ABSTRACT E 479.3
Epoxyeicosatrienoic acids (EETs) are metabolized by soluble epoxide hydrolase (sEH). Targeted disruption of the sEH gene enhances post-ischemic contractile function in isolated hearts and augments administration of EETs limits myocardial infarct size in vivo. We tested the hypothesis that sEH gene deletion is cardioprotective.

CONCEPT
Arachidonic acid (AA) is released from membrane phospholipids in response to a variety of pathophysiological stimuli, including myocardial ischemia. Free AA is metabolized by three pathways: cyclooxygenase, lipooxygenase and cytochrome P450 monooxygenase (CYP). CYP metabolizes AA to four biologically active eicosanoids (epoxyeicosatrienoic acids EET): 5,6-EET; 6,7-EET; 8,9-EET; and 11,12-EET. EETs have anti-inflammatory and anti-thrombotic effects, are coronary vasodilators, and reduce myocardial ischemic injury (1,4). The biological activity of EETs is terminated by hydration into less active dihydroeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH). Functional recovery is improved in isolated hearts from sEHKO mice after global ischemia (5).

BACKGROUND
Male C57BL/6J wild-type (WT) mice or sEH knockout (sEHKO) mice were subjected to 40 min coronary artery occlusion and 120 min reperfusion. Area-at-risk (AAR) and infarct size (I) were assessed using fluorescent microscopes and high-performance liquid chromatography. Infarct size is expressed as %AAR (mean SEM). Myocardial sEH protein content was assayed by immunoblot and its %I/AAR was reduced by 29% in sEHKO compared to WT (37% ± 10% vs. 49.3% ± 8.05%). Western blot confirmed the absence of sEH in sEHKO and its presence in WT hearts. HIF was positive for sEH in cardiomyocytes of WT but not sEHKO hearts. Captopril from targeted deletion of sEH is likely due to increased endogenous myocardial EET levels. Inhibition of sEH or injection of EETs may serve as a novel therapeutic option for cardioprotection during coronary revascularization and cardiac transplantation. Support: VA Merit Review 317 (M.EV) and ROI N01-RR-8023 (NIA).

HYPOTHESIS
Augmentation of endogenous EETs by inactivation of sEH reduces infarct size/AAR in vivo.

METHOD
Animals: Male C57BL/6J or sEHKO mice with targeted deletion of the sEH encoding gene EP2H02 received treatment in compliance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, National Research Council, National Academy Press, 1996) and with IACUC approval.

Regional Myocardial Ischemia-Reperfusion Injury
Mice were anesthetized with isoflurane and intubated with a 20G plastic IV catheter and ventilated. ECG and rectal temperature were monitored. The animals were positioned in a right lateral decubital position and rectal temperature was maintained at 37ºC. A PE-10 catheter was inserted into the jugular vein for drug infusion. A left-sided thoracotomy was performed in the 4th intercostal space. A ligature was placed around the LCA occlusion was confirmed with persistent ECG changes during occlusion and visual paling of the left ventricle (LV). Reperfusion was confirmed with visual hyperemia of the LV and return of ECG to baseline. After LCA re-oxygen, fluorescent microscopics were infused via needle puncture of the LV apex and the heat was excised. The ventricles were sliced into seven sections (0.1 mm) for imaging and staining. The microscopics delineate the non-perfused area (AAR). The infarct size (I) was determined by staining in 1% 2,3,5 triphenyl tetrazolium chloride (TTC) followed by formalin bath overnight.

Immunohistochemistry
Samples (LV) were fixed with 4% paraformaldehyde, embedded in paraffin, cut into 5-µm sections and mounted on super frosted glass slides. Sections were kept at 60ºC overnight and de-paraffinized with xylene following by washing in 100%, 95%, 80%, and 70% ethanol. Heat unmasking of epitope was done by boiling the samples three times in citrate buffer (pH-4) (600 W microwave). After cooling down to room temperature and 1 h of blocking in 5% dry milk, slides were incubated overnight at room temperature with primary antibodies (rabbit anti-sEH antibody, 1:50; Santa Cruz sheep anti-TH antibody, 1:100, Pel-Freprieve). After washing in PBS, sections were incubated with secondary FITC-conjugated goat anti-rabbit IgG (1:100 in 5% dry milk, Invitrogen) and Alfa Fluor 560 donkey anti-sheep IgG (1:200 in 5% dry milk. Invitrogen) for 4 h. After washing, sections were placed in an autofluorescence-reduction solution (10 mM CuSO4, 50 mM ammonium acetate) for 30 min, and then mounted with Slow Fade Gold Antifade Reagent with DAPI (Invitrogen). The sections were viewed on Zeiss Axiovert 200 fluorescence microscope. Negative controls included sections in which the primary antibodies were omitted, incubated with secondary antibodies only, or sections in which both primary and secondary antibodies were added. Further negative controls included use of sEH antibody after preincubation with its specific blocking peptide. Western Blot
Heart or cardiomyocyte proteins in SDS sample buffer (2% SDS, 10% glycerol, 80 mM Tris, pH 6.8, 0.15 M β-mercaptoethanol, 0.02% bromophenol blue) were separated on 4-20% linear gradient SDS-polyacrylamide gels (Bio-Rad) in a minigel apparatus (Mini-PROTEAN 3, Bio-Rad) and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% nonfat dry milk in TBST (10 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 60 minutes at room temperature and incubated overnight at 4°C with primary rabbit anti-sEH antibody (1:2000 in 5% dry milk, Santa Cruz). The antigens were detected by the luminescence method (ECL plus Western blotting detection kit, Amersham) with peroxidase-linked anti-rabbit (1:2000 in 5% dry milk).

RESULTS
Figure 1: Effect of sEH deletion on infarct size
Myocardial infarct size data expressed as a percent of area at risk (I/AAR) are shown (A). I/AAR was reduced in sEHKO mice compared to wild-type (WT). (B) AAR as percent of biventricular volume was similar in both groups.

Figure 2: Images of wild-type (WT) and sEHKO hearts
Top row: fluorescent images for AAR analysisBottom row: TTC staining for infarct size analysis

Figure 3: Physiological parameter WT and sEHKO mice (mean ± SEM, * p<0.05 WT versus sEHKO)

Figure 4: sEH expression via Western Blot
sEH is absent in LV tissue from sEHKO mice in both sexes

CONCLUSION
- sEH inactivation by targeted gene deletion is cardioprotective.
- Enhancing endogenous EET level during and after myocardial ischemia may prevent a novel pharmacological therapy against myocardial ischemia-reperfusion injury.

REFERENCES:
4) Granu et al. J Mol Cell Cardiol 2007;43: 865-876