

Diesel Exhaust and Atherosclerotic Plaque Stability R01 ES13434

Michael Rosenfeld – PI

Dan Luchtel – Co-PI

Terry Kavanagh – Co-Investigator

Joel Kauffman – Co-Investigator

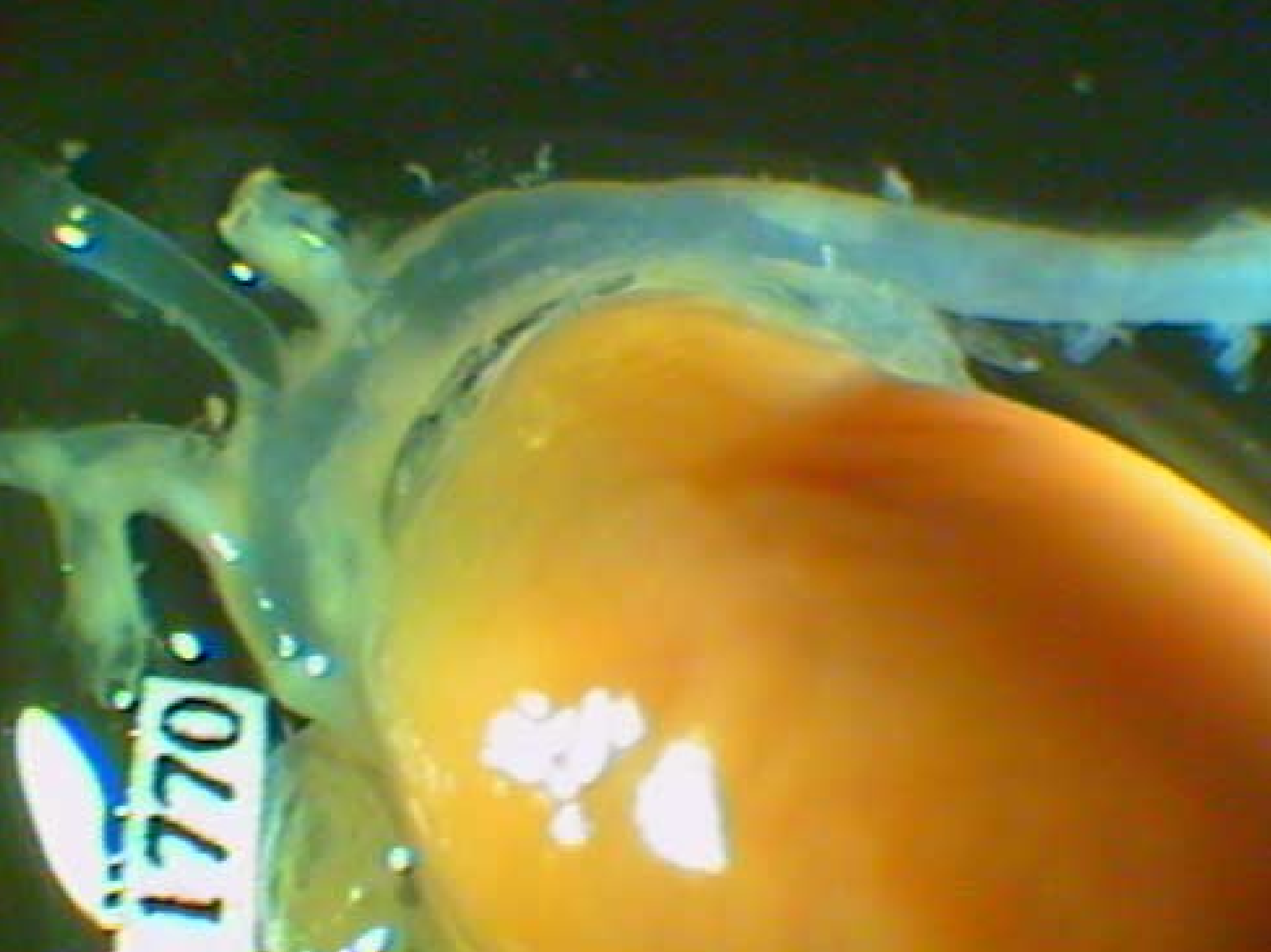
Stephan Van Eden (UBC) – Co-Investigator

Specific Aim 1: To determine whether both acute and chronic exposure to diesel exhaust increase plasma cytokines and contribute to the progression and destabilization of advanced atherosclerotic lesions in the innominate arteries and to changes in cardiovascular function (heart rate variability, blood pressure, and ECG) and NO mediated dilation in older apolipoprotein E deficient mice.

Specific Aim 2: To determine whether both acute and chronic exposure to diesel exhaust induce comparable systemic cytokine responses and progression and destabilization of advanced atherosclerotic lesions in the innominate arteries, and changes in cardiovascular function and NO mediated dilation, in apolipoprotein E deficient mice that have increased or decreased capacities for producing the main endogenous antioxidant, glutathione.

Apolipoprotein E Deficient Mice

- Plump, A.S., Smith, J.D., Hayek, T., Aalto-Setälä, K., Walsh, A., Verstuyft, J.G., Rubin, E.M., and Breslow, J.L. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 71:343-353.
- Zhang, S.H., Reddick, R.L., Piedrahita, J.A., and Maeda, N. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258:468-471.



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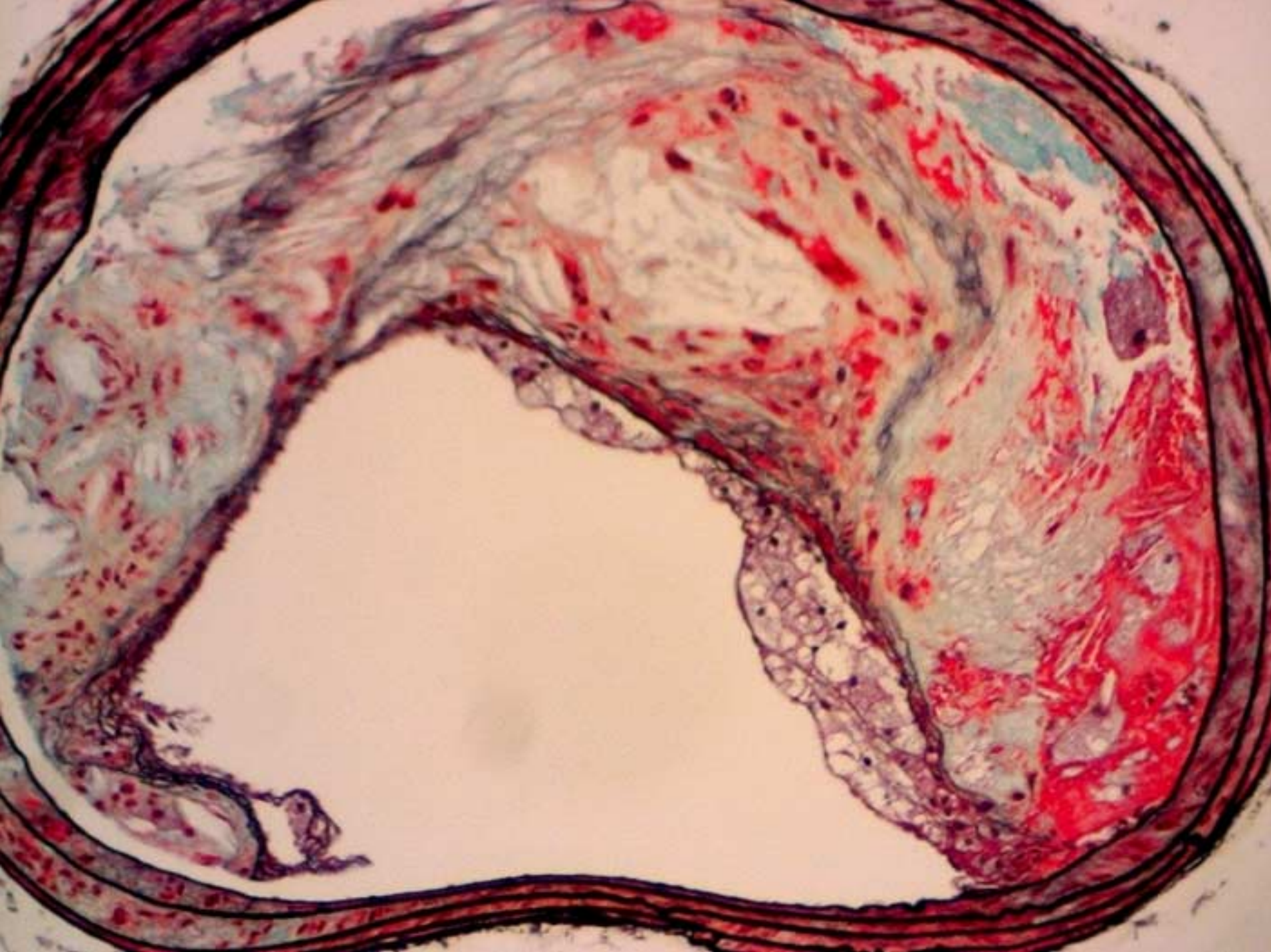
Characteristics of Unstable Plaques

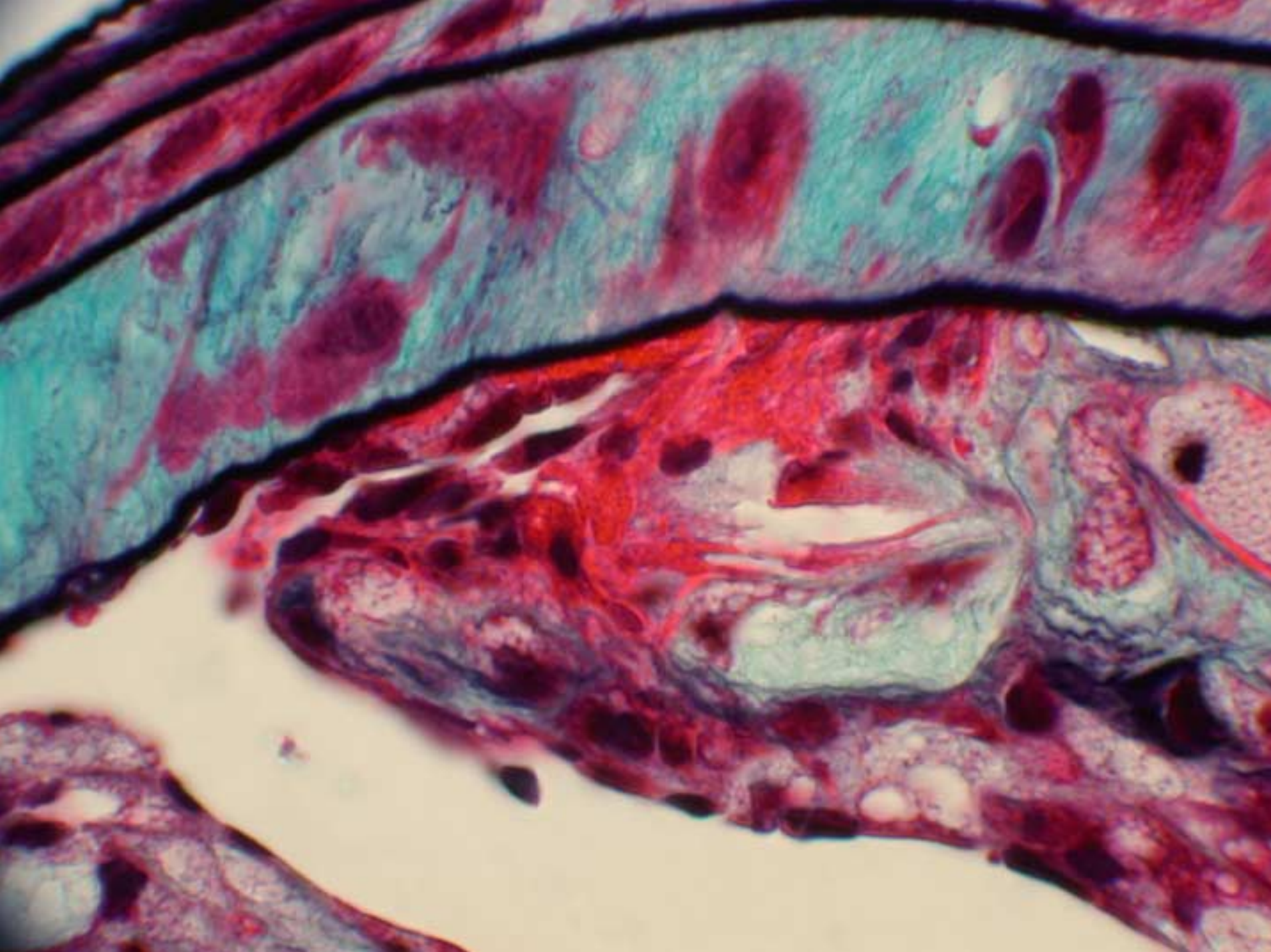
- Large Necrotic Core
- Thin Fibrous Cap
- Rupture/Fissure at Lateral Margins
- Intra-plaque Hemorrhage
- Thrombosis
- Calcification

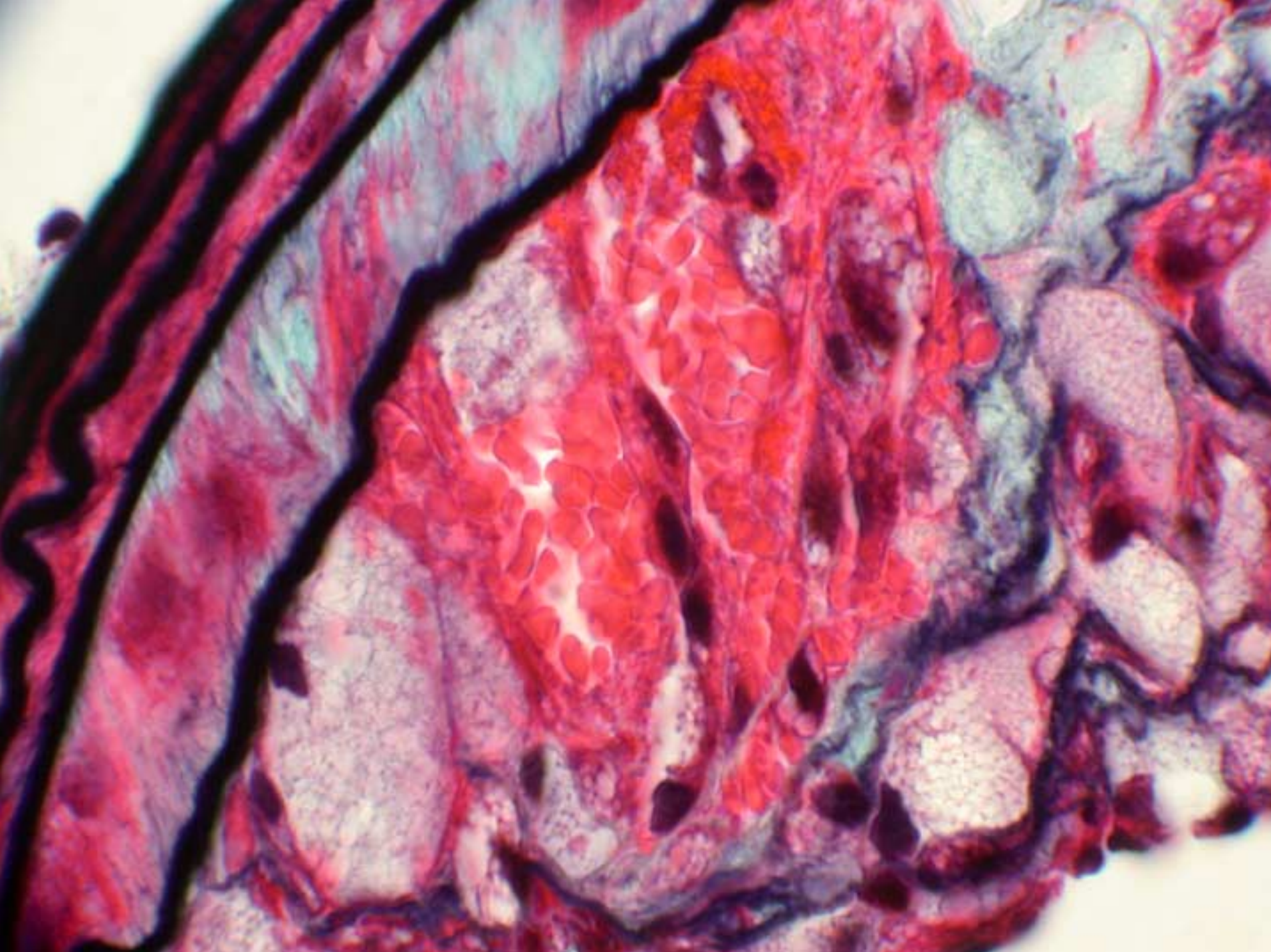
Movat's Pentachrome Stain

- Yellow = Collagen
- Turquoise Blue = Proteoglycan
- Black = Elastin
- Purple = Nuclei
- Red = Cell Cytoplasm, Plasma Proteins (fibrin), RBCs

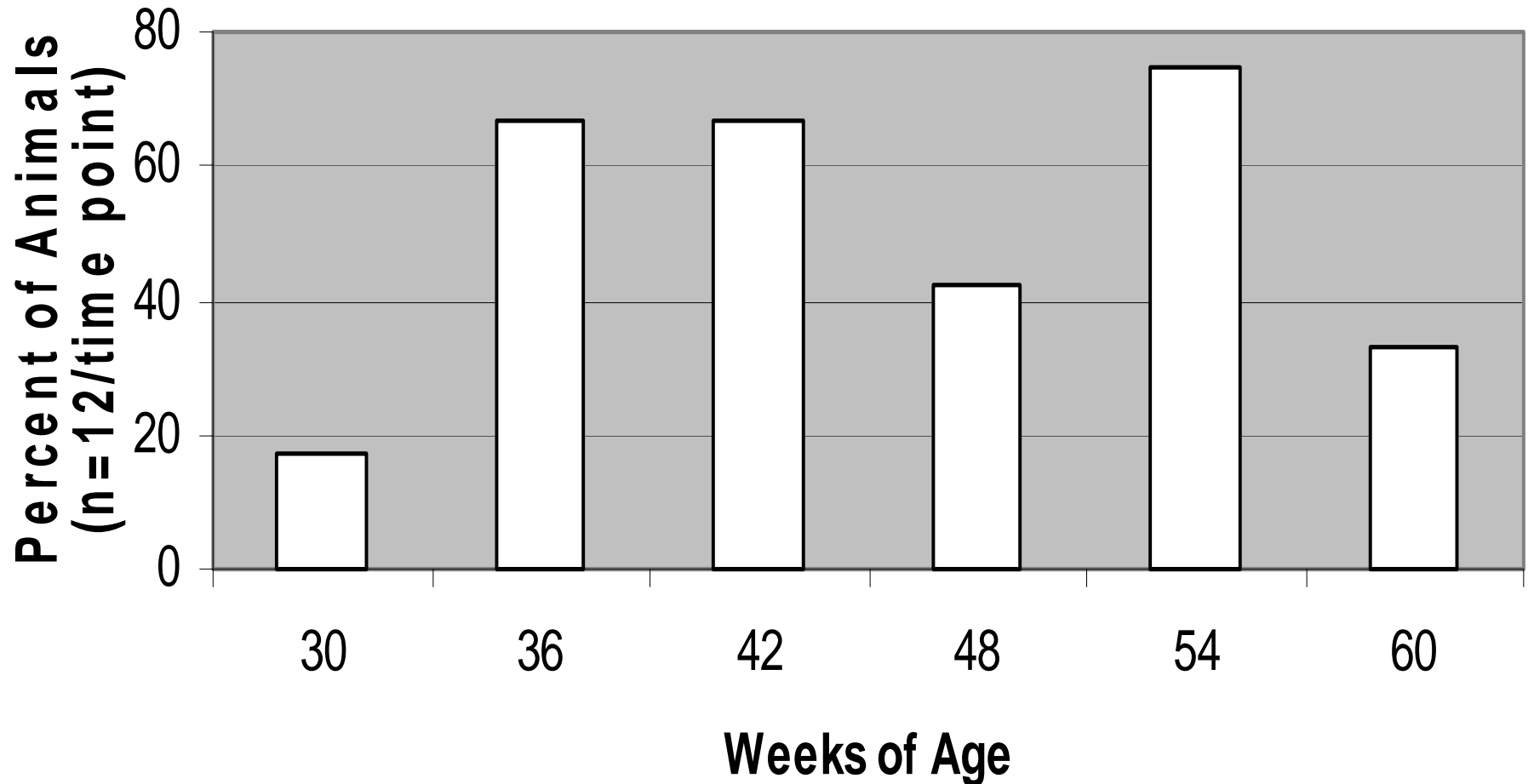


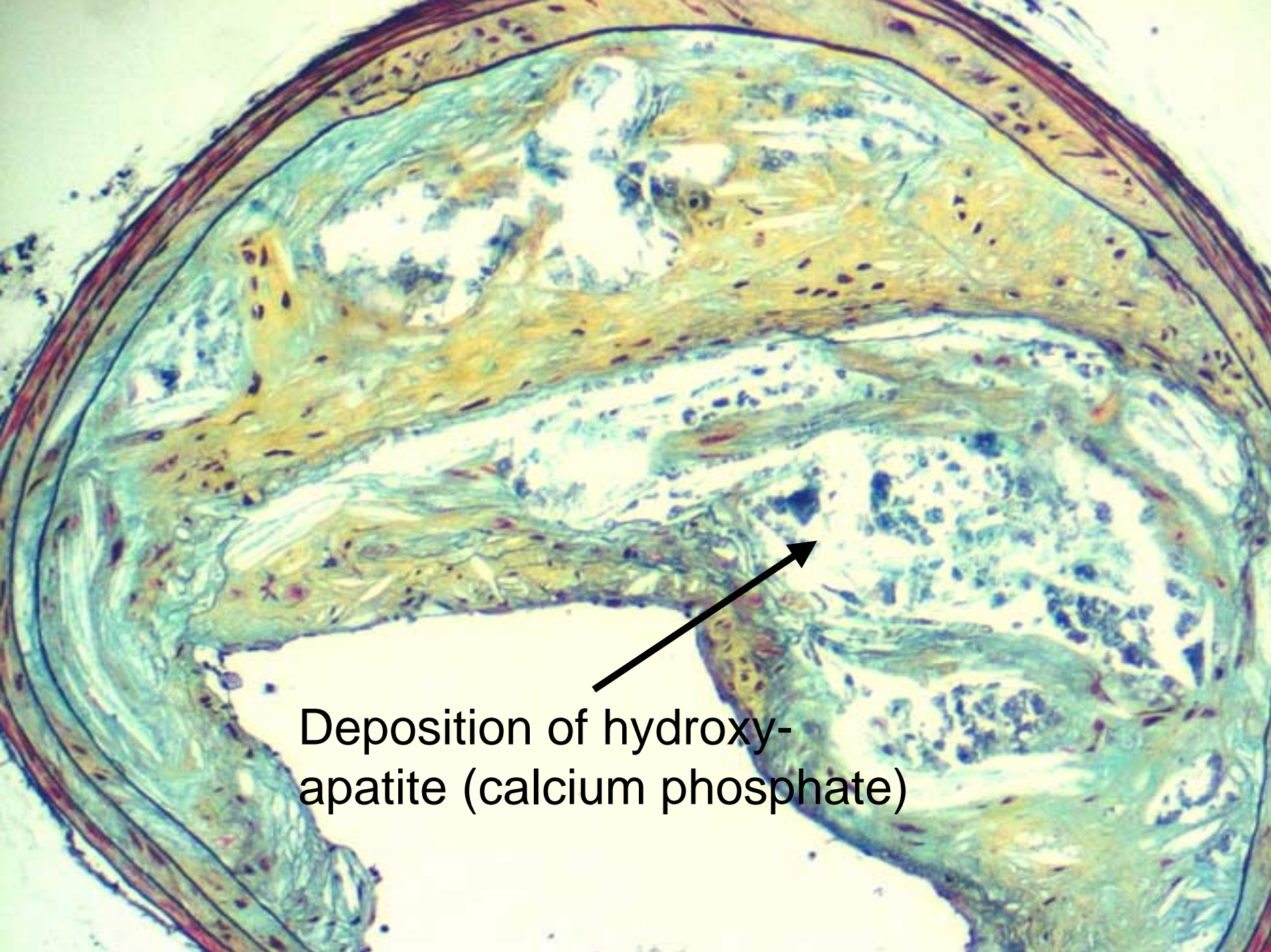






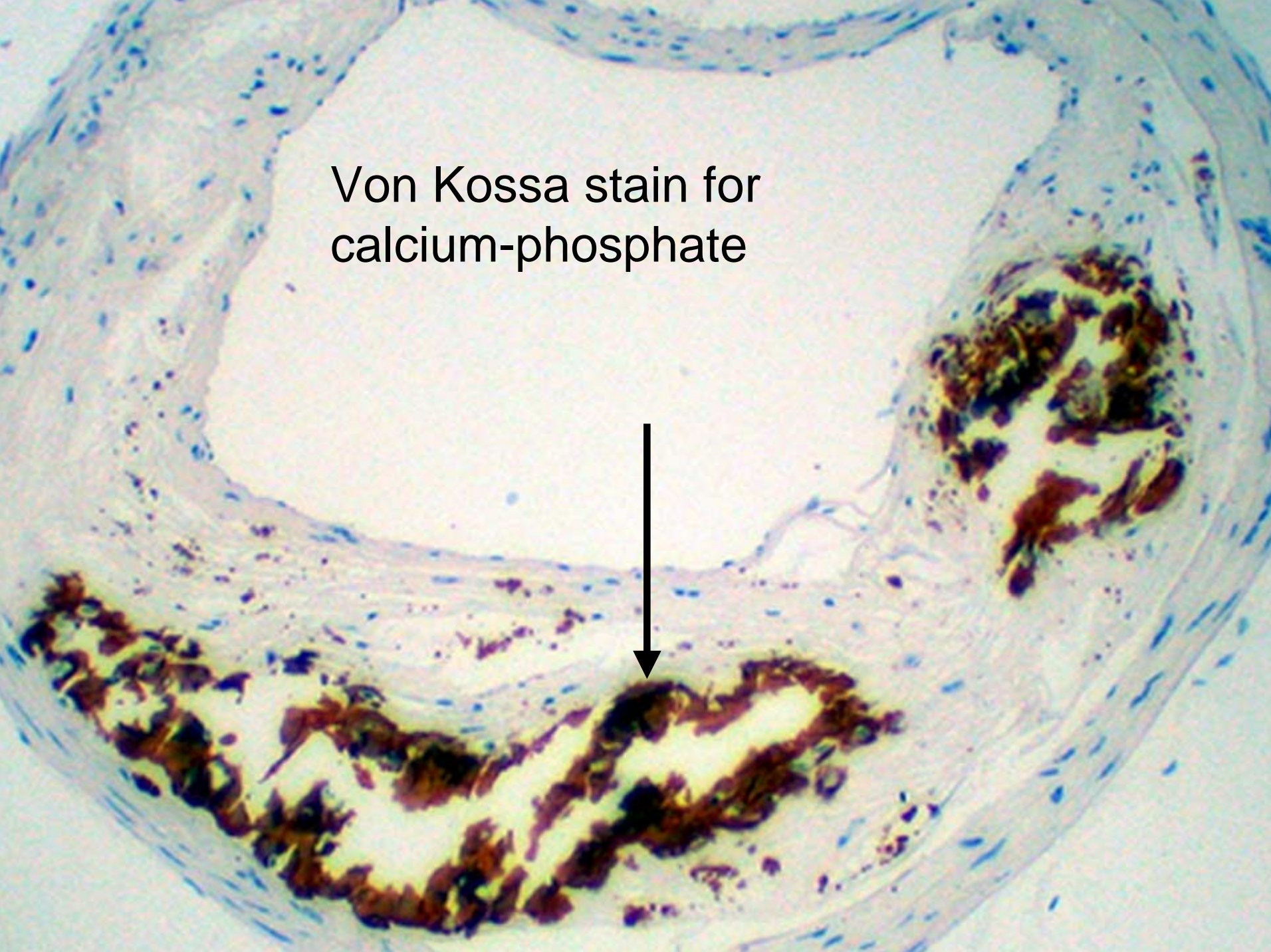
Frequency of Hemorrhage in Atherosclerotic Lesions in the Innominate Artery of Older Apo E^{-/-} Mice





Deposition of hydroxy-apatite (calcium phosphate)

Von Kossa stain for
calcium-phosphate



Subaim 1A. To determine whether acute exposure to diesel exhaust stimulates the progression of advanced atherosclerotic lesions and contributes to measures of plaque instability in older apo E^{-/-} mice with established lesions.

Exposure to: 100 ug/m ³	6 hours – 1 day	6 hours- 3 days	6 hours – 7 days
Sacrifices – time post last exposure	1 day	1 day	1 day
	3 days	3 days	3 days
	2 weeks	2 weeks	2 weeks
Exposure to: 200 ug/m ³	1 day	3 days	7 days
Sacrifices – time post last exposure	1 day	1 day	1 day
	3 days	3 days	3 days
	2 weeks	2 weeks	2 weeks
Exposure to: 400 ug/m ³	1 day	3 days	7 days
Sacrifices – time post last exposure	1 day	1 day	1 day
	3 days	3 days	3 days
	2 weeks	2 weeks	2 weeks

Time Course and Dose Response for Acute Exposure to Diesel Exhaust

Lesion characteristic	Quantitation	Procedure
Lesion and lumen size	Area	Movat's pentachrome stain
Thin fibrous cap (<2 cell layers)	Frequency	Movat's
Fibrous cap breaks	Frequency	Movat's
Central necrotic core with cholesterol clefts (>50% of lesion area)	Frequency	Movat's
Luminal occlusion	% and frequency	Movat's
Elastin content	Area as % total lesion area	Verhoeff-Van Gieson stain
Collagen content	Area as % total lesion area	Sirius red and polarized microscopy
Intra-plaque hemorrhage	Frequency and location	Movat's and Perls' iron stain
Lateral xanthomas	Frequency	Movat's
Macrophage content	Area as % total lesion area	Immunocytochemistry (anti-Mac-2, Pharmingen)
SMC content	Area as % total lesion area	Immunocytochemistry (anti-alpha actin, Dako)
Cell death	Frequency and area as % total lesion area	TUNEL and Immunocytochemistry (anti-cleaved caspase 3, Cell Signaling Technology)
Tissue factor expression	Frequency and area as % total lesion area	Immunocytochemistry (binding of digoxigenin labeled factor VIIa, American Diagnostica)
MMP expression	Area as % total lesion area	Immunocytochemistry (anti-MMP 3,7,9, Santa Cruz)
Calcification	Frequency and area as % total lesion area	von Kossa stain
Activated NFkB and AP-1	Area as % total lesion area	Immunocytochemistry (anti-active p65, Roche)

Subaim 1B. To determine whether chronic exposure to diesel exhaust stimulates the progression of advanced atherosclerotic lesions and contributes to measures of plaque instability in older apo E^{-/-} mice with established lesions.

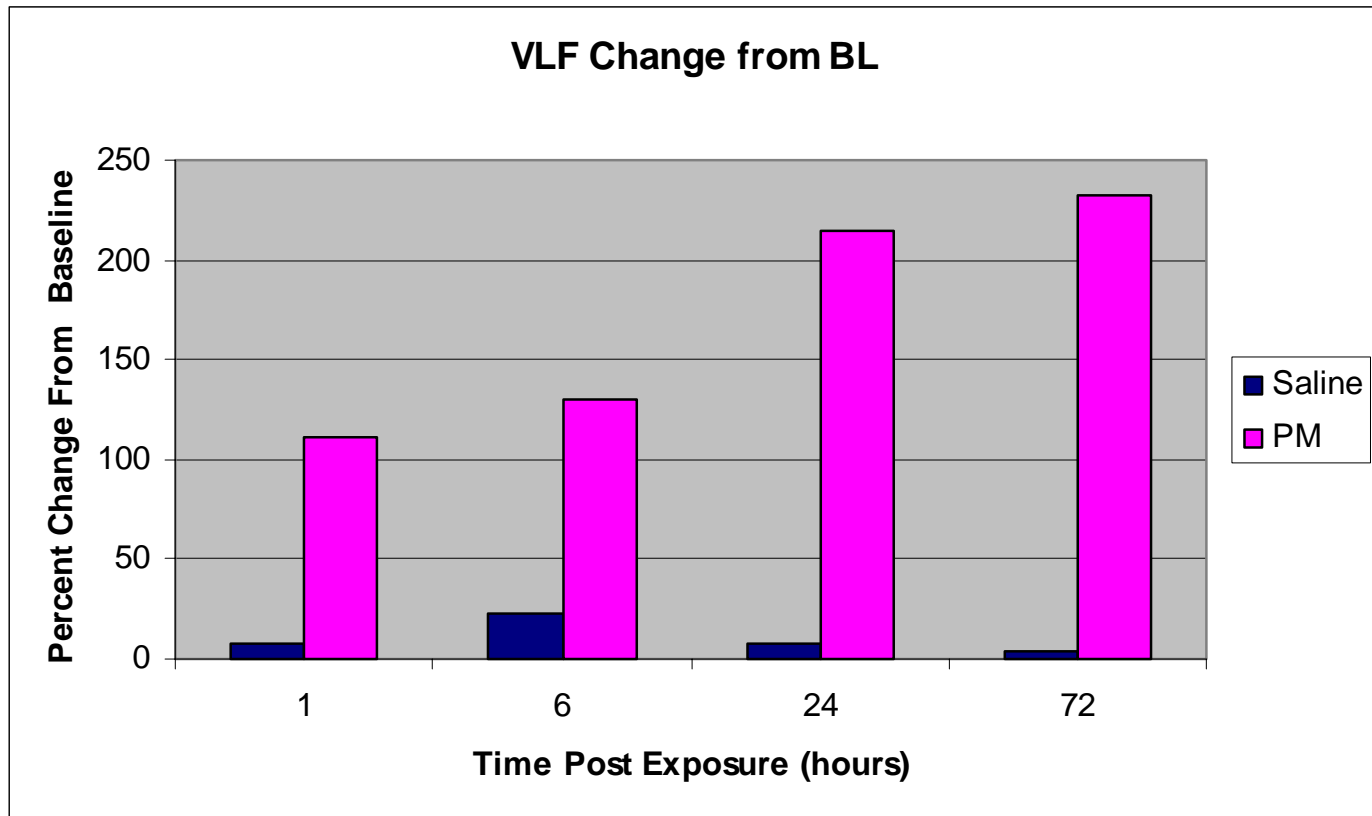
Exposure to: 100 ug/m ³	5 days/wk for 4 weeks	5 days/wk for 8 weeks
Sacrifices – time post last exposure	1 day	1 day
	3 days	3 days
	2 weeks	2 weeks
Exposure to: 200 ug/m ³	5 days/wk for 4 weeks	5 days/wk for 8 weeks
Sacrifices – time post last exposure	1 day	1 day
	3 days	3 days
	2 weeks	2 weeks
Exposure to: 400 ug/m ³	5 days/wk for 4 weeks	5 days/wk for 8 weeks
Sacrifices – time post last exposure	1 day	1 day
	3 days	3 days
	2 weeks	2 weeks

Time Course and Dose Response for Chronic Exposure to Diesel Exhaust

Subaim 1C. To determine the types and amounts of pro- and anti-inflammatory cytokines and other acute phase stress proteins secreted into the lungs and blood following acute and chronic exposure of older apo E-/- mice to diesel exhaust.

Subaim 1D. To determine the effects of acute and chronic diesel exhaust exposure on cardiovascular function (heart rate variability, changes in blood pressure and ECG) in older apo E-/- mice.

Heart Rate Variability in ApoE^{-/-} Mice Based on Frequency Domain Analysis



Increase in VLF band (0.05-0.4 Hz) following exposure to Seattle PM. Suggested that an increase in the VLF reflects neurohumoral influences.

Subaim 1E-1. To determine the effects of acute and chronic exposures to diesel exhaust on endothelial dependent and independent vasodilation of the innominate arteries and aorta in older apo E-/- mice.

Subaim 1E-2. To determine the endothelial dependent and independent vasodilation response of innominate arteries and aorta of apo E-/- mice following **ex vivo exposure to the particulate and soluble components of diesel exhaust.**

PM₁₀-induced endothelial dysfunction

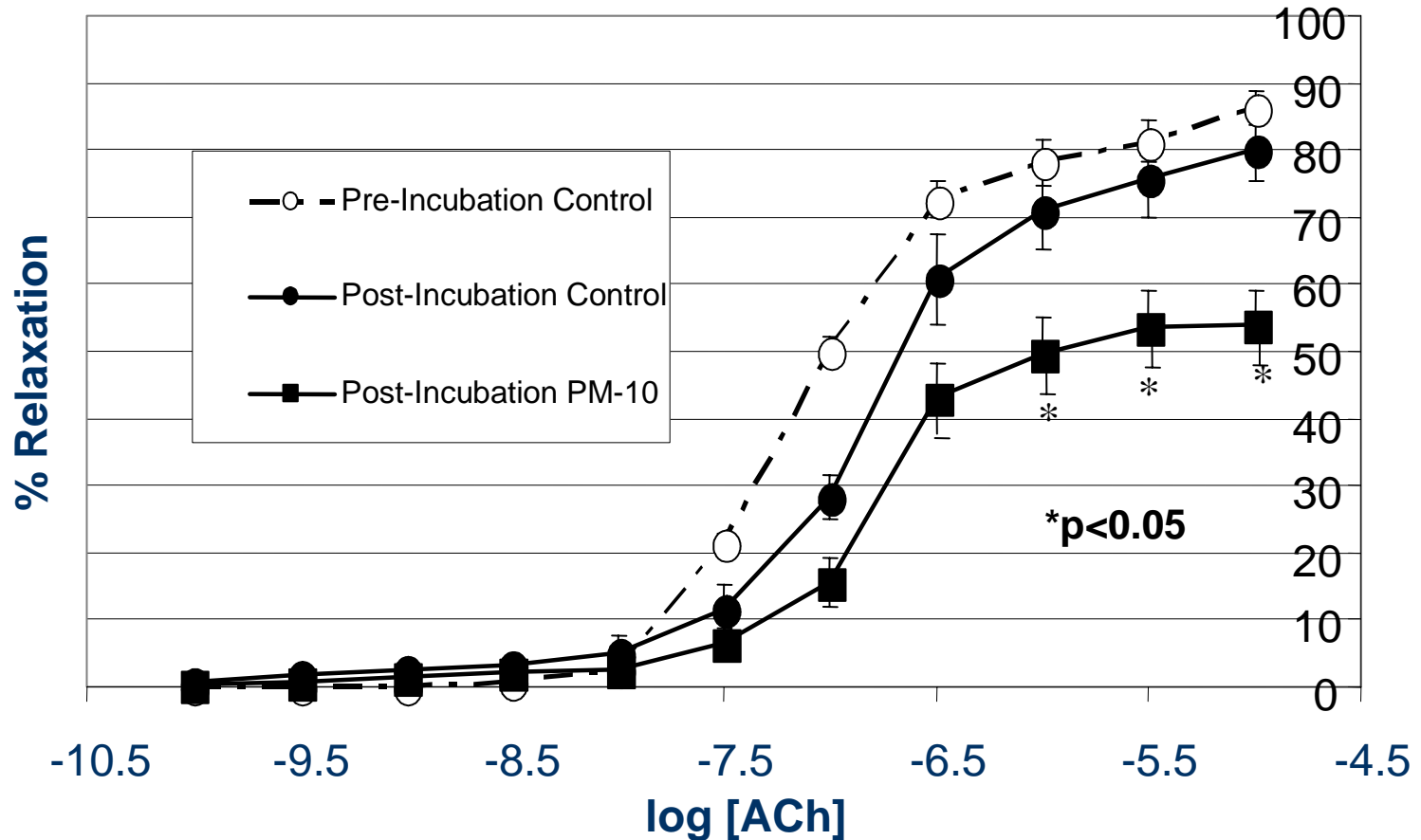
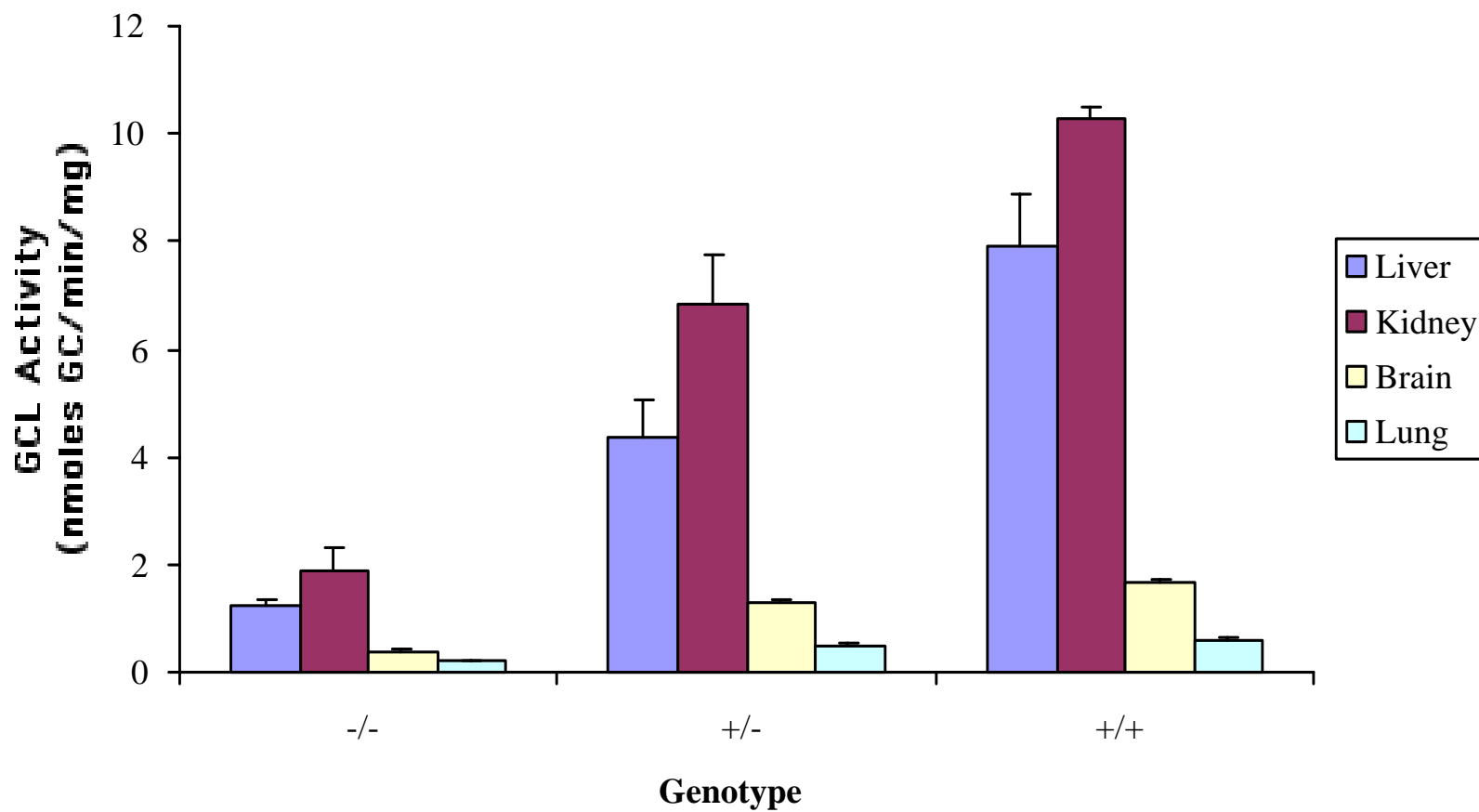


Figure 1: PM₁₀-induced (EHC93 particles <0.1 μ m) reduces acetylcholine mediated relaxation of pre-constricted (phenylephrine) aortic strips.

Subaim 2A. To determine whether acute and/or chronic exposure to diesel exhaust stimulates an inflammatory response in the lungs and the progression of advanced atherosclerotic lesions and contributes to measures of plaque instability in older apo E-/- mice that have a **reduced capacity to produce glutathione** due to a homozygous deficiency in the modulatory subunit of glutamate cysteine ligase.

Mean GCL Activities in Female GCLM KO Mice



Subaim 2B. To determine whether acute and/or chronic exposure to diesel exhaust stimulates an inflammatory response in the lungs and the progression of advanced atherosclerotic lesions and contributes to measures of plaque instability in older apo E-/- mice that have **an increased capacity to produce glutathione** due to over-expression of the catalytic subunit of glutamate cysteine ligase in macrophages.

TgCD681040 +/- ApoE +/- Expression of GCL-c
Normalized to GAPDH

