

BRIDGING CARDIOVASCULAR *IN VITRO* AND *IN VIVO* MODELS

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Presentation Outline

1. Introduction

2. *In Vivo* » ApoE-/- mouse model

- Description of the model
- Pathology and biochemistry
- Gene expression changes in thoracic aorta

3. *In Vitro* » Acute and Chronic

- Gene expression changes

4. Connections between *in vivo* and *in vitro* models

Introduction: Why Study CVD?

- CVD contributes to a third of deaths per year in the USA
- Atherosclerosis is an inflammatory disease characterized by cholesterol accumulation in medium and large arteries
- Several risk factors contribute to the development of atherosclerosis; for example:
 - Smoking, Atherogenic Diet, Exercise
 - Genetics, Gender, Age

Pre-clinical Model for Cardiovascular Disease

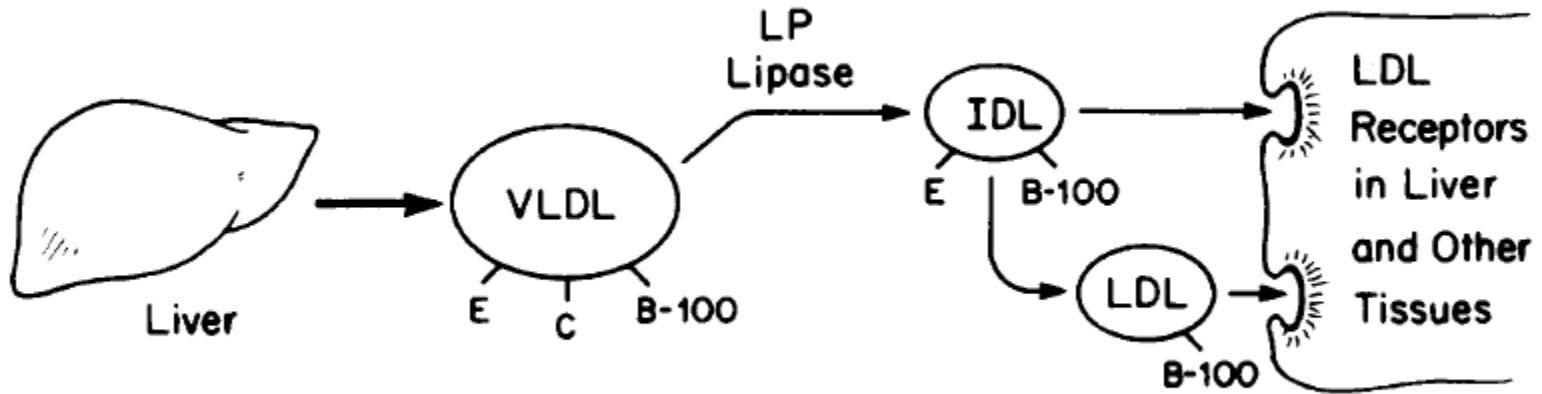
ApoE $-/-$ Mouse and Mainstream Cigarette Smoke



<http://jaxmice.jax.org/images/jaxmicedb/featuredImage/002052.jpg>

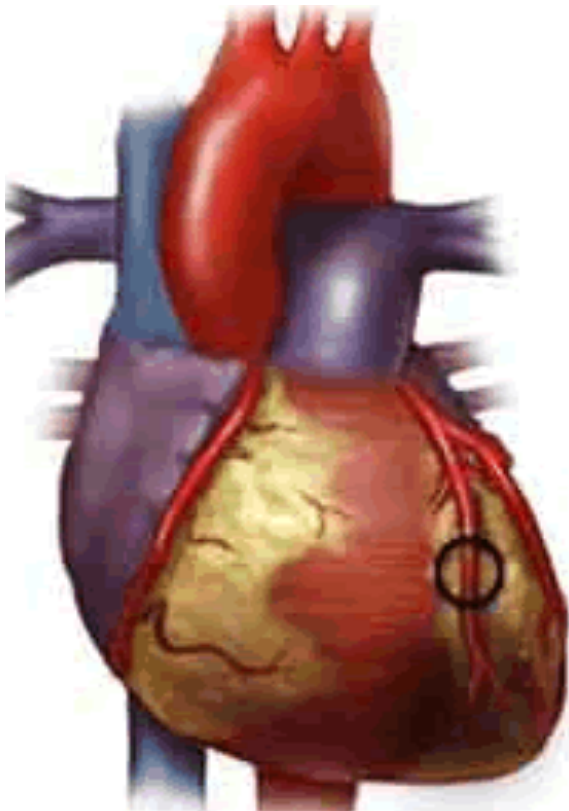
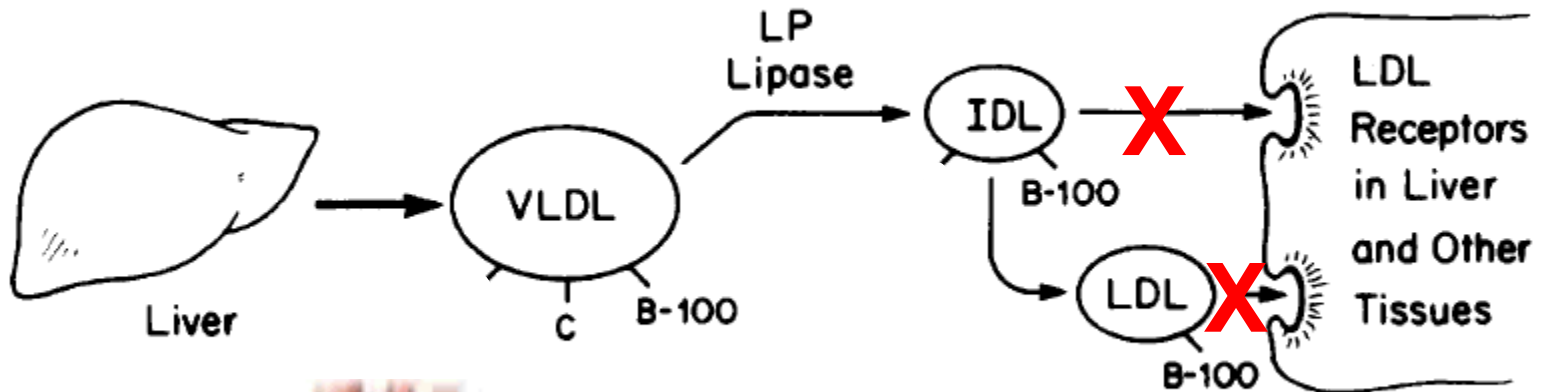
With permission from The Jackson Laboratory

Apolipoprotein E



Republished with permission
Brown and Goldstein
J Clin Invest. 1983 Sep;72(3):743-7

Apolipoprotein E



Normal coronary artery



Atherosclerosis



Atherosclerosis with blood clot



In Vivo Study Design Summary

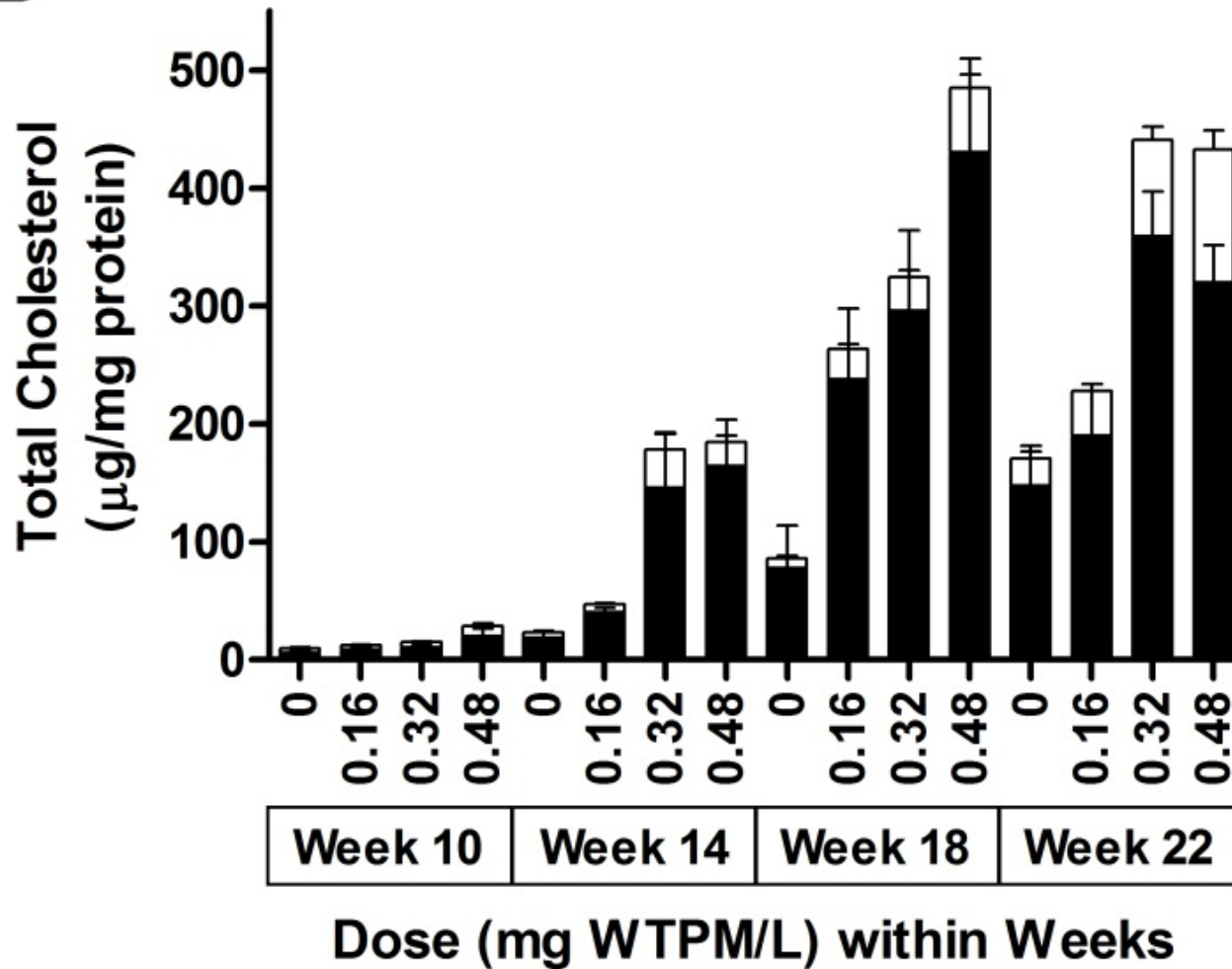
- **Nose-only exposure**
- **Exposure concentrations ranged from 0 - 0.48 mg WTPM/L**
 - Duration: 3 hrs/day, 5 days/week
- **Diet**
 - Chow or Atherogenic
- **Measured various endpoints**
 - Arterial cholesterol
 - Serum lipids
 - Histology (traditional and virtual)
 - Aortic gene expression changes



Arterial Cholesterol

Thoracic Aorta

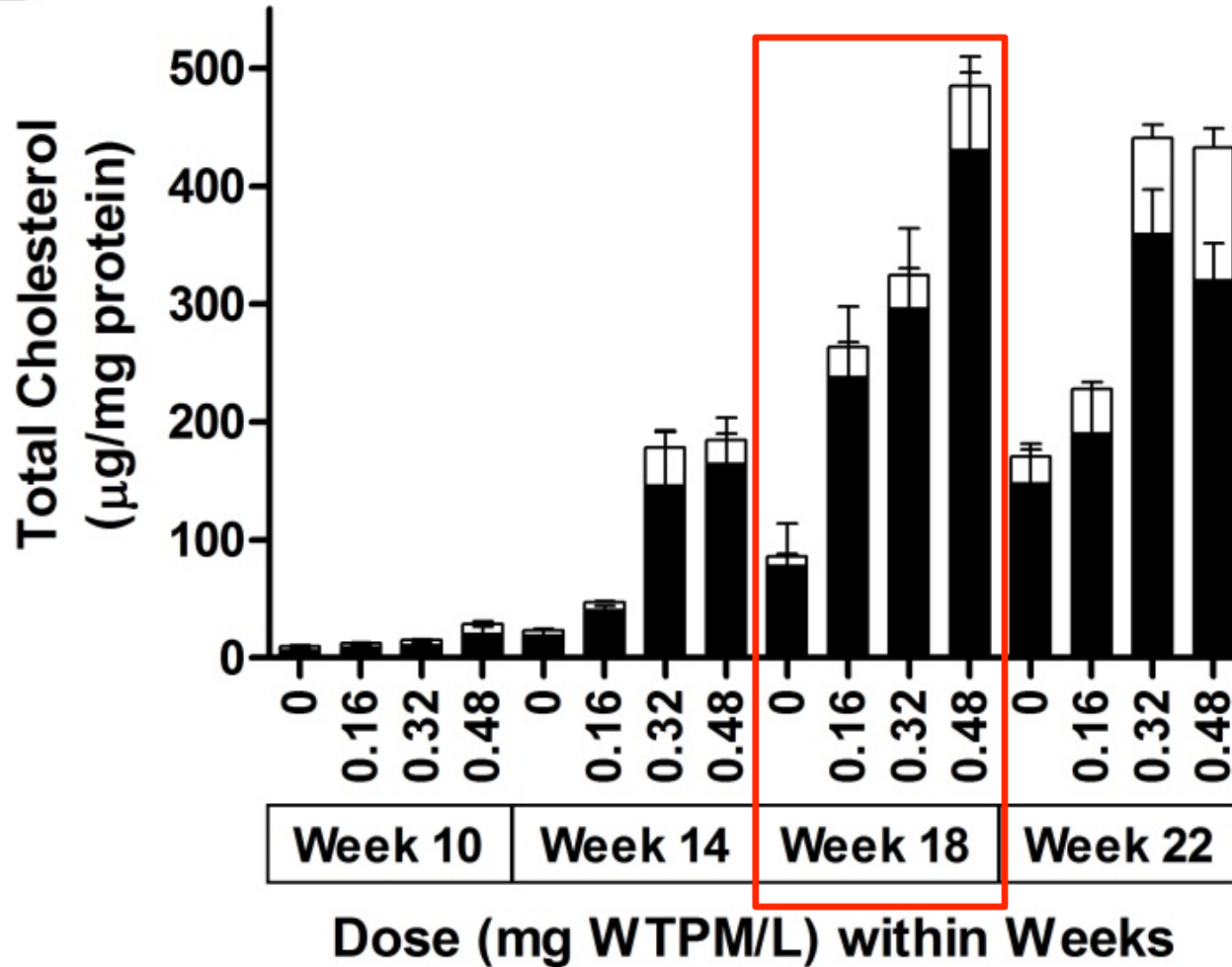
B



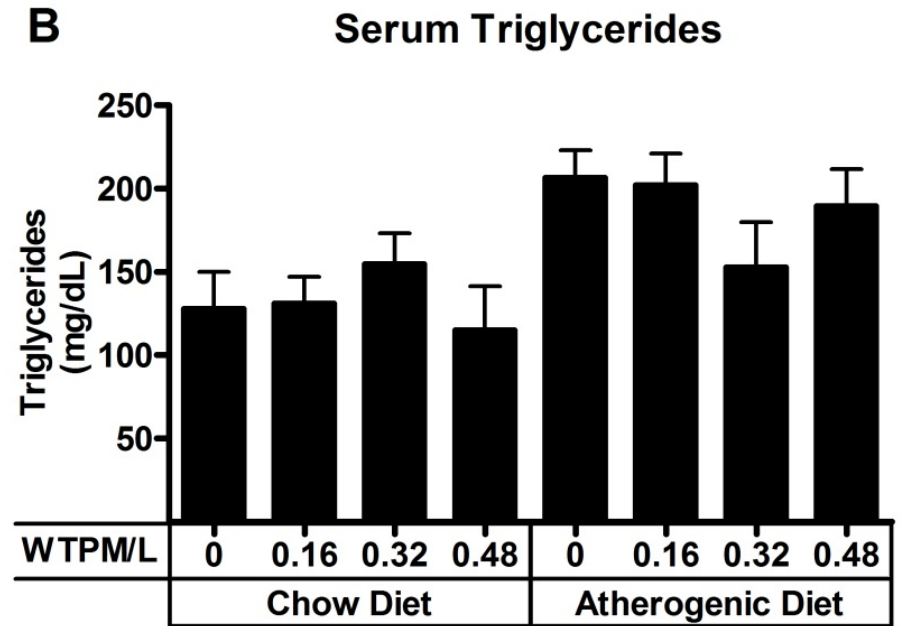
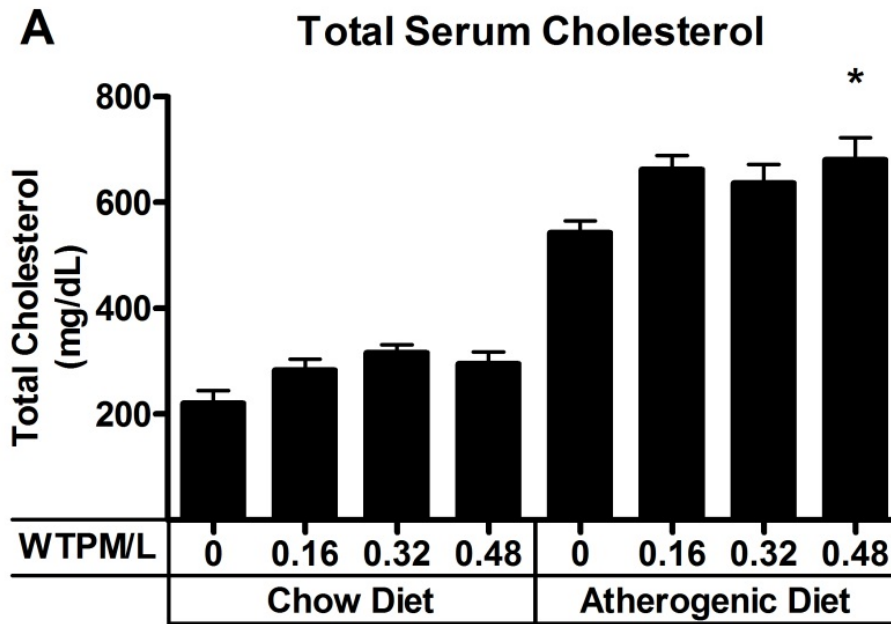
Arterial Cholesterol

Thoracic Aorta

B

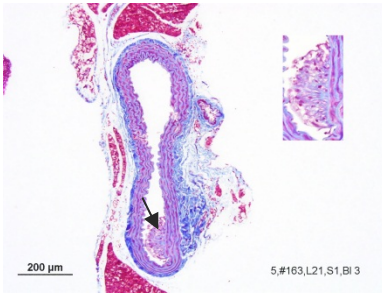


Serum Lipids

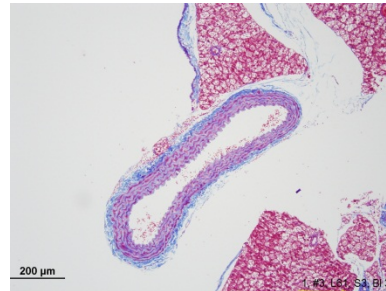


Histology (18 weeks)

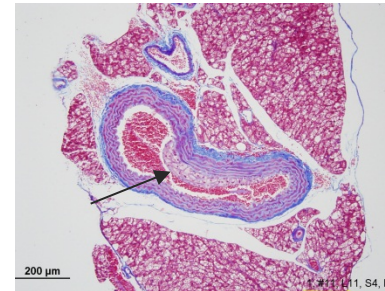
A. Chow Diet



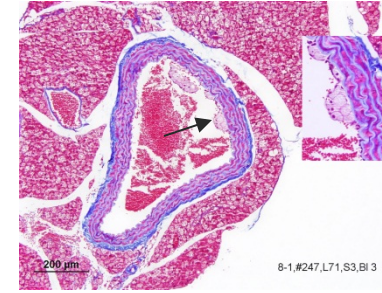
B. Chow Diet, 0.16 mg WTPM/L



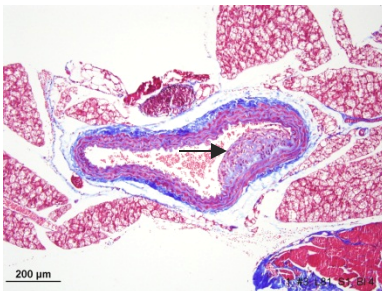
C. Chow Diet, 0.32 mg WTPM/L



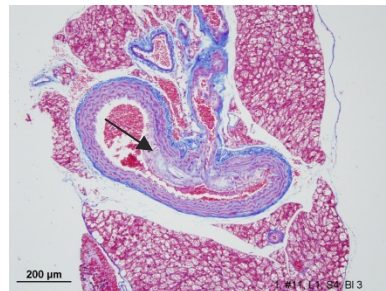
D. Chow Diet, 0.48 mg WTPM/L



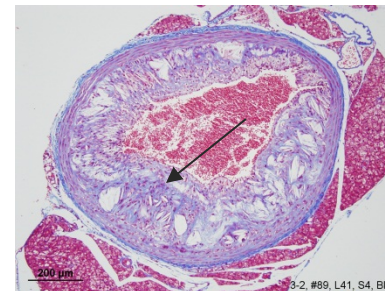
E. Atherogenic Diet



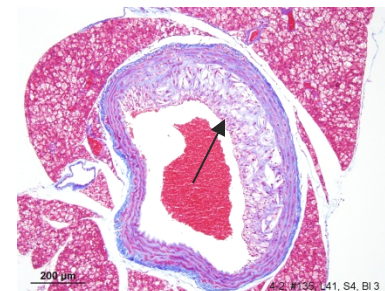
F. Atherogenic Diet, 0.16 mg WTPM/L



G. Atherogenic Diet, 0.32 mg WTPM/L

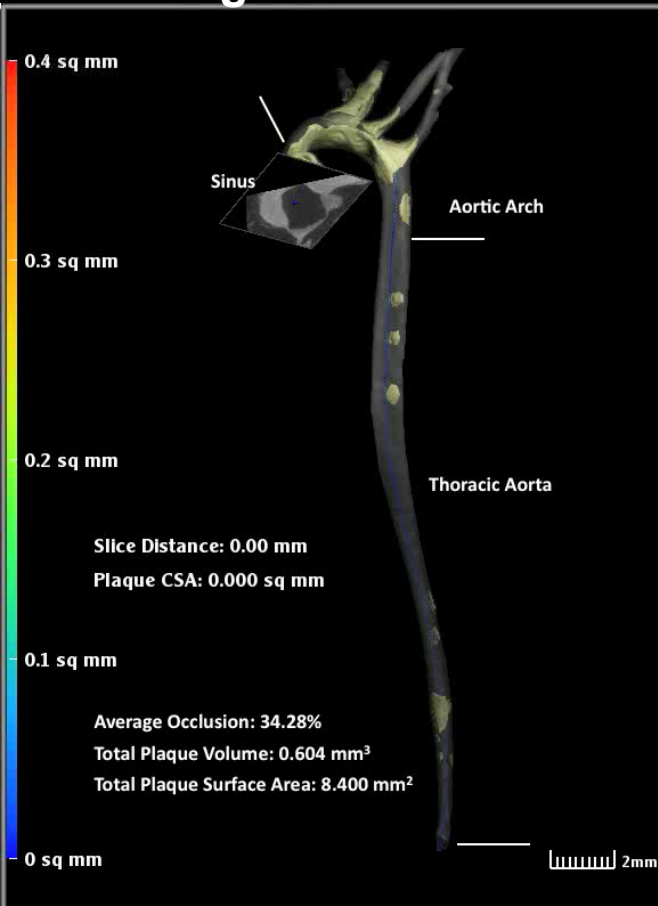


H. Atherogenic Diet, 0.48 mg WTPM/L

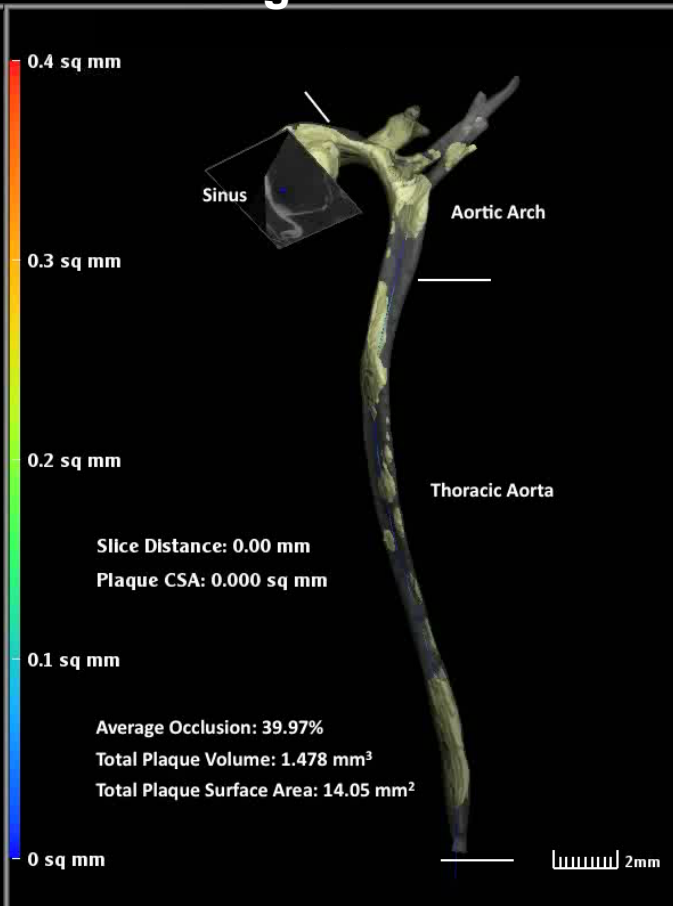


Virtual Histology

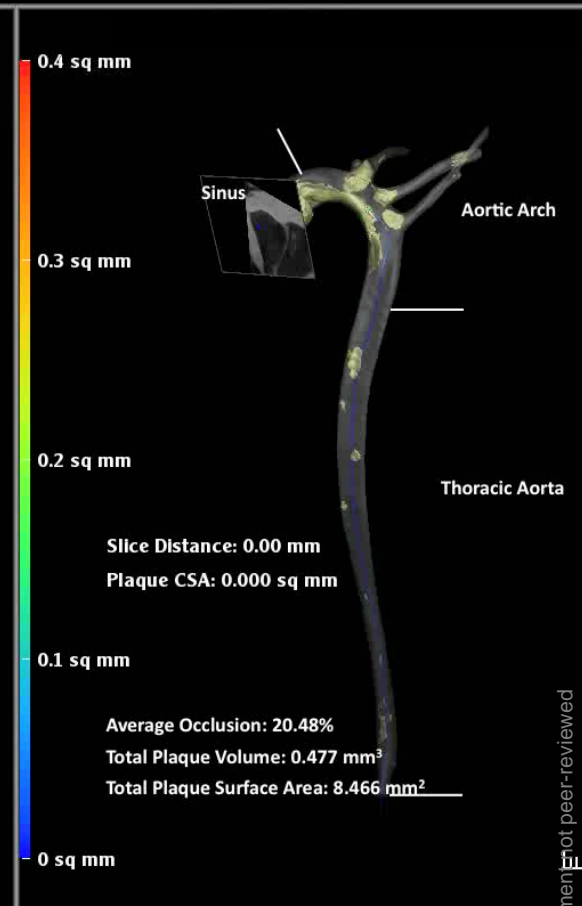
Atherogenic Diet/Sham



Atherogenic Diet/MCS



Chow Diet/MCS



Gene Expression Analyses

Thoracic Aorta (Atherogenic Diet)

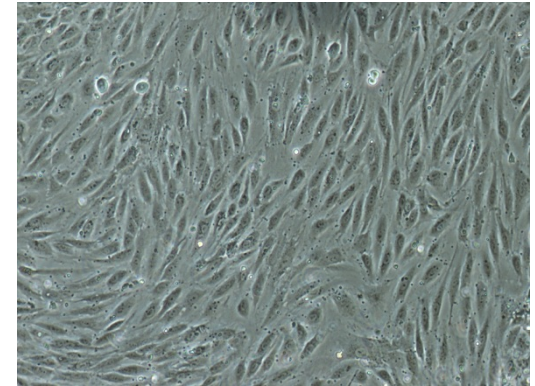
mg WTPM/L	Total	Up Regulated	Down Regulated
0.32	2909	1970	939

Fold change threshold +/- 2

Gene Identifier	Gene Title	Chow Diet				Atherogenic Diet			
		Fold Change							
		MTS (mg WTPM/L)							
		0	0.16	0.32	0.48	0	0.16	0.32	0.48
BC015293	Gremlin 1	1	1.29	2.69	1.66	3.97	37.46	95.97	95.82
NM_031167	Interleukin 1 receptor antagonist (Il1rn), transcript variant 1	1	4.57	4.45	3.24	10.84	44.21	85.58	45.90
BC008107	Tissue inhibitor of metalloproteinase 1	1	7.21	10.21	5.57	5.92	37.65	74.43	45.64
X14607	SV-40 induced 24p3	1	3.05	3.98	2.79	2.34	27.94	90.56	45.05
NM_020001	C-type lectin domain family 4, member n	1	2.56	4.00	5.19	6.36	15.58	35.15	40.32
BC018397	Cellular retinoic acid binding protein II	1	4.68	5.50	4.64	7.32	30.28	52.92	38.67
BC013271	Annexin A8	1	4.74	5.83	4.12	6.68	26.60	49.68	38.55
AK004847	E3 ubiquitin ligase (Greul1)	1	2.31	3.26	3.13	2.62	23.51	45.27	35.86
BB311924	G protein-coupled receptor 176 (Gpr176)	1	1.45	2.68	1.85	3.58	16.37	31.63	32.48

In Vitro model of CVD

- **Primary cultures of human aortic endothelial cells (HAEC)**
- **Acute and chronic models of tobacco exposure**
- **Exposed to aqueous extracts of tobacco**
 - **Acute model: 24 hrs continuously**
 - **Chronic model: 6 hrs/day; 5 days/week**
- **Endpoint – gene expression**



Chronic HAEC Model

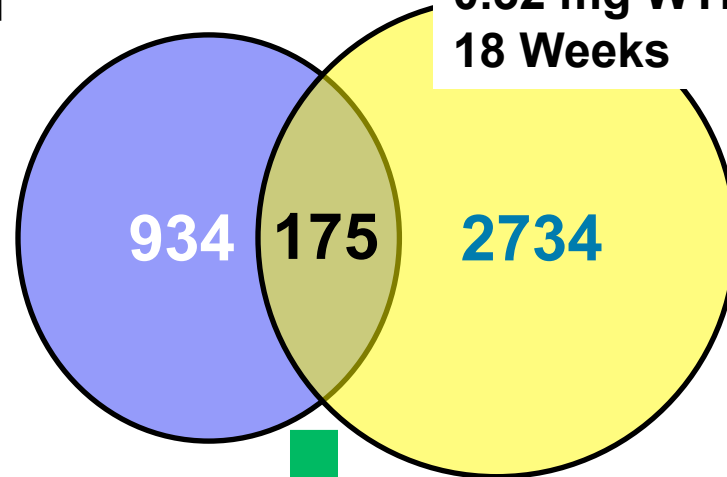
Days of Exposure	Nicotine Eq. Concentration (ng/mL)	Total	Up Regulated	Down Regulated
19	50	1109	818	291

Gene Title	Day 1			Day 3			Day 5			Day 12			Day 19			Day 26		
	Ctl	50	500	Ctl	50	500	Ctl	50	500	Ctl	50	500	Ctl	50	500	Ctl	50	500
Cytochrome P450, family 1, subfamily B, polypeptide 1	1	47.1	126.0	1	73.5	439.6	1	141.1	891.2	1	228.0	592.9	1	145.0	630.1	1	108.5	531.1
Cytochrome P450, family 1, subfamily B, polypeptide 1	1	37.7	104.0	1	47.2	264.3	1	83.1	492.8	1	163.8	385.7	1	86.0	401.4	1	60.4	264.2
Transcribed locus	1	27.9	67.0	1	28.1	155.6	1	72.1	376.3	1	129.5	311.6	1	72.1	337.8	1	35.8	172.7
SPARC-like 1 (hevin)	1	-1.0	1.9	1	1.1	10.9	1	-1.1	-33.4	1	-2.2	-111.0	1	1.1	-38.5	1	-1.3	-146.1
Cytochrome P450, family 1, subfamily A, polypeptide 1	1	12.5	18.5	1	24.8	43.3	1	27.6	42.3	1	13.5	17.1	1	14.2	20.0	1	16.4	14.7
MRNA; cDNA DKFZp686B14224 (from clone DKFZp686B14224)	1	3.5	18.9	1	4.1	43.2	1	1.9	60.9	1	14.4	41.8	1	3.3	35.5	1	2.0	10.5
Periostin, osteoblast specific factor	1	1.4	-1.2	1	-1.5	-6.1	1	-1.2	-8.2	1	-1.9	-18.3	1	-1.5	-46.8	1	-1.7	-138.3
Endothelin receptor type B	1	2.9	91.1	1	1.1	22.9	1	2.4	29.5	1	2.9	15.4	1	2.6	23.5	1	2.0	29.0

Linking *In Vitro* and *In Vivo* models

HAEC
50 ng/mL NicEq
19 Days

Thoracic Aorta
0.32 mg WTPM/L
18 Weeks



~20 genes



89 Genes expressed in same direction (both up- or down-regulated)

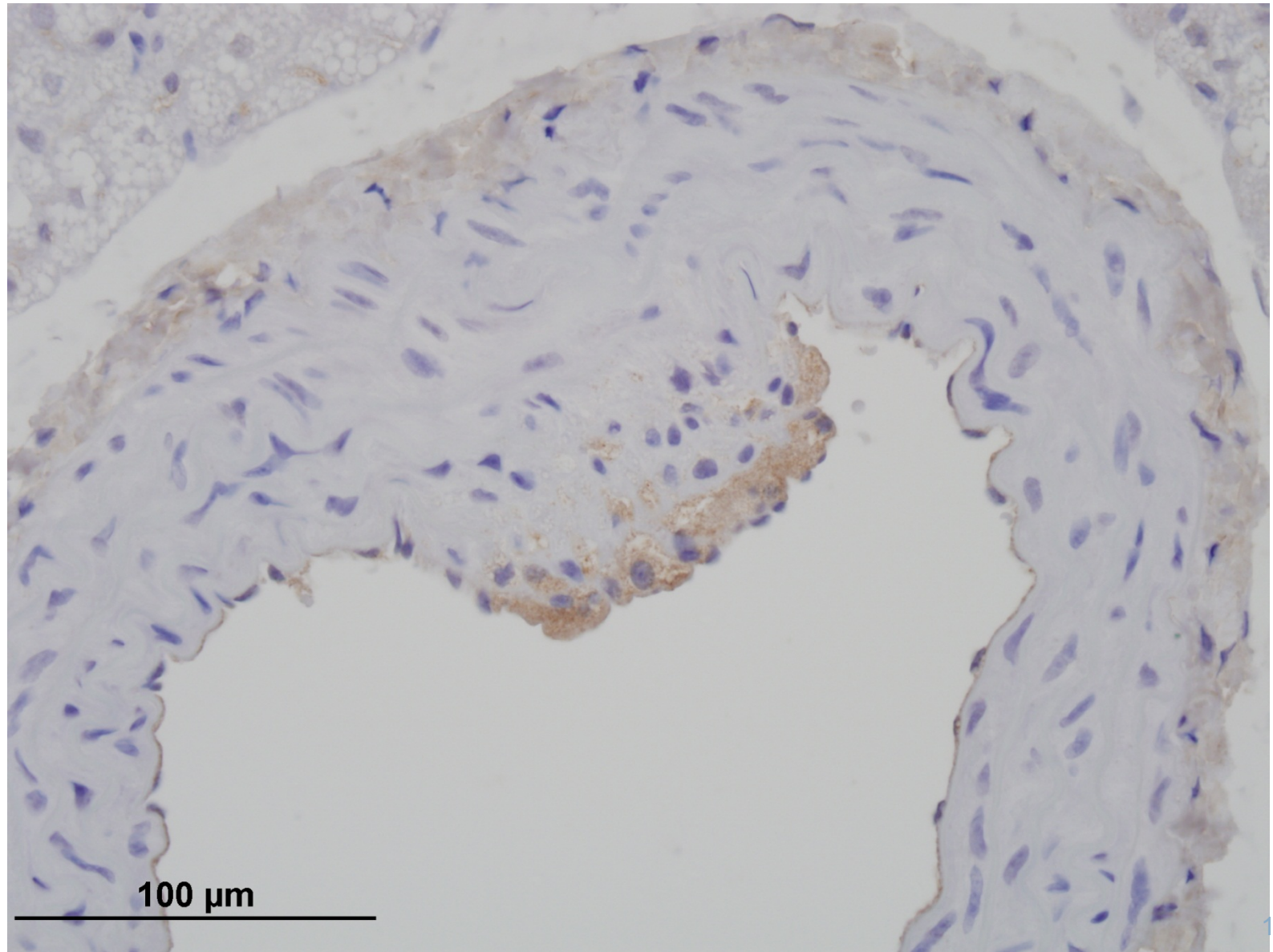
Thoracic Aorta Gene Expression

Gene Identifier	Gene Title	Chow Diet				Atherogenic Diet			
		Fold Change							
		MTS (mg WTPM/L)							
		0	0.16	0.32	0.48	0	0.16	0.32	0.48
BC015293	Gremlin 1	1	1.29	2.69	1.66	3.97	37.46	95.97	95.82
NM_031167	Interleukin 1 receptor antagonist (Il1rn), transcript variant 1	1	4.57	4.45	3.24	16.64	44.21	85.98	45.98
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X14607	SV-40 induced 24p3	1	3.63	3.83	2.73	2.34	27.94	60.98	43.16
NM_020001	C-type lectin domain family 4, member n	1	2.86	4.90	3.16	6.34	15.66	35.13	40.32
BC018397	Cellular retinoic acid binding protein II	1	4.63	5.93	4.84	7.12	30.23	92.22	38.17

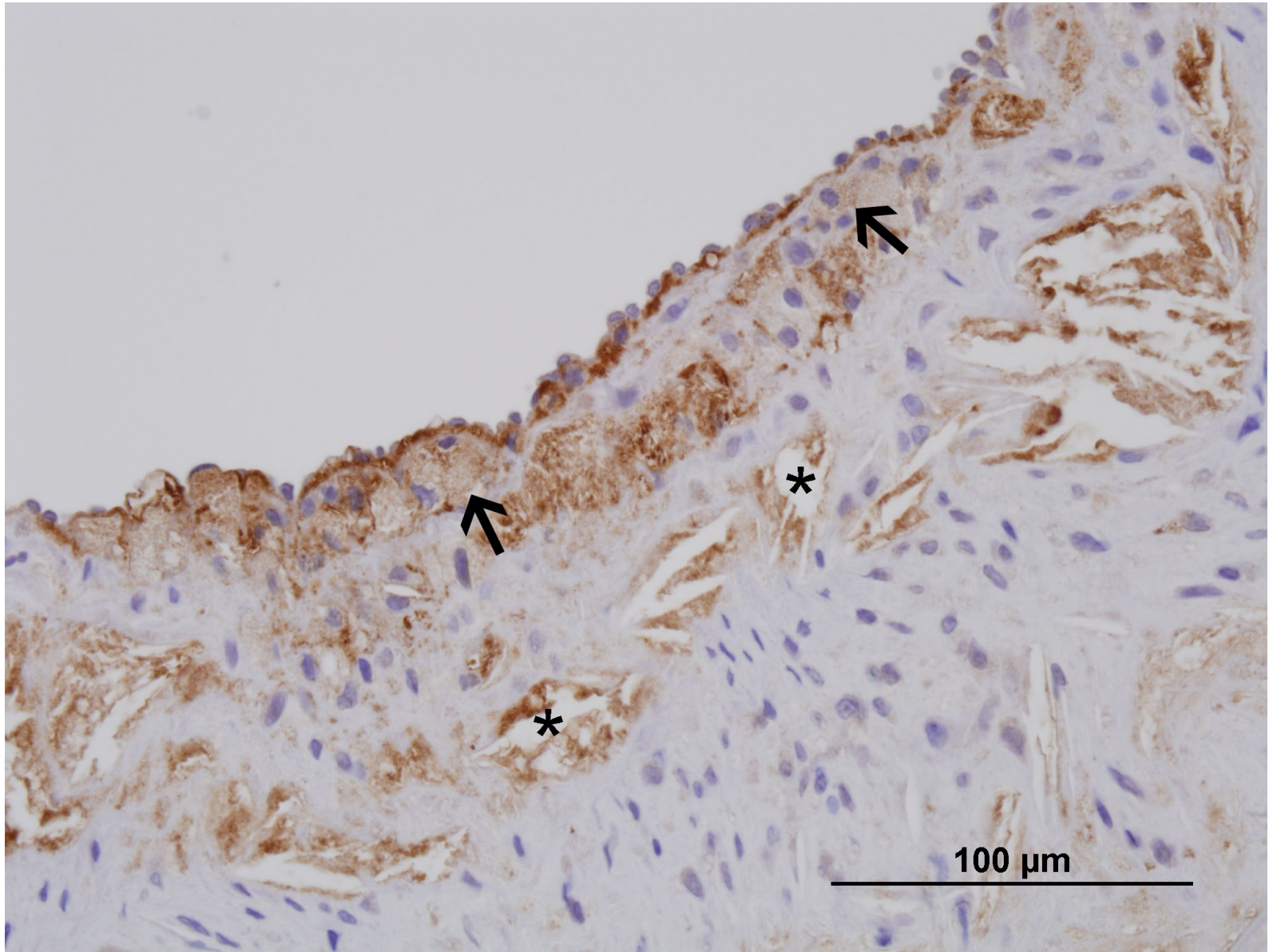
BC015293	Gremlin 1	1	1.29	2.69	1.66	3.97	37.46	95.97	95.82
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NM_031167	Interleukin 1 receptor antagonist (Il1rn), transcript variant 1	1	4.57	4.45	3.24	16.64	44.21	85.98	45.98
AK009736	Transcribed locus, strongly similar to XP_237907.2 PREDICTED: similar to transmembrane 7 superfamily member 1	1	3.92	3.49	3.38	6.11	24.69	49.31	31.37
M57525	Interleukin 1 receptor antagonist (Il1rn), transcript variant 1	1	2.84	3.63	3.73	8.33	22.11	44.79	29.73
NM_021334	Integrin alpha X (Itgax)	1	3.67	3.43	3.32	8.09	23.76	37.38	27.36
NM_009252	Serine (or cysteine) peptidase inhibitor, clade A, member 3K	1	6.63	7.94	4.48	4.76	21.91	33.17	28.16
BG917242	CDNA clone	1	5.18	4.93	3.45	5.75	24.52	43.97	23.31
U91905	Frizzled-related protein	1	6.35	6.23	3.66	5.39	26.62	46.48	35.44
AV249817	Transcribed locus	1	1.99	2.47	2.41	3.47	16.27	32.98	24.10
NM_009892	Chitinase 3-like 3 (Chi3l3)	1	-1.04	1.13	1.75	3.31	7.23	15.90	24.90
AF042856	Hepatocyte growth factor/scatter factor	1	2.56	3.54	2.09	3.98	21.41	36.72	23.91
D10212	Hepatocyte growth factor/scatter factor	1	2.69	3.93	2.14	3.02	19.98	34.44	23.19
NM_013473	Annexin A8	1	2.80	3.17	3.31	3.34	16.76	30.03	23.14

Gremlin 1



Gremlin 1



Do we see Gremlin 1 in our HAEC models?

YES - Gremlin 1 is expressed in the Chronic HAEC model around day 19

Summary

- **ApoE-/- mouse model is a promising *in vivo* model of MCS-induced CVD**
 - **Atherogenic diet and MCS act synergistically to stimulate atherosclerosis development in the thoracic aorta**
- ***In vitro* HAEC chronic exposure model shows molecular associations with *in vivo* results**
 - **This molecular profile/fingerprint may be predictive of atherosclerotic changes in the mouse model**
- **Gremlin 1 may be a novel biomarker for atherosclerosis**

Acknowledgements

- **Dr. John Caraway, RJRT**
- **Dr. Paul Harp, RJRT**
- **Dr. James Lin, RJRT**
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- **Dr. Gregory Shelness, WFU**
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- **Virtual Histology – Numira**
- **Covance**