SUPPLEMENTAL MATERIAL

Supplemental Methods.

Studies of Cerebral Arteriolar Function. Methods used to measure responses of cerebral (pial) arterioles (in vivo) have been described previously.[1,2] Briefly, a cranial window was made over the parietal cortex and a segment of a cerebral arteriole was exposed for measurement of vascular diameter. All drugs were applied topically over the cranial window. Diameter of a single arteriole per animal was measured under control conditions and during topical application of drugs. Baseline cerebral arteriolar diameter was similar in all groups of mice and averaged 31±1 microns in diameter. Changes in diameter were measured in response to the endothelium-dependent dilator, acetylcholine (1 and 10 μmol/L) and to the endothelium-independent dilator nitroprusside (0.1 and 1 μmol/L). In some experiments, responses to acetylcholine and nitroprusside were determined and then repeated in the presence of vehicle (saline) or Tempol (1 mM). Arterial pressure under anesthesia was similar in all groups and averaged ~ 70 mmHg. Arterial blood gasses were measured and averaged pCO_2, 32±1mmHg; pO_2, 160±5mmHg; and pH, 7.32±0.01 (Mean±SE). At the conclusion of each experiment, samples of arterial blood were saved for determinations of blood glucose (fasting) using an Accu-Chek Advantage glucometer (Roche; Indianapolis, IN).

Studies of Carotid Artery Function. Carotid artery responses (in vitro) were examined in isolated organ chambers as described previously.[3,4] Following a 45-minute equilibration period, vessels were precontracted (50-60% of maximum) with the thromboxane analogue, 9,11-dideoxy-11a, 9a-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619). After reaching a stable contraction plateau, concentration-response curves were generated to acetylcholine (0.01 to 100 μmol/L) and nitroprusside (0.03 to 30 μmol/L). In some experiments, carotid

arteries were incubated with either vehicle or 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (Tempol, 1 mM; a superoxide scavenger).

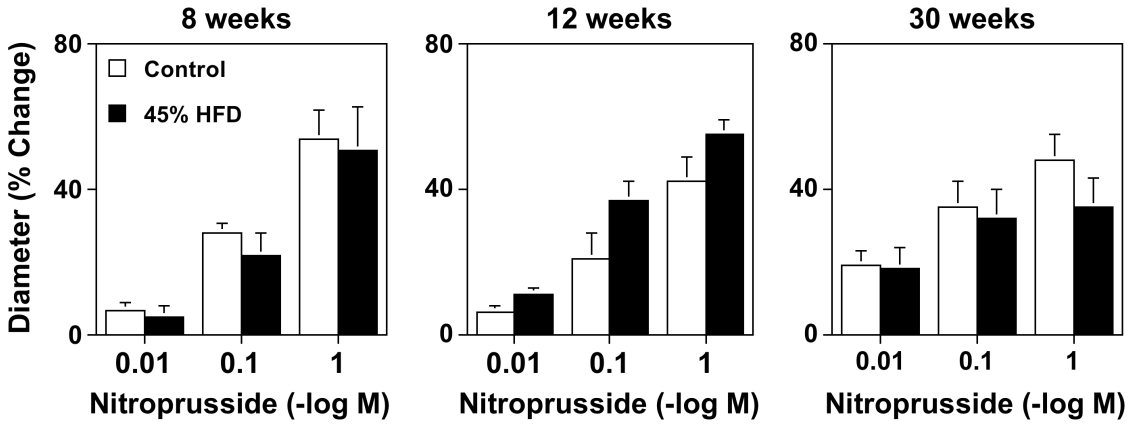
Glucose Tolerance Test. Glucose tolerance tests were performed in wild-type and *Nox2*$^{-/-}$ under baseline conditions (ie, before placement on a control or HFD) and in wild-type and *Nox2*$^{-/-}$ mice fed either a control or HFD for 12 wks as described previously.[5] Briefly, mice were fasted overnight (16 hrs). Baseline glucose levels (fasting) were measured in each mouse followed by a bolus intraperitoneal glucose (1 g/kg) injection. Glucose levels were then determined at 15, 30, 60, 90 and 120 min post-glucose injection.

Quantitative real-time RT-PCR. Gene expression was analyzed in wild-type mice fed either a control or HFD for 12 wks using quantitative real-time RT-PCR as previously described.[6] RNA from whole brain was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and purified using Mini RNeasy kit (Qiagen, Valencia, CA). cDNA was obtained by reverse transcription and gene expression for endothelial nitric oxide synthase and CD36 were evaluated using SYBR-green dye chemistry on a Bio-Rad CFX96 platform.

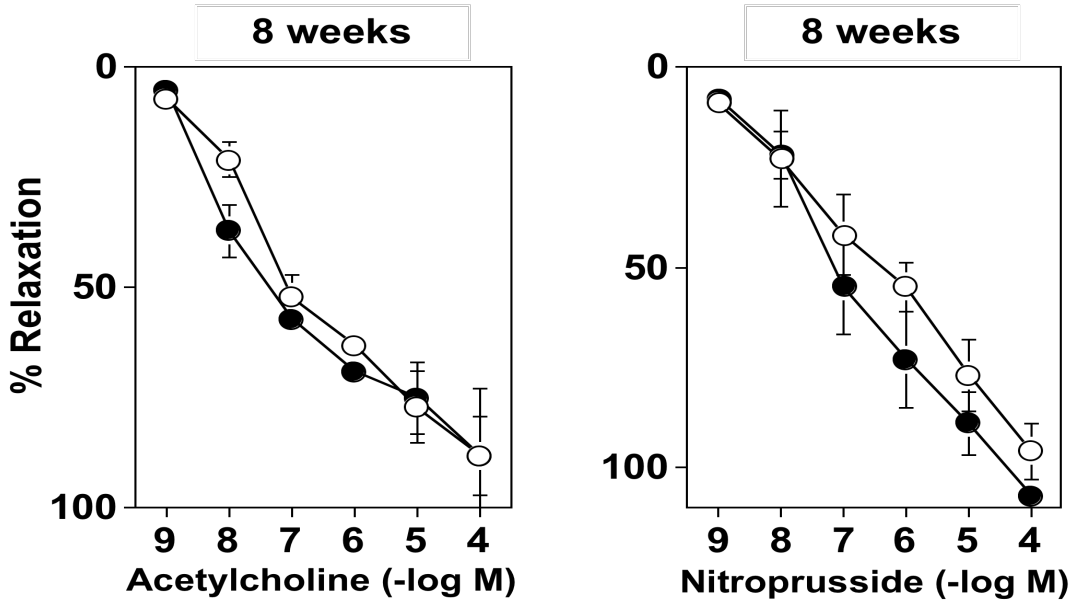
Drugs and Chemicals. Acetylcholine, nitroprusside, and Tempol were obtained from Sigma (St. Louis, MO) and all were dissolved with saline. U46619 was obtained from Cayman Chemical (Ann Arbor, MI) in peanut oil and dissolved in 100% ethanol with all subsequent dilutions being made with saline. All other reagents were of standard laboratory grade.

Supplemental Figures and Figure Legends.

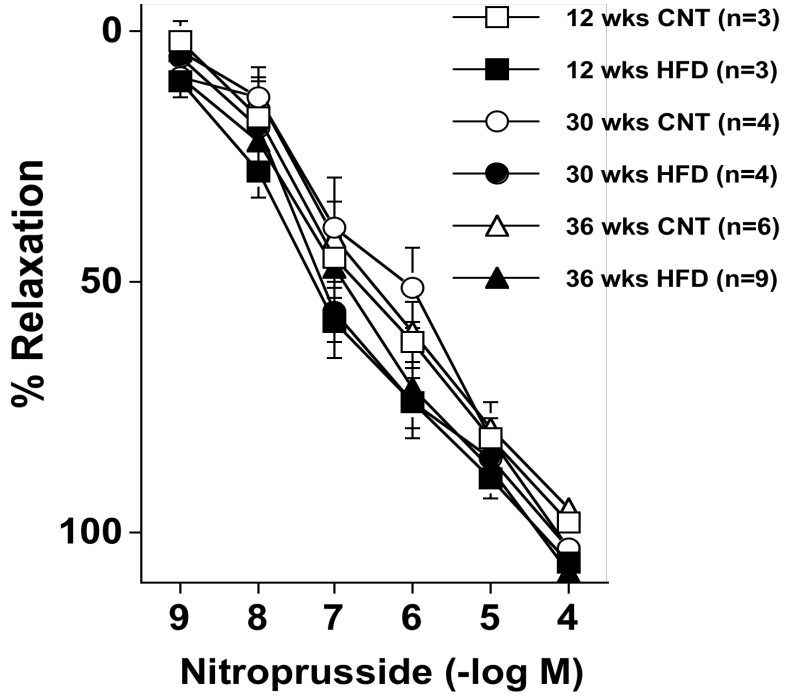
Supplemental Figure I. Endothelium-independent responses to nitroprusside were similar in cerebral arterioles from wild-type mice fed a control diet or a 45% HFD for 8, 12, and 30 wks. These findings suggest that the impairment of endothelium-dependent responses to acetylcholine produced by a HFD was selective for endothelium. Mean±SE; 8 wks, n=6; 12 wks, n=4-6; 30 wks, n=6; P>0.05.



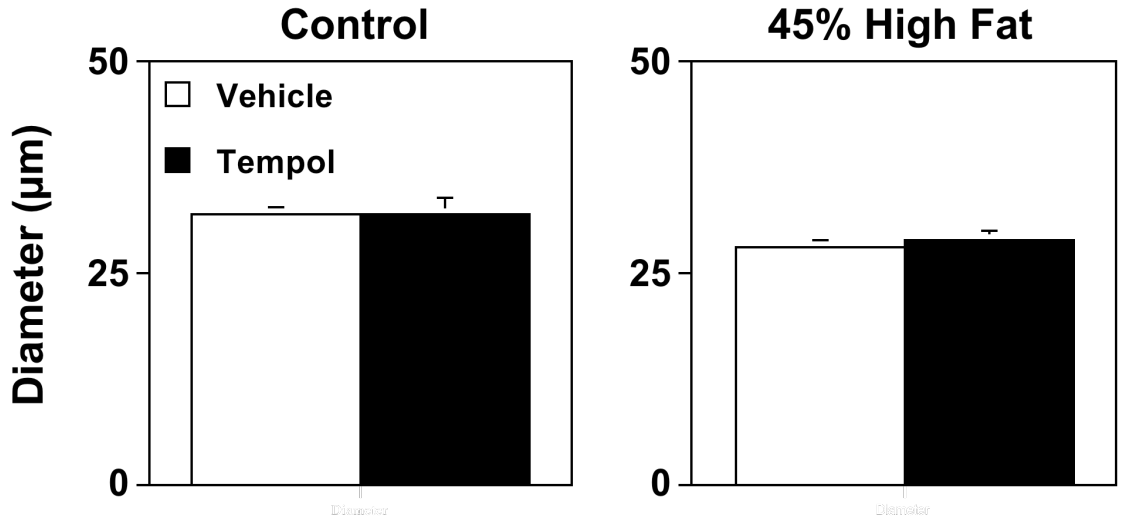
Supplemental Figure II. Responses to acetylcholine and nitroprusside were similar in carotid arteries from wild-type mice fed either a control diet or 45% HFD for 8 wks, suggesting that this diet and duration was not associated with impairment of function in the carotid artery. Mean±SE; n=3/group; P>0.05.



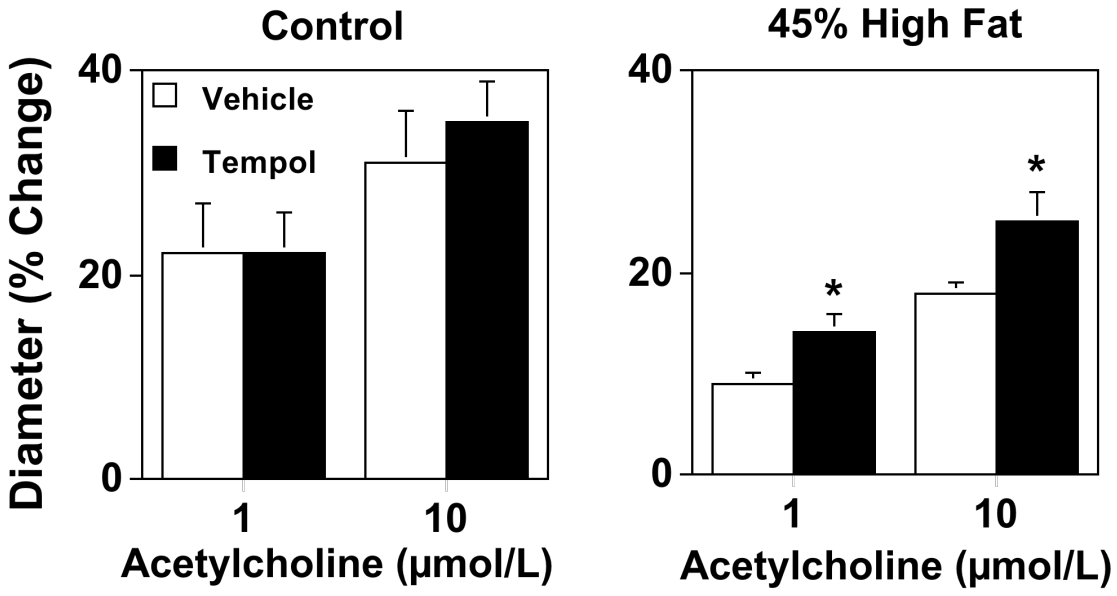
Supplemental Figure III. Responses to nitroprusside were similar in carotid arteries from wild-type mice fed either a control or a 45% HFD for 12-36 wks. These findings indicate that the effect of a 45% HFD on responses to acetylcholine in the carotid artery were selective for endothelium. Mean±SE; P>0.05.



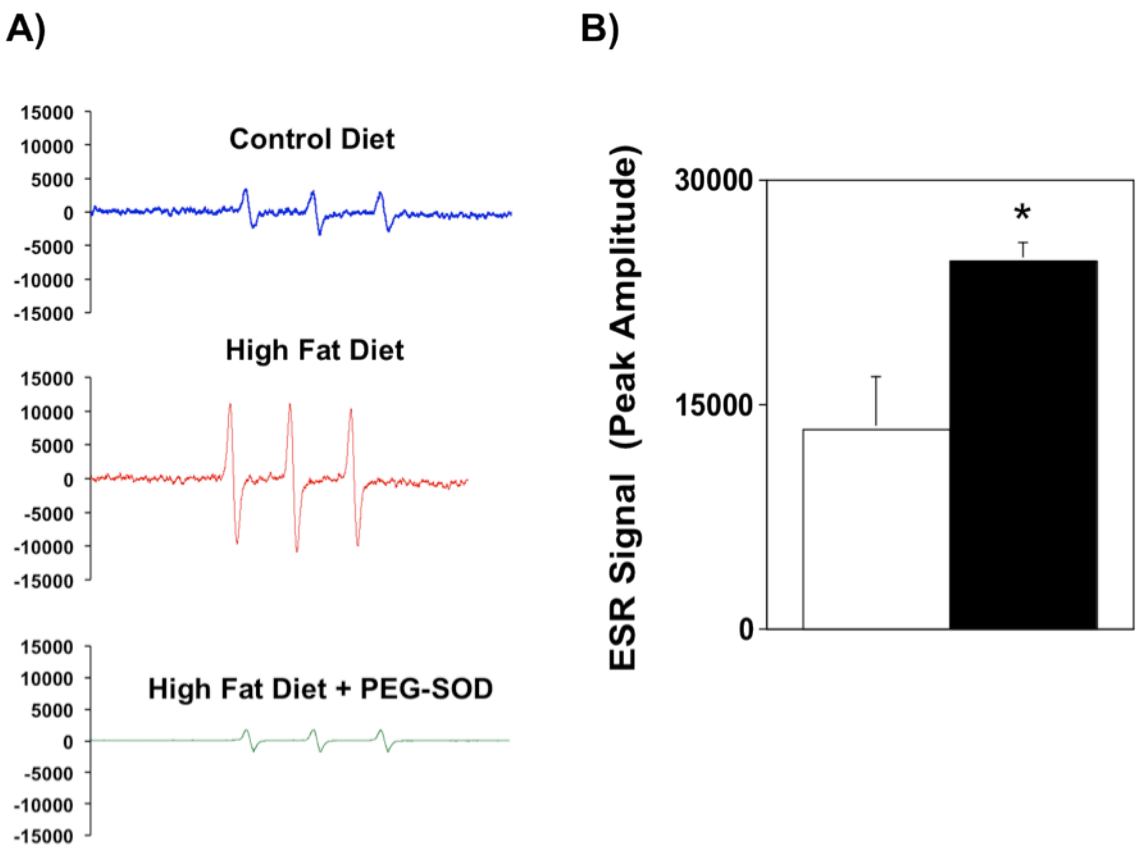
Supplemental Figure IV. Tempol had no effect on baseline diameter in cerebral arterioles of wild-type mice fed either a control or a 45% HFD for 12 wks. Arteriolar diameter was measured before (vehicle) and then re-measured following 30 min suffusion of the cranial window with Tempol (1 mM). Mean±SE; n=5/group; P>0.05.



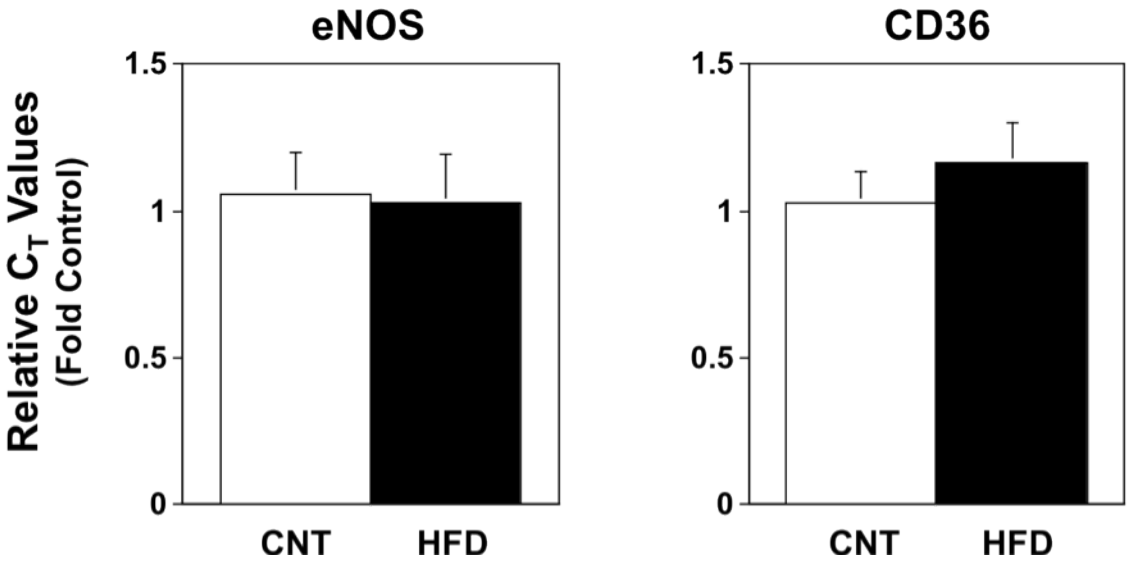
Supplemental Figure V. Dilatation produced by acetylcholine was similar in cerebral arterioles following suffusion with vehicle or Tempol in wild-type mice fed a control diet for 12 wks. Dilatation in response to acetylcholine was significantly improved in cerebral arterioles from wild-type mice fed a 45% HFD for 12 wks treated acutely with Tempol. These findings implicate a role for superoxide in the impairment of endothelial function produced by a HFD. Mean±SE; n=5/group; *P<0.05 vs. vehicle.



Supplemental Figure VI. A) Representative ESR spectra from vascular (aortic) homogenates from wild-type mice fed a control or a 60% HFD for 36 wks. Superoxide levels were measured using the spin probe 1-hydroxy-3-methooxycarbonyl-2,2,5,5,-tetramethylpyrolidine•HCl (CMH; Alexis Biochemicals, San Diego, CA). Aortic homogenates (1 mg/ml) from each experimental group were incubated with 75 µl of spin-trap stock solution consisting of CMH (20 µM in DPBS plus 25 µM desferrioxamine). ESR spectra were measured using a benchtop ESR (Magnettech) at a microwave power of 40 mW, modulation amplitude of 3,000 mG and modulation frequency of 100 kHZ. The ESR signal was significantly attenuated in the presence of PEG-SOD (100 U/ml) in vascular homogenates from mice fed a HFD providing confirmation that the signal was due to superoxide. **B)** The peak ESR amplitude was significantly greater in wild-type mice fed a HFD (n=4) as compared to wild-type mice fed a control diet (n=3). Mean±SE; *P<0.05 vs. control.



Supplemental Figure VII. Endothelial nitric oxide synthase (eNOS) and CD36 expression as assessed by real-time RT-PCR in whole brain from wild-type mice fed either a control (CNT) diet or HFD for 12 wks. Mean±SE; n=5/group; P>0.05.



Supplemental References.

1. Didion SP, Lynch CM, Faraci FM. Cerebral vascular dysfunction in TallyHo mice: a new model of Type II diabetes. *Am J Physiol Heart Circ Physiol*. 2007;292:H1579-1583.

2. Didion SP, Lynch CM, Baumbach GL, Faraci FM. Impaired endothelium-dependent responses and enhanced influence of rho-kinase in cerebral arterioles in type II diabetes. *Stroke.* 2005;36:342-347.

3. Schrader LI, Kinzenbaw DA, Johnson AW, Faraci FM, Didion SP. IL-6 deficiency protects against angiotensin II induced endothelial dysfunction and hypertrophy. *Arterioscler Thromb Vasc Biol.* 2007;27:2576-2581.

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5. Andrikopouplos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab.* 2008;295:E1323-E1332.

6. Williams JM, Johnson AC, Stelloh C, Dreisbach AW, Franceschini N, Regner KR, et al. Genetic variants in Arhgef11 are associated with kidney injury in the Dahl salt-sensitive rat. *Hypertension.* 2012;60:1157-1168.