Vascular Stiffening
(Maximum passive stiffness, MPS)

Version: 1
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Summary: One index of arterial function that is compromised to a varying degree in individuals with cardiovascular disease is vascular stiffness. This protocol can be utilized to analyze vascular function as an index for cardiovascular disease. This protocol directly measures the stress and strain on an arterial vessel as an index of vascular function.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Krebs- Henseleit Solution		
Force transducer model FT10	Grass Instruments	
Stainless steel rods (200µM outside diameter)		

Protocol:

WARNING:
All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

1. Mice are anesthetized with an intraperitoneal injection with 50 mg pentobarbital/kg weight.
2. Either common carotid arteries or aorta are dissected as described in **Macrovascular Permeability and Lipoprotein Flux** protocol. Artery explants can be:
    a. Immediately utilized for analysis of vascular stiffness
    b. Stored in Krebs-Henseleit at 4°C overnight.

   c. Snap-frozen in liquid nitrogen and stored at -80°C, samples will be thawed overnight in Krebs-Henseleit at 4°C prior to analysis.
3. Two stainless steel rods (200-µm outside diameter) qre inserted through the lumen of a 1-mm section of carotid artery in a parallel manner while the vessel is immersed in Krebs-Henseleit buffer. One rod is attached to a motorized controller, while the other to a force transducer. (As the motorized controller pulled the rods apart, the vessel tension is recorded.)
4. Arterial segments are preconditioned three times at [almost equal to] 10% of their maximal load.
5. Stress (vessel tension development divided by vessel area, N/mm^2) versus strain (vessel circumference) curves is generated so that maximum strain (ie, strain at vessel breakage), maximal stress (vessel tension at maximum strain), and failure energy (area under the stress-strain curve) can be calculated.
   a. MPS is defined as the maximal slope of the maximal slope of the load vs. strain relationship.
6. Experiments are performed on three 1-mm segments of each artery, and the results are averaged.

Reagent Preparation:

Reagent 1: Krebs-Henseleit Solution

116 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2*H_2O$, 1.2 mM $MgCl_2$, 1.2 mM NH_2PO_4, and 11mM glucose