Methods Supplement

Cell culture and shear stress experiments

Pooled HUVEC or single individual HAEC (Lonza, Walkersville, MD, USA) were grown in M199 media (Mediatech, Manassas, VA, USA) supplemented with 20% (v/v) FBS (Hyclone), 50 ug/ml endothelial mitogen (Biomedical Technologies, Stoughton, MA, USA), 2mM L-glutamine (Mediatech), 2.5 U/ml heparin sodium (American Pharmaceutical Partners, Schaumburg, IL, USA), 50 U/ml penicillin, and 50 μg/ml streptomycin (Mediatech) at 37°C in humidified 5% CO_2/95% air. Different lots of pooled HUVEC were used for each experimental replicate.

Passage 3 to 5 HUVEC or passage 6 HAEC were seeded (20,000 cells/cm^2) onto 0.5 M NaOH treated glass slides (Corning, Corning, NY, USA) coated with 1% bovine gelatin (Sigma, St. Louis, MO, USA) and crosslinked with 0.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, USA). After 36-48 hours confluent slides were mounted in parallel plate chambers and subjected to shear stress using a constant head flow loop. Shear stress experiments were performed at 37°C, in 5% CO_2/95% air, using the same media as for cell culture.

For the reversing flow system, two syringes were mounted in parallel on a continuous flow pump (33 Twin Syringe Pump, Harvard Apparatus, Holliston, MA, USA) providing a steady shear stress of 1 dyne/cm^2 across a parallel plate chamber. The oscillating portion of the flow was provided by a 1 mL glass syringe, in line with the syringe pump, driven by a linear stage motor (MX80L linear motor, Parker Motion, Rohnert Park, CA, USA).

RNA and protein isolation

Immediately after exposure to shear stress, total RNA and protein were extracted using TRI-zol (Invitrogen); RNA was further purified with DNase (Qiagen, Valencia, CA, USA) and RNeasy MinElute Cleanup Kit (Qiagen), according to manufacturers' instructions. The integrity and quantity of the RNA were verified with UV spectrophotometry, accepting only RNA with a 260/280 ratio greater than 1.9. Protein concentration was quantified with DC Protein Assay (Bio-Rad) using BSA standards.

Quantitative Real-time PCR

For quantitative real-time PCR (qRT-PCR), total RNA was reverse transcribed into cDNA with SuperScript II (Invitrogen) according the manufacturer's instructions. The resulting cDNA was purified through Micro Bio-Spin P-30 Chromatography Columns (BioRad) and diluted 1:20. qRT-PCR primers used were CYP1A1 (forward: 5'-cactgtcaaggatgagccagcagtatg-3', reverse: 5'-gctgggtcagaggcaatggagaaactt-3'); CYP1B1 (forward: 5'-ggtgaccagcccaacctgccctatg-3', reverse: 5'-tctgctggtcaggtccttgttgatgag-3'); thrombospondin-1 (forward: 5'-gggggcgtcaatgacaatttc-3', reverse: 5'-gccaatgtagttagtgcggat-3'); and 18S (QuantumRNA Classical II, Ambion). qRT-PCR was performed using iQ SYBR Green Supermix (BioRad) according to the manufacturer's instructions. The qRT-PCR reactions and analysis were performed on a MyiQ (BioRad) with a 3 min initial denaturation step at 95°C; 45 cycles (30 cycles for 18 s) at 95°C for 5 s, 60°C for 10 s, 72°C for 60 s/kbp product and a ramped melting cycle. Absolute concentrations were quantified from CT values with a linear standard curve.

Western Blotting

Equal amounts of protein were resolved by SDS-PAGE in 7.5% Tris–HCl precast polyacrylamide gels (Bio-Rad) under reducing conditions with Laemmli sample buffer and electro-transferred onto polyvinyl difluoride membranes. After blocking with 5% nonfat dry milk, membranes were probed with primary antibody. Bound primary antibodies were labeled with either sheep anti-mouse or donkey anti-rabbit immunoglobulin conjugated with horseradish peroxidase (GE Healthcare, Buckinghamshire, UK) and detected with enhanced chemiluminescence (ECL Plus, GE Healthcare).

Mouse Aorta Immunohistochemistry

Immunoreactive AhR and CYP1A1 were detected with goat anti-rabbit IgG (1:250, Alexa Fluor 568, Invitrogen). Along with primary antibody, all samples were counterstained with antibody against CD31 using rat anti-mouse IgG (1:100, Santa Cruz), followed by counterstaining with goat anti-rat IgG (1:250, Alexa Fluor 488).

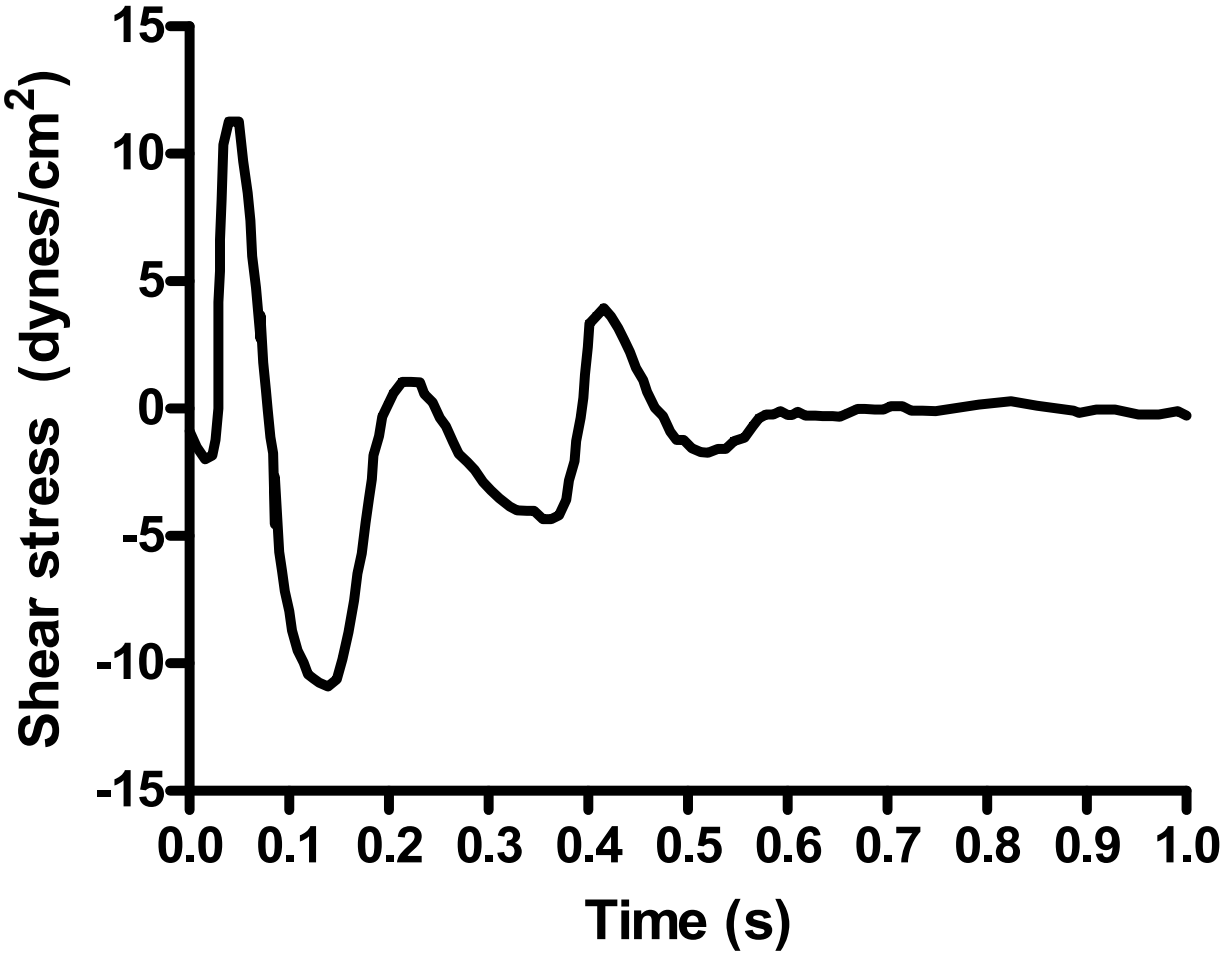
Human Coronary Artery Immunohistochemistry

Along with primary antibody, all samples were counterstained with antibody against mouse anti-human CD31 (1:500, Clone WM59, BD Biosciences, San Jose, CA USA) for endothelial cell identification. Immunoreactive CYP1A1 and CYP1B1 were detected with goat anti-rabbit IgG (1:250, Alexa Fluor 568, Invitrogen). CD31 was detected with goat anti-mouse IgG (1:1000, Alexa Fluor 488, Invitrogen). Nuclei were counterstained with Hoechst. Images were viewed with a Nikon Eclipse TE2000-U fluorescence microscope and acquired with a Retiga EXi cooled CCD camera (Qimaging, Surrey, BC Canada).

Supplemental Figure 1. Reversing shear stress waveform. A shear stress waveform for a parallel flow chamber was designed based on simulated data of the internal carotid sinus . The waveform has a time-averaged shear stress of –1 dyne/cm^2, a maximum shear stress of +11 dynes/cm^2, a minimum shear stress of –11 dynes/cm^2, and a cycle frequency of 1 Hz.

Supplemental Figure 2. Expression of CYP1A1 and CYP1B1 protein in human coronary artery endothelial cells in vivo. Sectioned whole human coronary arteries were stained for either CYP1A1 or CYP1B1. Endothelial cells were identified using CD31. Merged images were counterstained with Hoechst (blue). CYP1A1 and CYP1B1 staining was strongest in the endothelium (E) with additional CYP1B1 expression in the media (M) and adventitia (A). Negative controls (N.C.) deleted the primary antibody. Bar is 20 micrometers. Lumen is at top of image (L).

Supplemental Figure 1 Conway



Supplemental Figure 2 Conway