Supplemental Information

Supplemental Materials and Methods

Measurement of plasma nitric oxides and aortic cGMP

To measure total nitrite plus nitrate, plasma samples were thawed and deproteinized by incubation in 95% ethanol at 4°C for 30 minutes, followed by centrifugation for 10 minutes at 13,000 *g*. NO metabolites were measured using the NO fluorometric assay kit (BioVision Inc., Milpitas, CA, USA) according to the manufacturer's protocol. Briefly, 2 μl plasma was used to assay NO (measured as the concentration of nitrite and nitrate as an index of NO levels) by 2, 3-diaminonaphthalene (DAN) reaction and fluorescence was determined at 360 nm excitation and at 450 nm emission using a Victor3 1420 Multilabel Counter (Perkin Elmer, MA, USA). Nitrate reductase was used to convert nitrate in nitrite. Nitrite concentrations (NO levels) were calculated from a standard curve derived from sodium nitrite (provided with the kit).

Aortic cGMP level was determined with the cGMP direct immunoassay kit (Biovision Inc.). In brief, aortic tissues were homogenized with 0.1 mol/L HCl at 4°C. Homogenates were centrifuged at 16,000 *g* for 15 minutes at 4°C. The resulting tissue supernatant (100 μl) was neutralized with neutralizing buffer (50 μl) and acetylated at room temperature for 10 minutes, and assay buffer (845 μl) was added to each sample. Standard cGMP provided by the manufacturer was serially diluted with 0.1 N HCl to concentrations from 2.5 to 0.039 pmol/L/50 μl and then neutralized and acetylated as described above to generate a standard curve. Acetylated standard (50 μl) or test samples (50 μl) were added to a protein G-coated assay plate, assay buffer (10 μl) containing anti-cGMP polyclonal antibody was added to each

well (except for the 0-pmol/L standard), and the mixtures were incubated for 1 hour at room temperature with agitation. Diluted cGMP-HRP (10 µl) was then added to each well, and the mixtures were incubated for a second time for 1 hour at room temperature with agitation. Wells were washed five times with assay buffer (200 µl) before the addition of HRP developer (100 µl). Reaction mixtures were finally incubated for 1 hour at room temperature with agitation, and reactions were stopped by the addition of 1 N HCl (100 µl) and read immediately at 450 nm, using a VERSAmax (Tunable) microplate reader (Molecular Devices, CA, USA). The cGMP levels were calculated using the cGMP standard curve after subtraction of the background reading (0-pmol/L cGMP well).

Supplemental Figure Legends

Supplemental Figure 1. βL increases both plasma NO level and aortic cGMP content. (A and B) SHR received either normal chow or a βL-supplemented diet (0.12% *wt/wt*) for 6 weeks. **(A)** Plasma was collected from each rat and NO levels were measured. **(B)** Thoracic aortas were removed and aortic cGMP levels were evaluated. Control group, n=5; βL group, n=6. Grouped quantitative data are presented as mean ± SEM. The βL group was compared with the control group using Student's t-test; *, $P<0.05$.

Supplemental Figure 1.