SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Endothelium-dependent dilation. Endothelium-dependent dilation was assessed as brachial artery flow-mediated dilation (FMD_{BA}) using an ultrasound machine. The right arm was adducted at heart level and the brachial artery was located 3-6 cm above the antecubital crease. The ultrasound probe was then clamped to improve stability and avoid movement. After obtaining baseline diameters, reactive hyperemia was produced by inflating a blood pressure cuff placed on the upper forearm to 250 mmHg for 5 minutes. After 5 minutes the cuff was rapidly deflated. Brachial artery diameter and blood velocity were measure during the first 2 minutes post-occlusion to obtain the peak dilatory response. FMD_{BA} was calculated as the percentage change in brachial artery diameter in response to the forearm hyperemic stimulus.

Reactive oxygen species-associated suppression of endothelium-dependent dilation. The infusion of ascorbic acid (vitamin C) at supra-physiological concentrations temporarily reduces superoxide/reactive oxygen species (ROS) bioactivity, thus removing the “tonic” influence of excessive ROS. The acute increase (or lack thereof) in “function” (FMD_{BA}) is a measure of the tonic influence of the ROS under normal conditions. If function improves from baseline control levels, then there is tonic suppression by ROS; if function does not change, then the interpretation is that there is little or no tonic suppression or function by ROS under normal conditions.

To determine whether superoxide plays a mechanistic roll in blood pressure-associated vascular endothelial dysfunction, FMD_{BA} was measured before (saline infusion) and after intravenous administration of ascorbic acid (American Regent Laboratories Inc., Shirley, NY). A priming bolus of 0.06 $g \cdot kg^{-1}$ fat-free mass dissolved in 100 ml of saline was infused in an antecubital vein at 5 $ml \cdot min^{-1}$ for 20 minutes. This was followed by a maintenance drip infusion

of 0.02 $g \cdot kg^{-1}$ fat-free mass dissolved in 30 ml of saline administered over 60 minutes at 0.5 $ml \cdot min^{-1}$; FMD_{BA} was measured during the maintenance drip infusion.

Endothelial cell protein expression via endovascular biopsy. Endothelial cells were collected from an antecubital vein with sterile J-wires briefly advanced (~4 cm beyond the tip of the catheter) and retracted through an 18-gauge catheter, and cells were recovered by washing and centrifugation. Cells were fixed with 3.7% formaldehyde and plated on poly-L-lysine coated slides (Sigma Chemical, St Louis, Mo). Cells were frozen at -70°C until analysis; thus, endothelial cells were collected at the same time as functional (i.e., blood pressure, FMD_{BA}) analyses were performed for each subject. Subjects were not recalled for collection of endothelial cells for the present analysis.

For immunofluorescence staining, cells were rehydrated with PBS and rendered permeable with 0.1% Triton X-100 and nonspecific binding sites were blocked with 5% donkey serum (Jackson Immunoresearch, West Grove, PA, USA). Cells were incubated with monoclonal antibodies for nitrotyrosine (Abcam, Cambridge, UK), NADPH oxidase subunit $p47^{phox}$ (Abcam, Cambridge, UK), MnSOD (Stressgen Biotechnologies, San Diego, CA), and NFκB p65 (Novus, Littleton, CO; Santa Cruz, Dallas, TX), as well as with an AlexaFluor fluorescent secondary antibody (Invitrogen, Carlsbad, CA). Cells were incubated with von Willebrand factor to identify endothelial cells, and with DAPI to confirm nuclear integrity. Cells were stored at 4°C overnight.

Slides were viewed using a fluorescence microscope (Eclipse 600; Nikon, Melville, NY). Fluorescence intensity of the primary antibody-dependent AlexaFluor staining (i.e., average pixel intensity) was analyzed using Metamorph Software (Universal Imaging, Downingtown, PA). Eight slides and two control cultured human umbilical vein endothelial cell (HUVEC; passage 6-9 processed identically to the sample cells) slides were selected for each staining batch. Values are reported as a ratio of sample endothelial cells to HUVEC average pixel

fluorescence intensity to reduce batch-to-batch variability. Reporting ratios of vascular endothelial cell protein expression to HUVECs is standard procedure in our laboratory[12,66–68] and others[69–72].