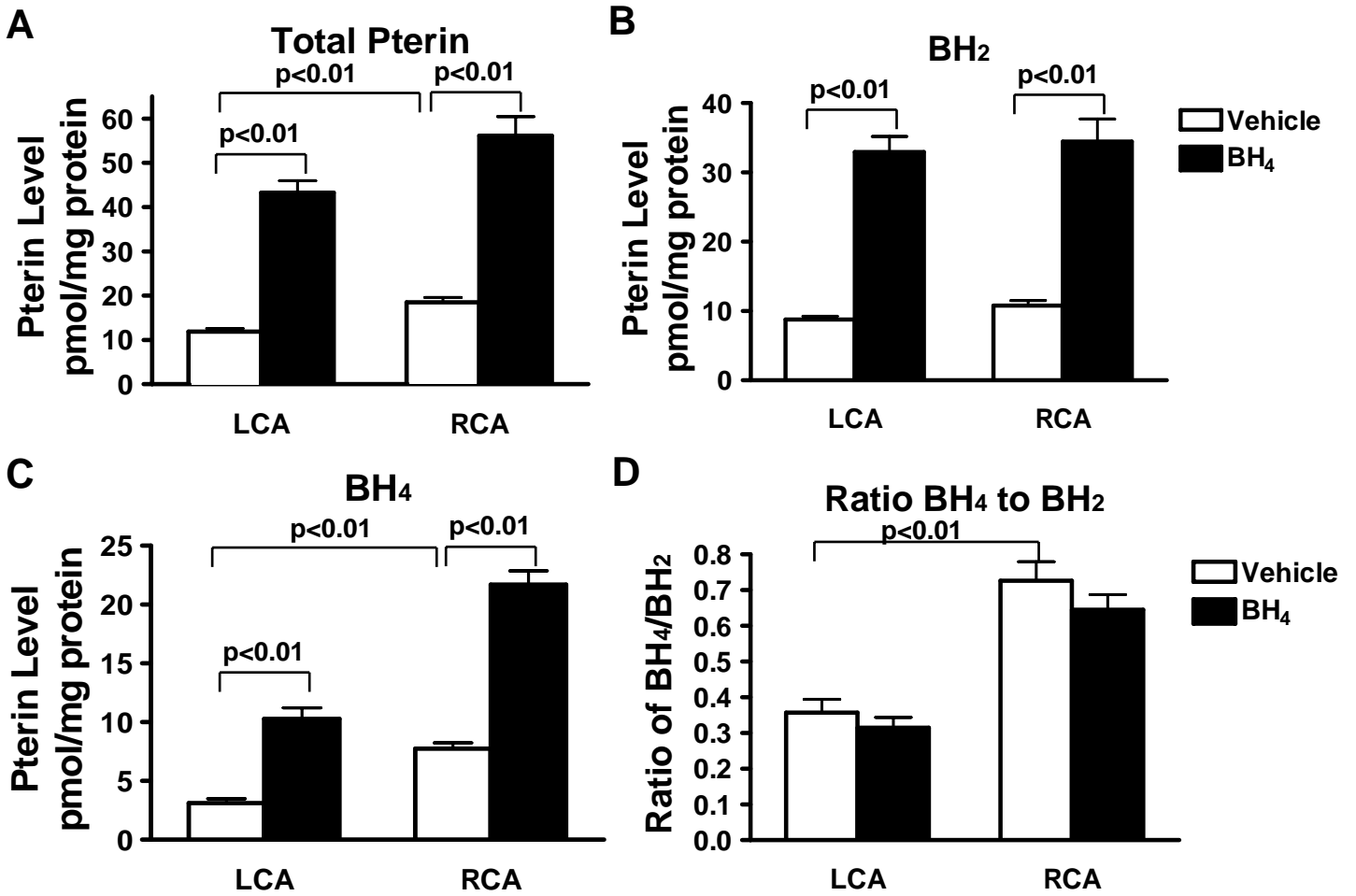


Supplemental Material

**Tetrahydrobiopterin Deficiency and Nitric Oxide Synthase Uncoupling Contribute to
Atherosclerosis Induced by Disturbed Flow**

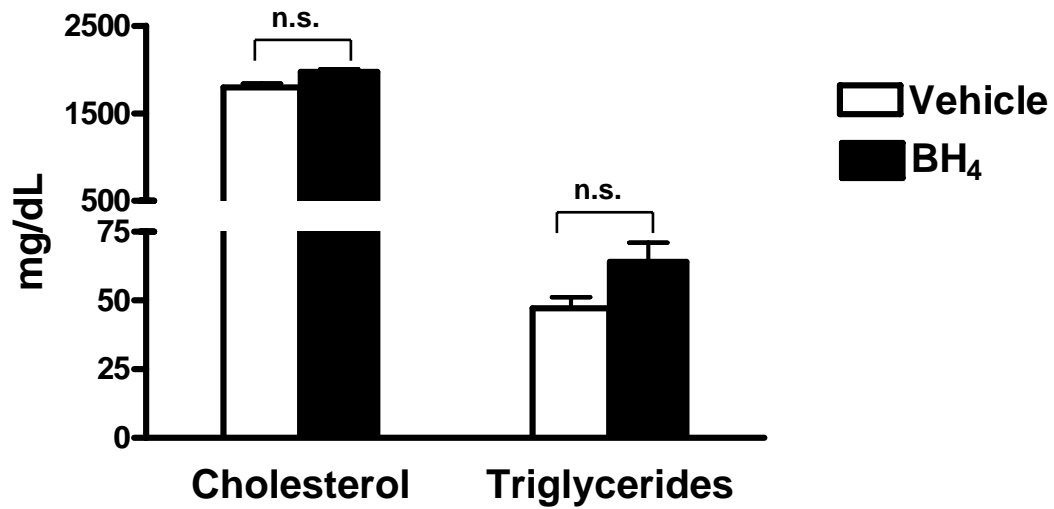
Li Li^{1,2}, Wei Chen¹, Amir Rezvan¹, Hanjoong Jo^{1,3}, David G. Harrison^{1,2,4}

Supplemental Figure I

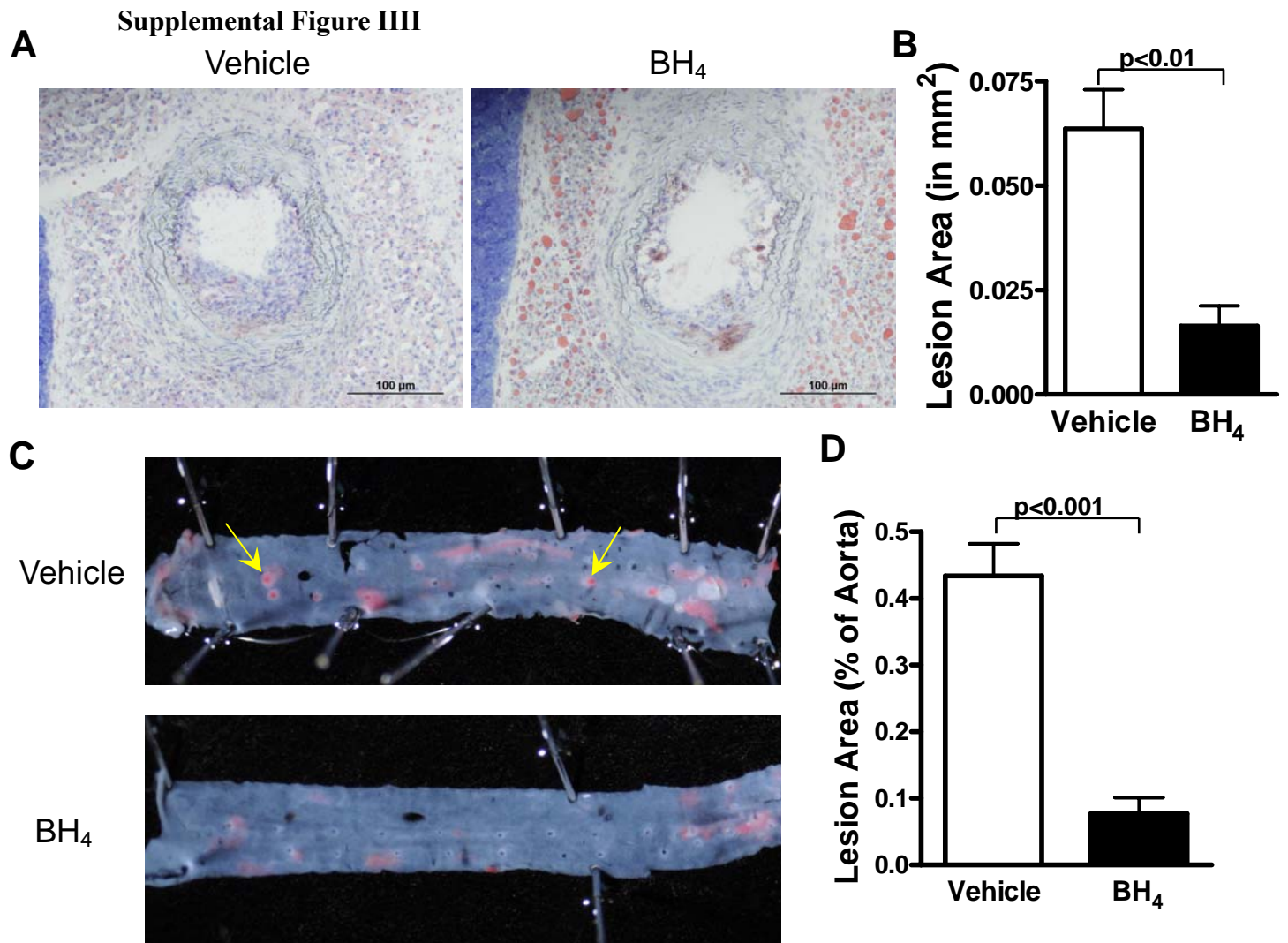


Supplemental Figure I: Pterin levels in the carotids that underwent partial ligation for 1 week. The left carotid arteries of ApoE^{-/-} mice were partially ligated and mice were treated with an approximate dose of 10 mg/kg per day BH₄ in the drinking water or vehicle for 1 week. Total biopterin (Panel A), BH₂ (Panel B), BH₄ (Panel C) levels and the ratio of BH₄/BH₂ (Panel D) in the carotids were shown (n=5). Values were compared using two-way ANOVA and selected comparisons were made using a Bonferroni post hoc test.

Supplemental Figure II

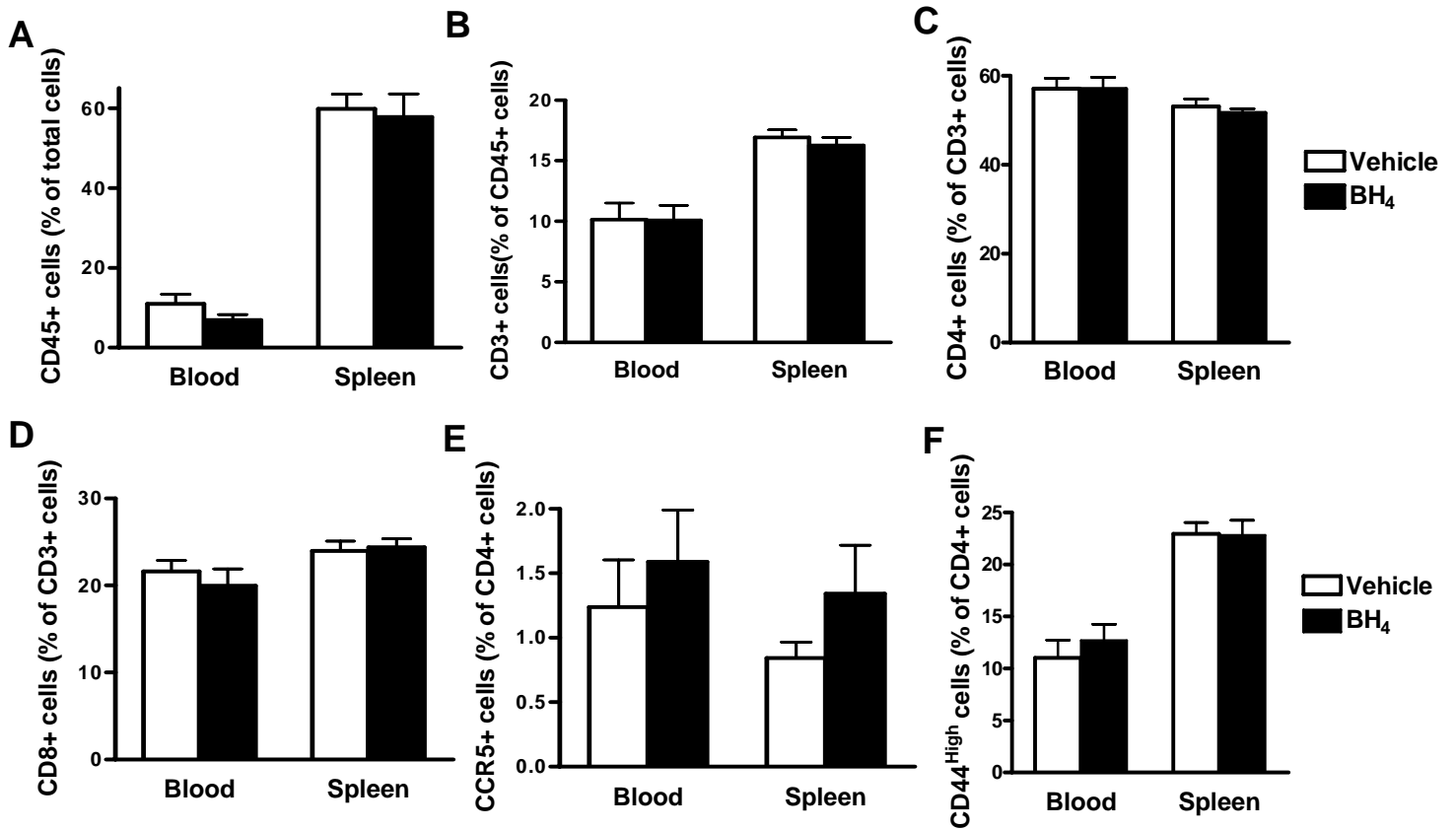


Supplemental Figure II: Effect of BH₄ supplementation on plasma lipid profile in ApoE^{-/-} mice. ApoE^{-/-} mice were treated with BH₄ or vehicle in the drinking water and fed a high fat diet for 3 weeks (n=4-5). Values were compared using ANOVA and selected comparisons were made using a Bonferroni post hoc test.



Supplemental Figure III: Effect of BH₄ treatment on atherosclerosis in the brachiocephalic and intercostal arteries of ApoE^{-/-} mice fed a high fat diet for 3 weeks. Animals also received BH₄ or vehicle in the drinking water for 3 weeks. A. Representative images of Hematoxylin and Oil red O staining of the brachiocephalic artery frozen sections. B. Quantification of the intimal lesion areas using Image J (n=5-6). Values were compared using an unpaired t-test. C. Representative images of en face Oil Red O staining of the aorta. Intercostal arteries were indicated by the yellow arrows. D. Quantification of the intercostal lesion area presented as percentage of entire aorta (n=5). Values were compared using an unpaired t-test.

Supplemental Figure IV



Supplemental Figure IV: Effect of BH₄ treatment on T cell numbers and activation in the peripheral blood and spleen after partial carotid ligation. ApoE^{-/-} mice were fed a high fat diet and underwent partial carotid ligation for 1 week. Animals also received BH₄ or vehicle in the drinking water. Percentages of CD45+ (Panel A), CD3+ (Panel B), CD4+ (Panel C), CD8+ (Panel D), CD4+CCR5+ (Panel E), and CD4+CD44^{High} (Panel F) cells were shown (n=6).