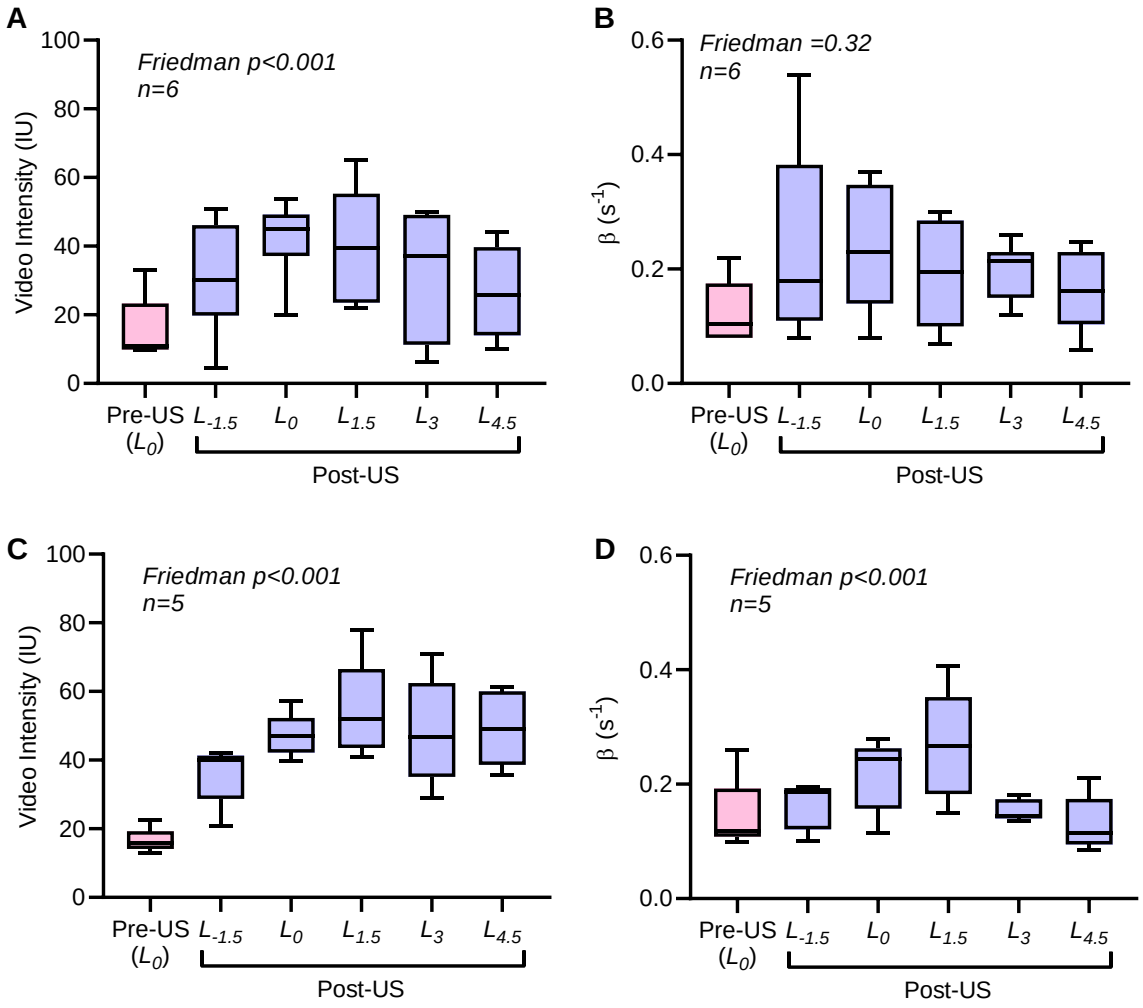
For NO release from RBCs, 4.7 mL aliquots of whole blood from normal human volunteers (within 1 hour of collection) and lipid-stabilized decafluorobutane MBs (5×10^8) were placed in acoustically transparent bulbs composed of low-density polyethylene (Thermo Fisher, Hampton, NH) for US exposure. For each experiment (n=3), calibrated amperometric electrochemical sensing elements (amino-IV; Innovative Instruments, Tampa, FL) interfaced with an A/D converter (inNO-T-II; Innovative Instruments) that detect only free extracellular NO were placed in two separate blood-containing bulbs. Continuous measurements of NO concentration were recorded during exposure of one of the tubes to US at a pulsing interval of 2 s for 1 minute.

Ex Vivo Arterial Dilation

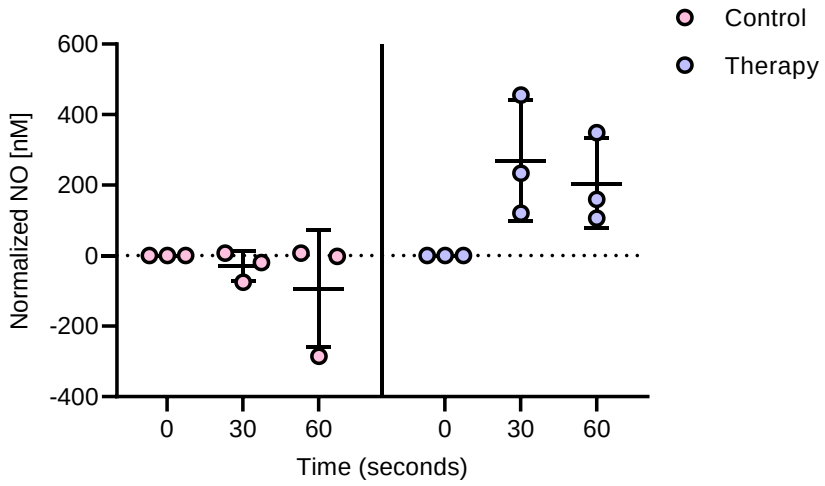
To determine the importance of RBCs as a source for vasodilator substances during conducted arterial dilation, *ex vivo* arterial dilation studies were performed. First-order mesenteric arteries (0.5-1.0 mm diameter) from Sprague Dawley rats (n=8) were excised from the omentum. The artery was cannulated at each end with glass pipettes, side branches were ligated, and the vessel was placed in an isothermic water bath and perfused at 0.6 ml/min. The following infusates and conditions were studied: (a) isothermic Krebs-Henseleit buffer, (b) buffer with MBs, (c) buffer with MBs and US, (d) isothermic Krebs-Henseleit buffer with RBCs (6:4 v:v), (e) buffer with RBCs and MBs, or (f) buffer with RBCs, MBs and US. For conditions with MBs, lipid-shelled decafluorobutane microbubbles were added to a final concentration of 5×10^7 mL^{-1}. For conditions with US, methods identical to those described in murine hindlimb protocols were applied using a PI of 5 seconds and positioning of the probe transaxial to the artery. Arterial diameter after two

minutes for each condition was measured by 2D US at 38 MHz using a linear-array transducer (Vevo 2100, Visualsonics Inc., Toronto, Canada) positioned co-axially with the artery. Data were expressed as resistance index derived from the Hagen-Poiseuille equation as $(\text{radius})^{-4}$ and normalized to control conditions of saline infusion alone.

SUPPLEMENTAL FIGURES



Supplemental Figure 1. Influence of 2-D versus 3-D cavitation on forearm perfusion parameters of microvascular blood volume (**A, C**) and microvascular flux rate (β) (**B, D**) from rhesus macaque cavitation experiments. Data are shown for 2-D (**A, B**) and 3-D (**C, D**) cavitation experiments using planes described in Figure 2 Legend.



Supplemental Figure 2. Raw data showing NO concentration normalized to the concentration at the time of US initiation (*Time 0)* during 1 minute of exposure to cavitation. Data from control experiments were performed by simultaneous measurement of blood not undergoing cavitation.