Supplemental Methods

Echocardiographic Measurement

Transthoracic echocardiograms were performed by a dedicated sonographer team at an Intersocietal Accreditation Commission laboratory. Two-dimensional images were acquired using Vivid 7 or E9 machines (GE Healthcare, Milwaukee, WI) and digitally archived. Echocardiograms were quantitated at the University of Pennsylvania Center for Quantitative Echocardiography (Philadelphia, PA) and performed using the TomTec Imaging Systems platform (Unterschleissheim, Germany). Left ventricular end-diastolic (LVEDV) and end-systolic volumes (LVESV) were calculated using Simpson's method of discs, and used to derive left ventricular ejection fraction (LVEF) with the standard formula (LVEDV – LVESV)/LVEDV. Echocardiography quantitation was performed in a blinded manner independent from clinical or biomarker data.

Measurements of Biomarkers

Plasma samples were collected in EDTA tubes, processed at 3353 RPM for 20 minutes at room temperature, aliquoted, and stored at -80°C until the time of assay. Measurement of arginine, ornithine, citrulline, ADMA, SDMA, and MMA were performed by stable-isotope-dilution HPLC with online electrospray ionization tandem mass spectrometry. All the isotope labeled internal standards were purchased from Cambridge Isotope Laboratories, Inc (Tewksbury, MA). Stable isotope labeled internal standards were added to plasma samples at time of protein precipitation by adding four volumes of methanol containing 13C6-Arg, d6-Orn, d4-Cit, d7-ADMA (each 10 uM final). Samples were chilled in ice water bath, protein precipitated and the

supernatant recovered after centrifugation at 13000 rpm for 10 minutes. For each LC/MS/MS assay, 10µL of supernatant was injected onto an HPLC column and the levels of arginine and related metabolites were quantified by LC/ESI/MS/MS analysis using an API 365 triple quadrupole mass spectrometer (Applied Biosystems, Foster, CA) with Ionics EP 10+ upgrade (Concord, Ontario, CA) and Cohesive Technologies Aria LX Series HPLC multiplexing system (Franklin, MA). Amino acids were separated with a 250 x 4.6mm Rexchrom S5-100-P phenyl column (Regis Chemical, Morton Grove, IL, USA) with a 1mm x 15mm Optimize technologies Opti-Guard Phenyl guard column (Optimize Technologies, Oregon City, OR, USA). Formic acid (0.1%, v/v) and ammonium formate (10mM) in water (solvent A) and formic acid (0.1%, v/v) and ammonium formate (10mM) in methanol (solvent B) were used as solvents. The gradient consisted of: 1) 100% solvent A at 800µL/min held for 0.5min after injection, 2) linear gradient run to 50% solvent B (50% solvent A) over 3min, 3) held at 50% B for 3.5min at a flow rate of 800µL/min, 4) flow rate increased to 1000µL/min with solvent composition changed to 100% B in linear fashion over 1min, 5) linear gradient run to 100% A at 1000µL/min over 0.5min, 6) held at this composition and flow rate for 6min. Overall an 8.5min data window was started at 3.5min after injection, and mass spectrometric analyses was performed using electrospray ionization tandem mass spectrometry in the positive ion mode with multiple reaction monitoring. Cone potentials and collision energy were optimized for each analyte, with each demonstrating quantitative recovery, linearity over multiple orders of magnitude in the concentration range, and intra- and inter-assay coefficients of variation <10%.