DATA SUPPLEMENT

Nuclear Factor-κB Activation Contributes to Vascular Endothelial Dysfunction via Oxidative Stress in Overweight/Obese Middle-Aged and Older Humans

Gary L. Pierce, Lisa Lesniewski, Brooke R. Lawson,

Stacy D. Beske and Douglas R. Seals

Department of Integrative Physiology, University of Colorado, Boulder, CO, 80309

Correspondence:

Gary L. Pierce, Ph.D.
Department of Integrative Physiology
UCB 354
University of Colorado at Boulder
Boulder, CO 80309
Tel: (303) 492-3010
Fax: (303) 492-6778
Email: gpierce@colorado.edu

Supplemental Methods.

Blood Analyses. The main assays were performed by the University of Colorado Adult GCRC core laboratory. Fasting plasma total cholesterol, LDL-C, HDL-C, triglycerides and glucose were determined using standard assays. Plasma concentrations of CRP were measured using a high-sensitivity Chemistry Immuno Analyzer (AU400e, Olympus America, Inc.). Plasma interleukin-6 (IL-6), tumor-necrosis factor-α (TNF-α; both R&D Systems, Inc), oxidized LDL (Alpco, Inc.), endothelin-1 (Peninsula Laboratories, Inc) were measured by ELISA. Serum adiponectin and leptin were measured by radioimmunoassay (Linco Research, Inc.) and plasma norepinephine by high performance liquid chromatography. Plasma salicylate concentrations were determined by the Boulder Community Hospital Clinical Chemistry laboratory using a validated colormetric assay (Ortho Diagnostics, Inc.). Plasma concentrations of 6-keto-prostanglandin $F_{1\alpha}$ and 11-dehydro-thromboxane B_2, major metabolites of prostacyclin and thromboxane A_2, respectively, were measured by the clinical laboratory of Cayman Chemicals, Inc. (Ann Arbor, MI) using ELISA.

Brachial Artery FMD and Endothelium-Independent Dilation. Responses are expressed as mm and % maximal change from baseline diameter per recent recommendations [1]. Baseline and hyperemic blood flow data were available on 11 subjects. Because blood viscosity was not available to calculate shear stress, baseline shear rate was calculated as baseline blood flow velocity/baseline diameter and peak shear rate as mean peak blood flow velocity/occlusion diameter (i.e., average diameter for 15 seconds prior to release of occlusion cuff pressure) [2]. In a subgroup of 8

subjects, FMD was measured during saline (control) and during supraphysiological intravenous infusion of vitamin C (ascorbic acid) on the same day as described previously by our laboratory [3]. Briefly, subjects received an intravenous saline bolus for 20 min followed by a 60 min saline drip infusion. Brachial artery FMD baseline measurements were obtained immediately after the saline bolus during the saline drip infusion. Next, an ascorbic acid priming bolus of 0.06 g/kg fat-free mass was infused for 20 minutes followed by a drip infusion of 0.02 g/kg fat-free mass administered over 60 minutes. Brachial artery FMD and endothelium-independent dilation were measured after the intravenous bolus of ascorbic acid during the drip infusion because peak plasma concentrations of ascorbic acid occur after the bolus infusion using this protocol [3].

Supplemental Results.

Supplemental Table I: Energy and macronutrient intake from 3-day research diet

	Placebo	Salsalate
Calories (kcal/day)	2382 ± 106	2316 ± 111
Carbohydrate (g/day)	333 ± 15	325 ± 16
Protein (g/day)	91 ± 4	90 ± 4
Fat (g/day)	82 ± 4	79 ± 4
Carbohydrate (% of kcal/day)	54.8 ± 0.2	54.6 ± 0.6
Protein (% of kcal/day)	15.0 ± 0.1	15.3 ± 0.2
Fat (% of kcal/day)	30.2 ± 0.2	30.1 ± 0.5

Values are mean ± standard error.

Supplemental References.

1. Donald AE, Halcox JP, Charakida M, Storry C, Wallace SM, Cole TJ, Friberg P, Deanfield JE. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. *J Am Coll Cardiol.* 2008;51:1959-1964.

2. Pyke K, Tschakovsky M. The relationship between shear stress and flow-mediated dilation: implications for the assessment of endothelial function. *J Physiol.* 2005;568.2:357-369.

3. Eskurza I, Monahan K, Robinson J, Seals D. Effect of acute and chronic ascorbic acid augmentation on flow-mediated dilation with physically active and sedentary aging. *J Physiol.* 2004;556:215-224.