



# Mesenteric Reactivity

Version: 1

Modified from: Zhao *et.al.* Am J Physiol Regul Integr Comp Physiol. 2005 Jan;288(1):R188-96.

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**Summary:** Impaired endothelium-dependent relaxation is associated with hypertension and diabetes. This protocol measures mesenteric microvessel endothelium-dependent dilation.

## Reagents and Materials:

| Reagent/Material                             | Vendor                      | Stock Number |
|--|-----------------------------|--------------|
| Dissecting microscope, Stereo Zoom 5         | Leica microsystems          |              |
| Microvessel myograph                         | Jules Osher                 |              |
| Force transducer: Fort10 transducer          | World Precision Instruments |              |
| acetylcholine                                | Sigma                       | A2661        |
| <i>N</i> <sup>G</sup> -monomethyl-L-arginine | Sigma                       | M7003        |

## 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. The mesenteric bed, including arteries and veins, is cut away from the intestinal wall.
3. Small mesenteric arteries (150–200  $\mu$ M passive ID at 60 mmHg) are dissected free from connective tissue and fat in cold Krebs-physiological salt solution
4. Individual arteries are mounted on glass micropipettes, pressurized to 60 mmHg in Krebs-physiological salt solution at 37°C, and allowed to equilibrate for 30 min.
5. Experimental protocols were performed, each separated by 20 to 30 minutes during which the vessel-bathing medium was exchanged with NPSS several times.
  - a. Endothelium-dependent vasodilator (acetylcholine;  $10^{-8}$  to  $10^{-4}$  mol/L)

- b. Single dose ( $5 \times 10^{-4}$  mol/L) of *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA; an inhibitor of nitric oxide synthase)
6. Percent relaxation curves are expressed as the difference in diameter at each dose relative to the baseline, pre-constricted diameter (0%), and the passive diameter (100%).

## Reagent Preparation:

### Reagent 1: Krebs-Physiological Salt Solution

(in mM, pH 7.4): 119.0 NaCl, 25.0 NaHCO<sub>3</sub>, 4.6 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.2 MgSO<sub>4</sub>, 1.8 CaCl<sub>2</sub>, and 11.0 glucose