Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs
R01 HL 069364

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AMDCC

THE ANIMAL MODELS OF DIABETIC COMPLICATIONS CONSORTIUM

RATIONALE FOR USING PIGS TO MODEL HUMAN TYPE 2 DIABETES MELLITUS

Phenotypic similarities between pigs and humans

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3. Coronary & peripheral atherosclerosis
4. Lipoproteins
5. Genomic information
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7. Pancreas size, morphology, & GI physiology
8. Omnivorous
9. Diurnal
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6. **Pharmacokinetics - subcutaneous**
7. **Pancreas size, morphology, & GI physiology**
8. **Omnivorous**
9. **Diurnal**
10. **Obesity**
11. **Sedentary Lifestyle**
12. **Willingness to exercise**

VALIDATION CRITERIA - Human-like Insulin Resistant Type 2 Diabetes Mellitus & ↑ Atherosclerosis in Pigs

1. **Insulin Resistance - oral and iv GTT**
2. **Hyperglycemia - present or absent**
3. **Body Fat -**
4. **Dyslipidemia - increased LDL, reduced HDL, ↑TG**
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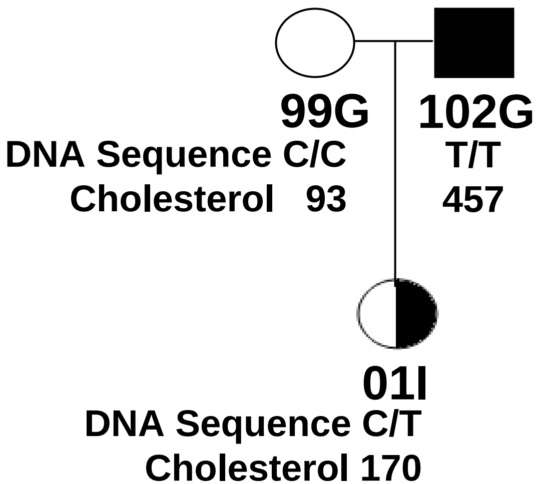
8. **Other complications - renal, myocardial, nerve, opthalmic,**
9. **Reduced complications with successful treatment -**

   Metabolic (↓ IR or glucose), devices (e.g., stents), other new rx

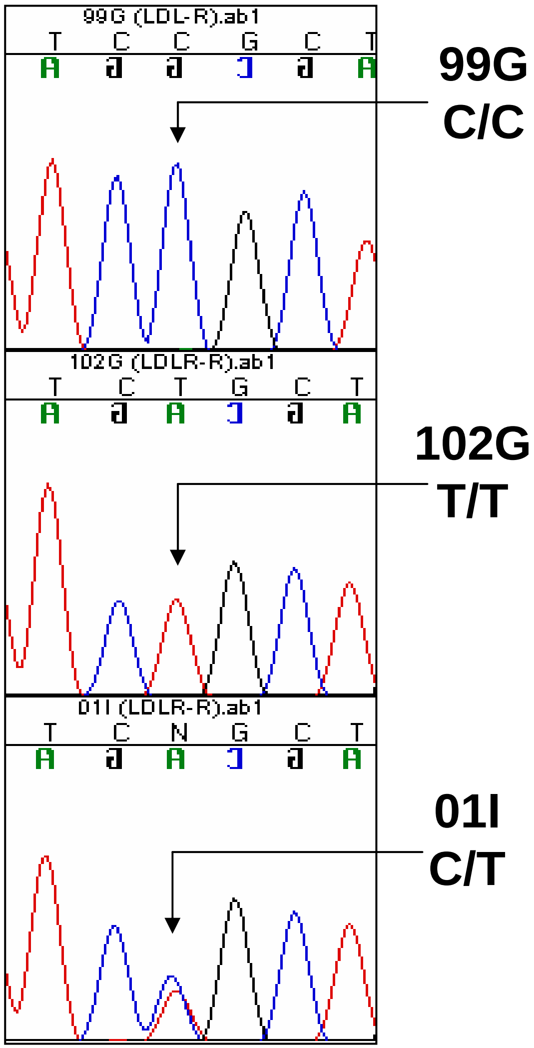
LDL Receptor Sequence in NL and FH and DownSized(DS)-FH Pigs

FH Pigs have a Missense Mutation ($C_{253} \rightarrow T_{253}$) Substituting $Arg_{94} \rightarrow Cys_{94}$

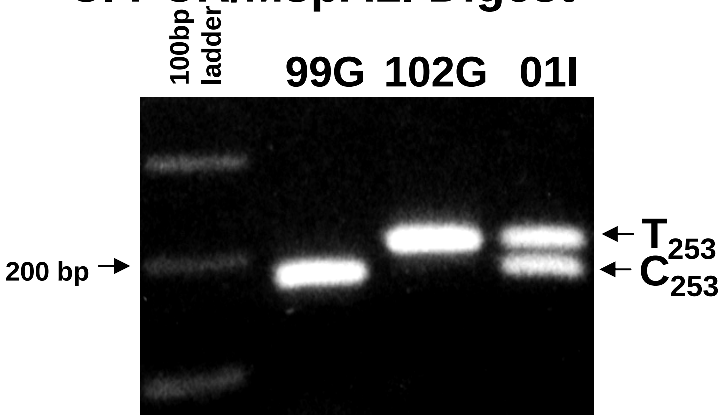
A. Pedigree



B. DNA Sequencing



C. PCR/MspA1I Digest



Hasler-Rapacz et al. Am J Med Genet. 1998;76:379-386
Grunwald et al. J Lipid Res. 1999;40:475-485.

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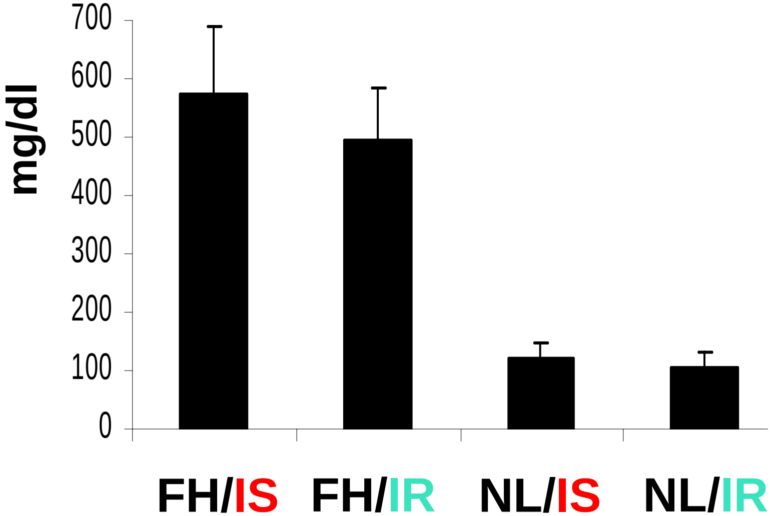
Oral Glucose Tolerance Test

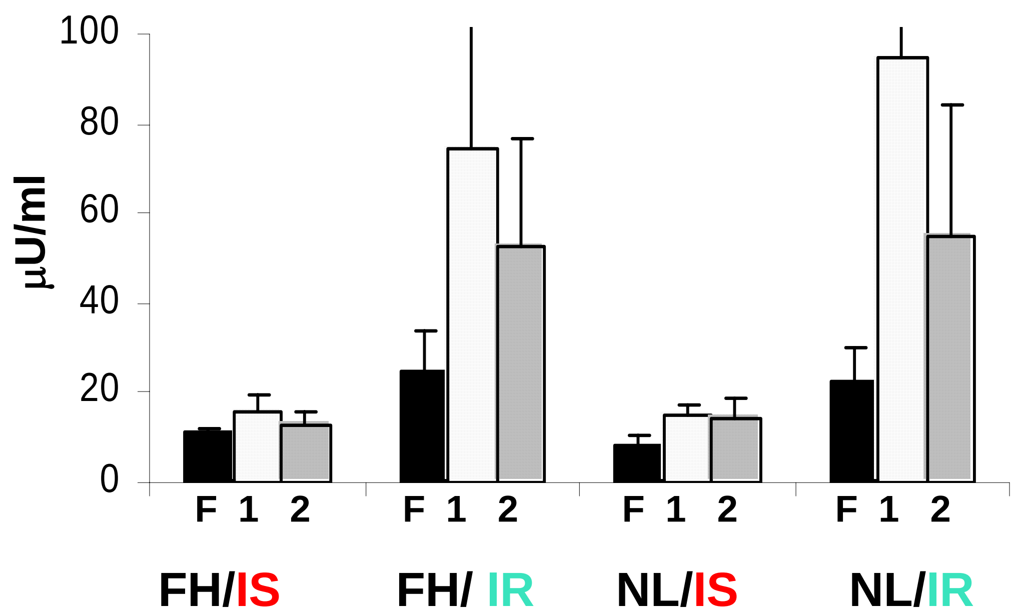
IS – insulin sensitive
IR – insulin resistant

CHOLESTEROL



SERUM INSULIN

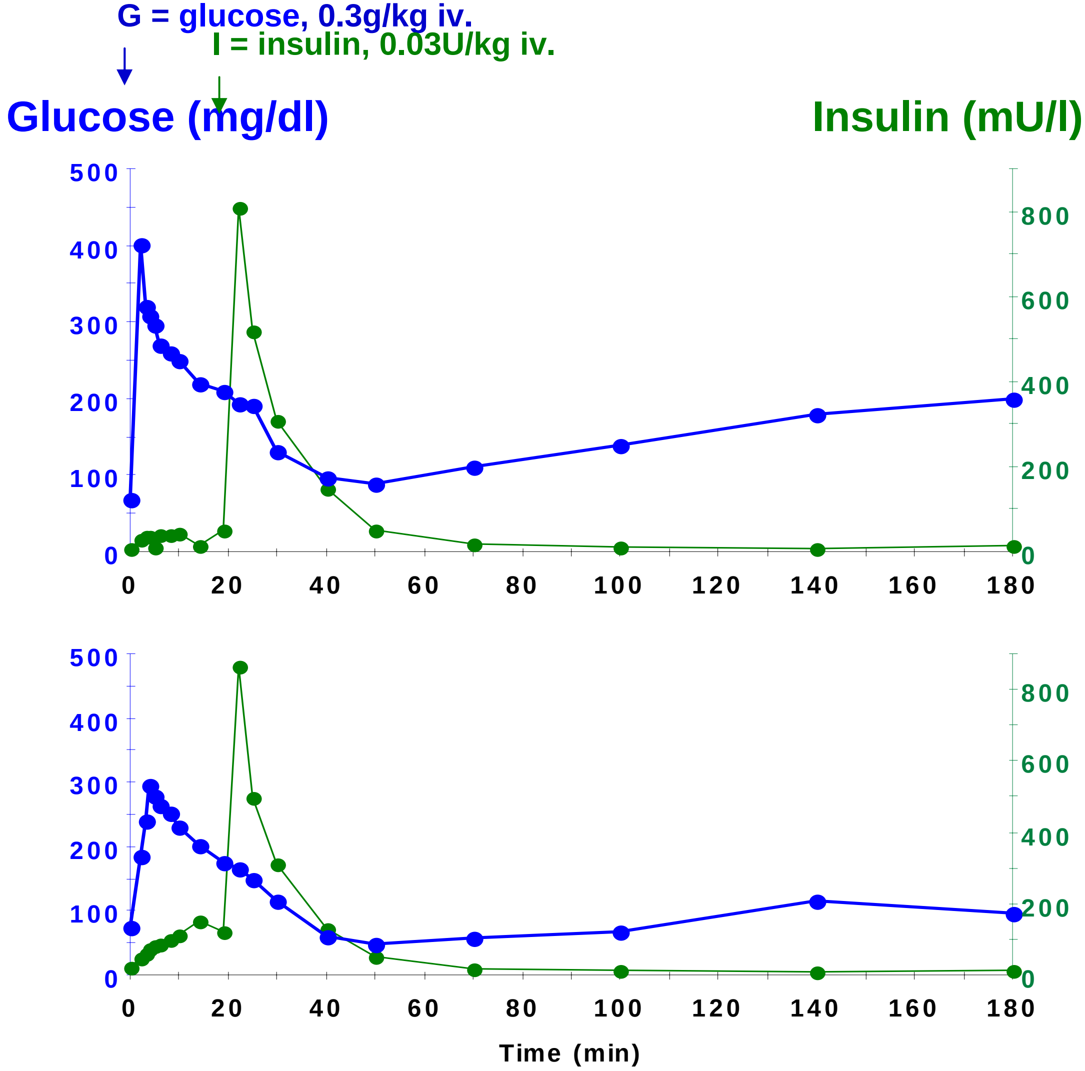
(Fasting, 1 & 2 post prandial)



Bergman FSIGT- 11G NL/IR

- Full 180 min Study

33G
Baseline
590 lb
Fat Back 8.66 cm
Chol 91 mg/dl
S_i 4.2

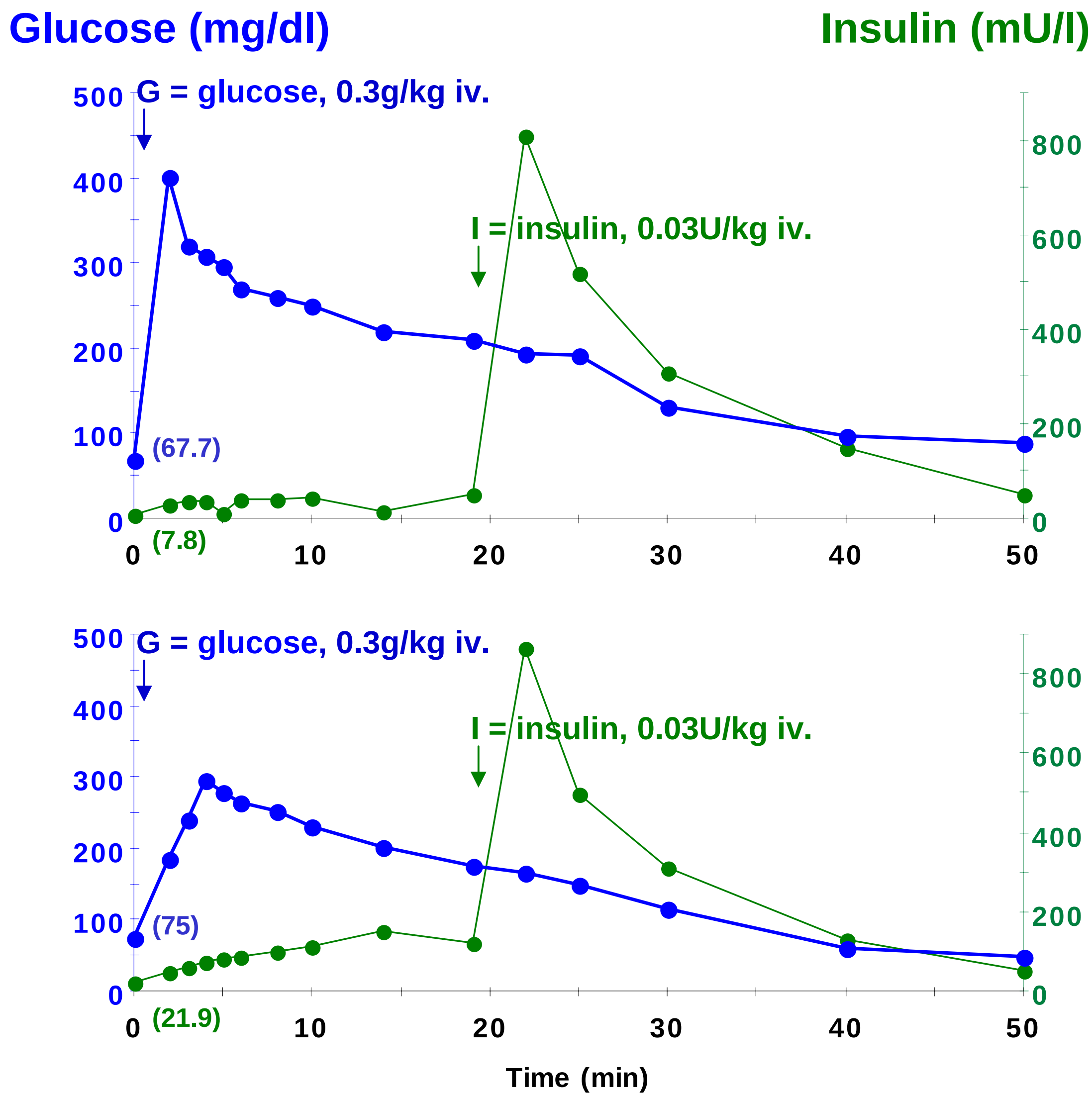


33G
2 month
665 lb
Fat Back 8.95 cm
Chol 366 mg/dl
S_i 3.3

Bergman FSIGT- 33G NL/IR
- First 50/180 min of Study

33G Baseline 590 lb Fat Back 8.66 cm Chol 91 mg/dl S_i 4.2

33G 2 month 665 lb Fat Back 8.95 cm Chol 366 mg/dl S_i 3.3



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Hyperglycemia in NL/IR & NL/IS Pigs

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7 NL pigs entered in year-long study

- **4/7 had hyperglycemia at baseline (127 ± 15 mg/dl)**
- **On the high fat atherogenic diet**
  - **4/7 show ↓ S_i**
  - **1/7 is showing ↑ S_i**
  - **1/7 has stable S_i**
  - **1/7 died of renal failure**

Weight, Back Fat, Bergman S_i, Fasting Glucose & Insulin in NL/IS and NL/IR Pigs

	Pig ID	Time on Study	Weight (lbs)	Back Fat (cm)	S_i	Fasting glucose (mg/dl)	Fasting Insulin (μU/ml)
A. NL Pigs with ↓ S_i							
1.	69F	Baseline	414	-	4	110 ± 3.2	15.1 ± 2.1
		12 mos	540	3.2	3.7	74 ± 5.5	18 ± 5.5
2.	06F	Baseline	380	2.3	4.2	118 ± 12	7.5 ± 1.2
		12 mo	538	3.2	2.8	165 ± 8.2	19.8 ± 4.8
3.	02F	Baseline	478	-	4	102 ± 4	10.2 ± 1.3
		12 mo	580	3.1	3.8	144 ± 5	12.8 ± 1.3
4.	33G	Baseline	590	8.6	4.2	67.7 ± 5.7	7.9 ± 0.6
		2 month	665	8.9	3.3	75.0 ± 2.5	21.9± 5.9
B. NL Pig with stable S_i							
5.	11G	Baseline	464	4.6	3	133.9 ± 9.8	34.5 ± 4.1
		2 month	515	5.2	2.9	120.8 ± 2.4	15.3 ± 3.1
C. NL Pig with ↑ S_i							
6.	69G	Baseline	445	3.6	3.9	146.5± 22.2	39.0 ± 24.7
		6 month	522	6.6	4.7	65.6 ± 2.5	5.9 ± 6
D. NL Pig with Renal Failure							
7.	76G	Baseline	448	-	3.2	72 ± 5.5	10.8 ± 3.9
		2 mo	463	-	2.9	120 ± 19.3	17.7 ± 4.5

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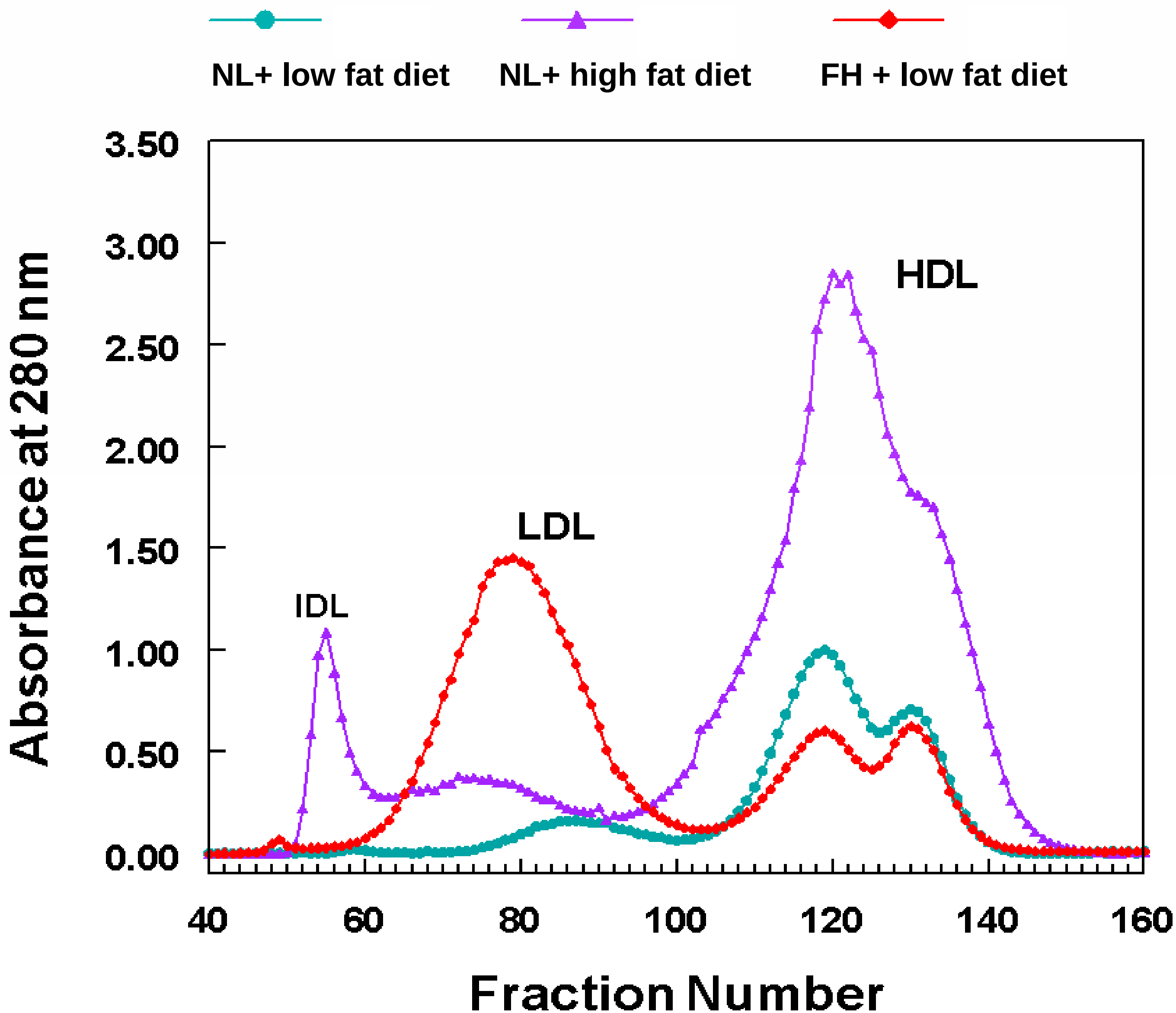
PIG LIPOPROTEIN CHARACTERIZATION BY PHYSICAL CHEMISTRY

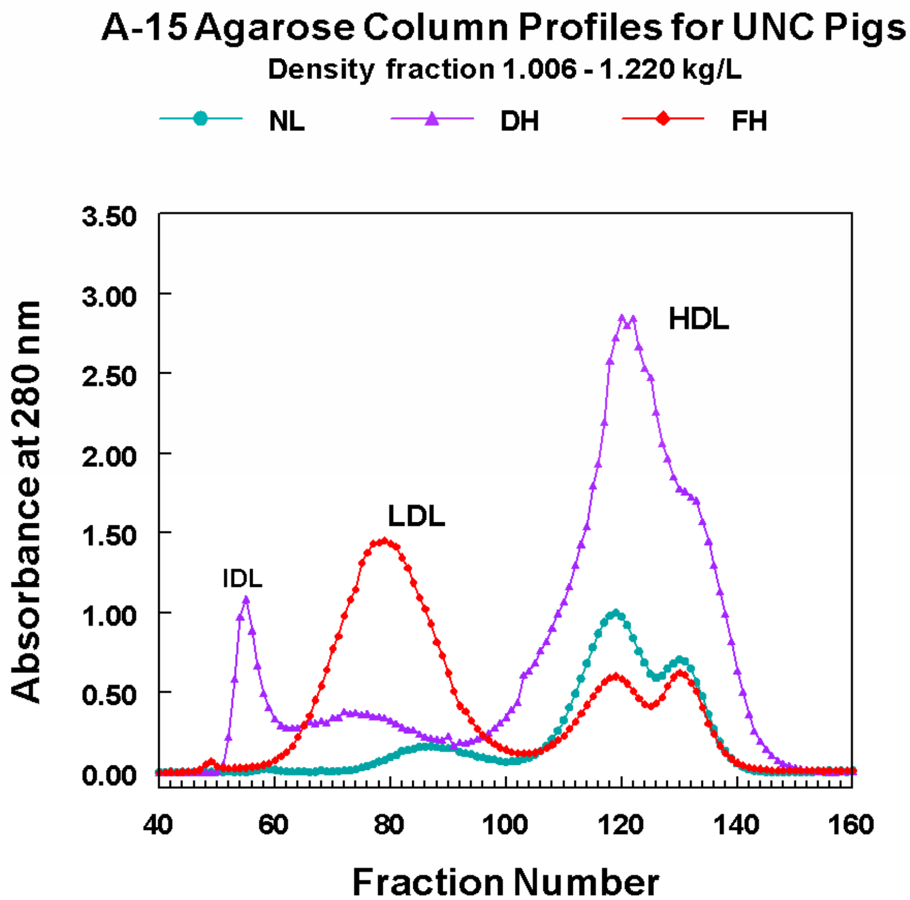
A-15 Agarose Column Profiles for UNC Pigs

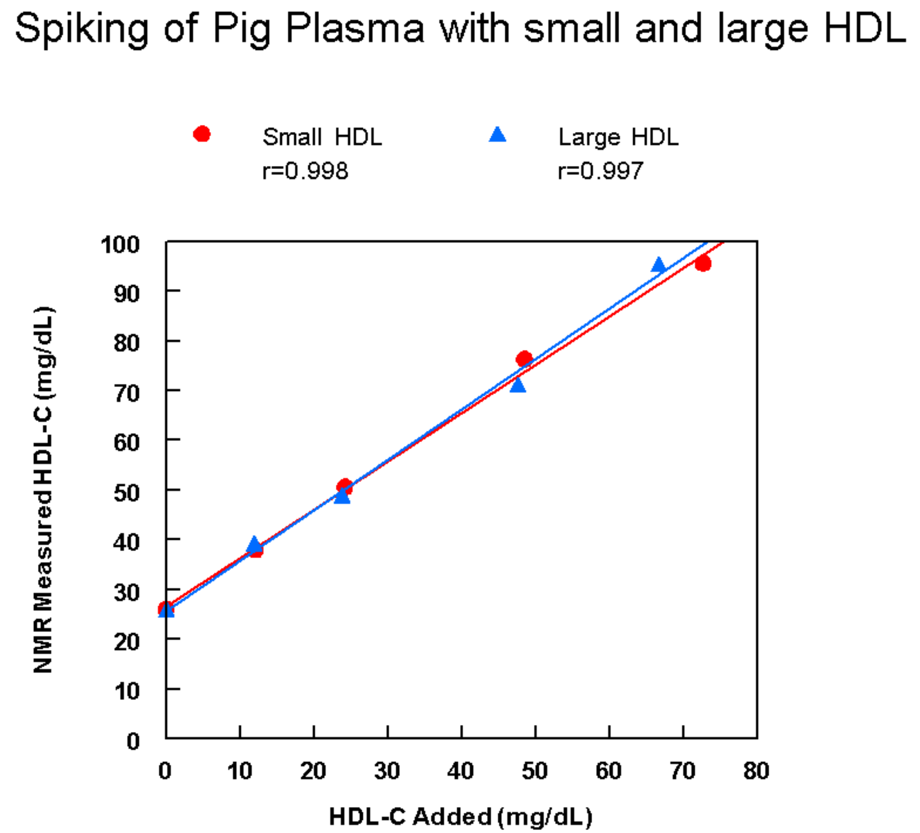
Density fraction 1.006 - 1.220 kg/L



PIG LIPOPROTEIN CHARACTERIZATION BY NMR

Validation by concurrent physical chemistry and NMR measurements





LDL Particle Concentration (nmol/L) in in NL/IS and NL/IR Pigs on Low Fat Chow

	n	Total LDL Particles	23-27 nm* IDL	21.3-23 nm* Large LDL	Total Small LDL	19.8-21.2 nm* Medium Small LDL	18.3-19.7 nm* Very Small LDL
NL/IS	16	236 ± 137	21 ± 60	43 ± 66	172 ± 104	161 ± 97	11 ± 22
NL/IR	8	246 ± 56.5	0	23 ± 24	223 ± 45	212 ± 55	12 ± 200

* Range of Particle Diameter

Goff et al. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. Metabolism. 2005;54:264-270

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Blood Pressure - Pig Tail-Cuff

Phenotype	n	Systolic	Diastolic	MAP	Pulse
NL/IS	5	154.61 ± 30.72	99.37 ± 26.41	122.52 ± 27.78	90.48 ± 19.31
NL/IR	4	153.24 ± 22.22	104.57 ± 19.90	123.14 ± 20.57	98.75 ± 14.65
FH/IS	4	129.48 ± 22.25	83.70 ± 20.57	102.89 ± 22.01	85.76 ± 18.59
FH/IR	1	162.80 ± 17.26	121.8 ± 23.95	133.7 ± 24.43	101.7 ± 8.87
DS-FH/IS	4	146.83 ± 27.91	93.70 ± 21.85	112.79 ± 25.34	95.97 ± 25.33
DS-FH/IR	2	168.79 ± 23.56	115.05 ± 26.64	136.71 ± 29.58	91.86 ± 15.38

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6. Serum Markers - C-reactive protein, MCP-1, IL-6, MMP-9, MMP-2, TNF-α, leptin, PAI-1, fibrinogen, VCAM-1, ICAM-1, E-selectin, and adiponectin
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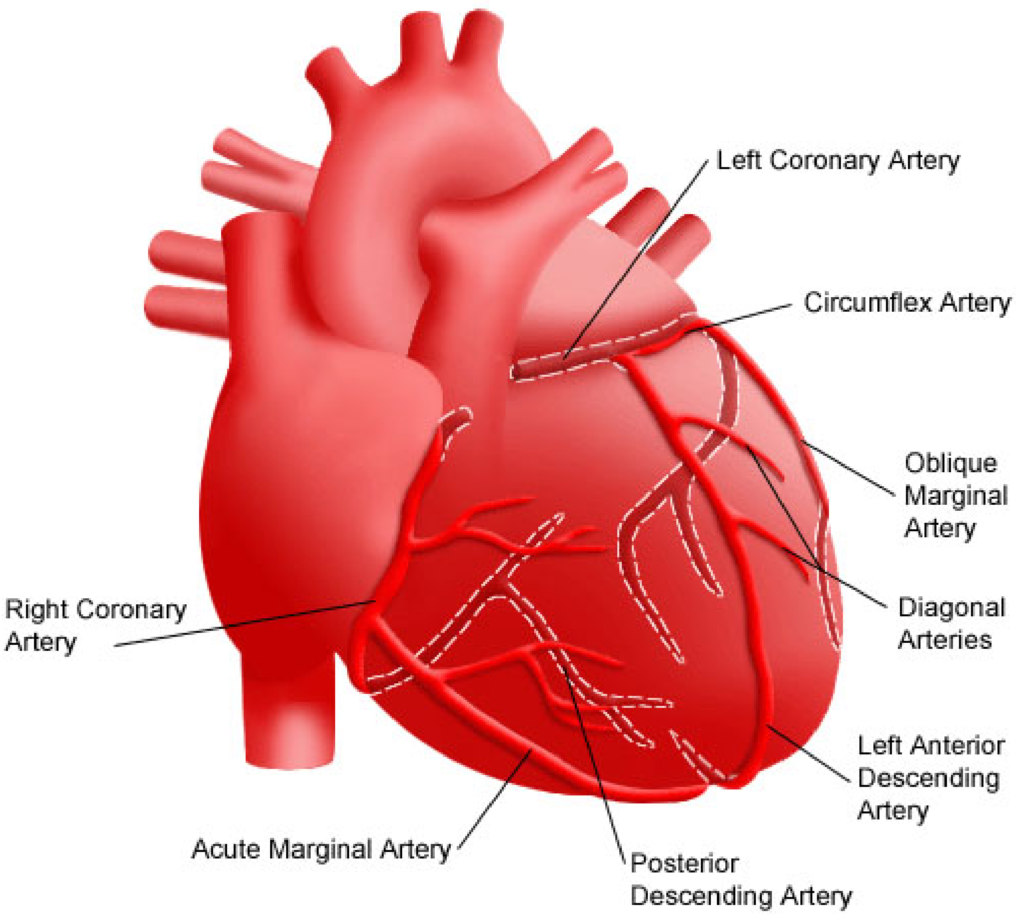
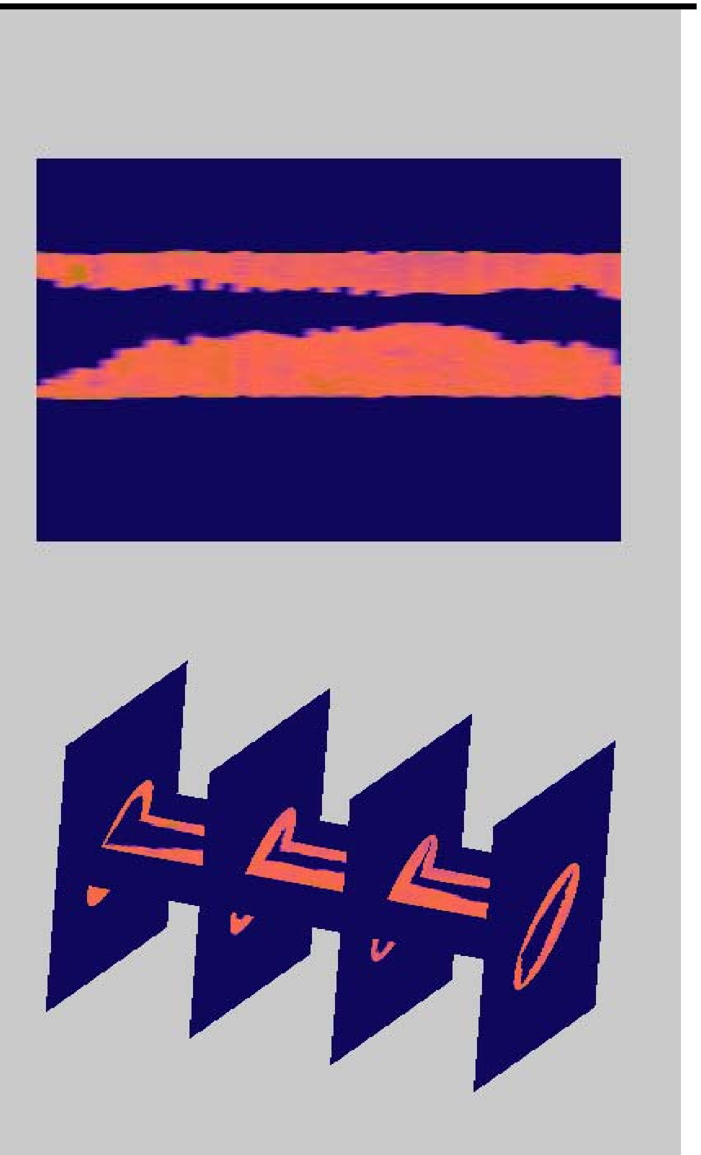
Computer-Assisted Morphometry of of Coronary and Aortic Atherosclerosis (Image J/ NIH)





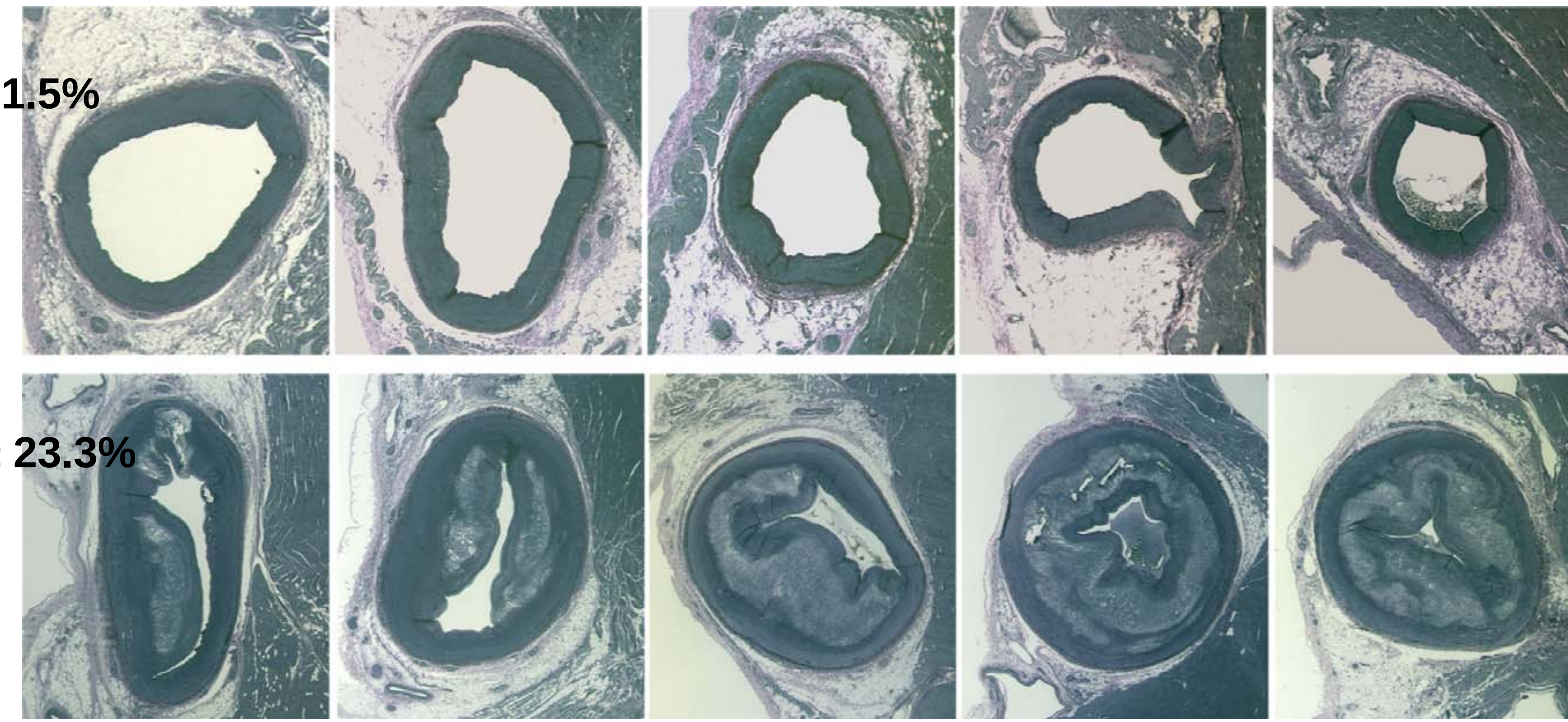
Percent Coronary Artery Stenosis in NL/IS and NL/IR Pigs

69F

19 ± 21.5%

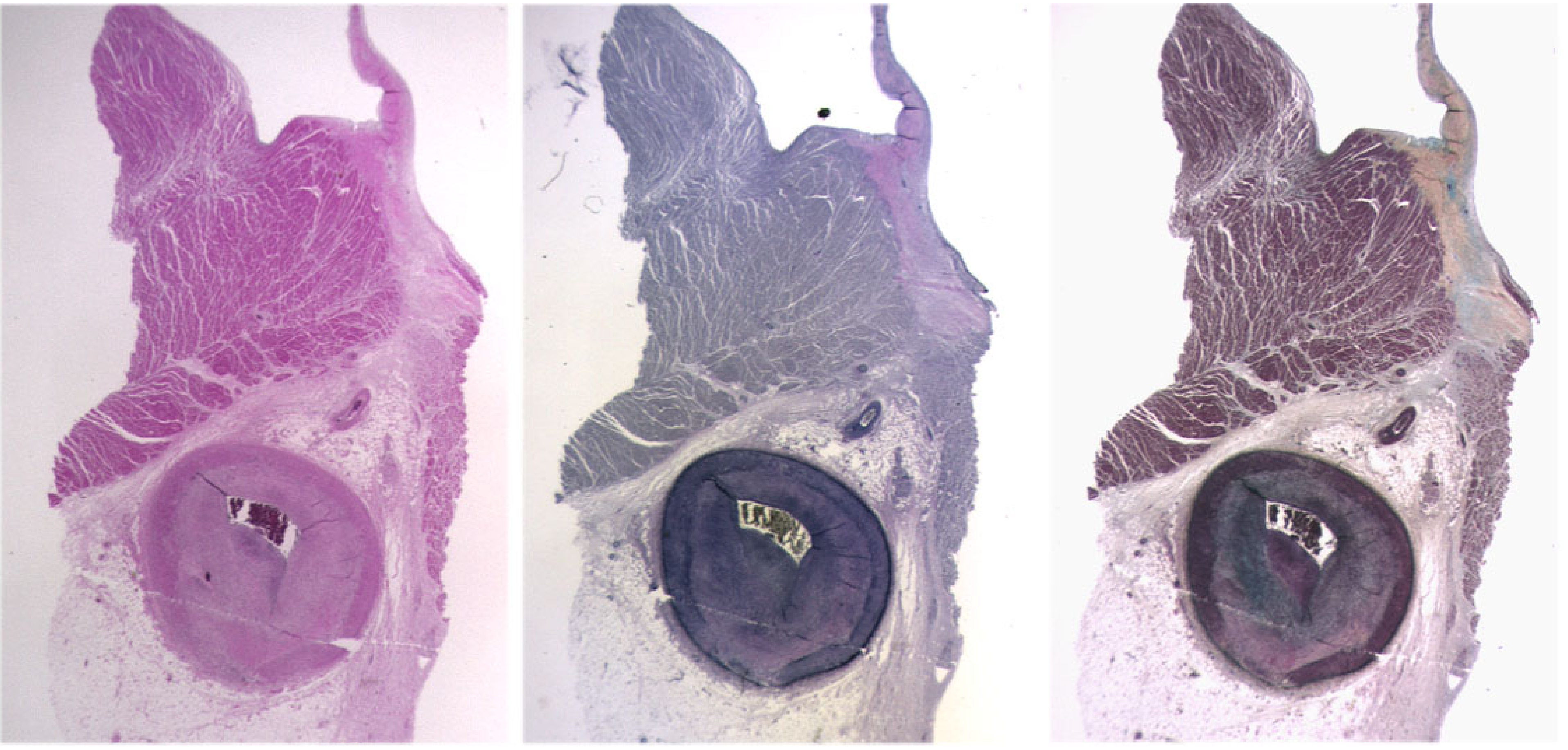
02F

72.3 ± 23.3%



Coronary Atherosclerosis in IR pigs

H & E VVG Movats



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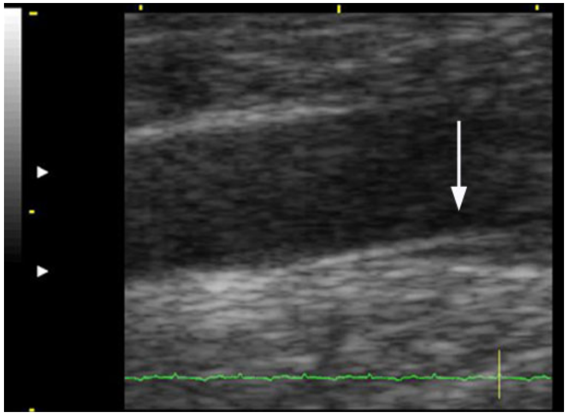
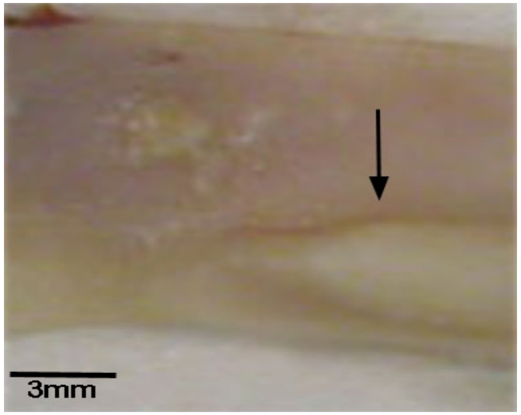
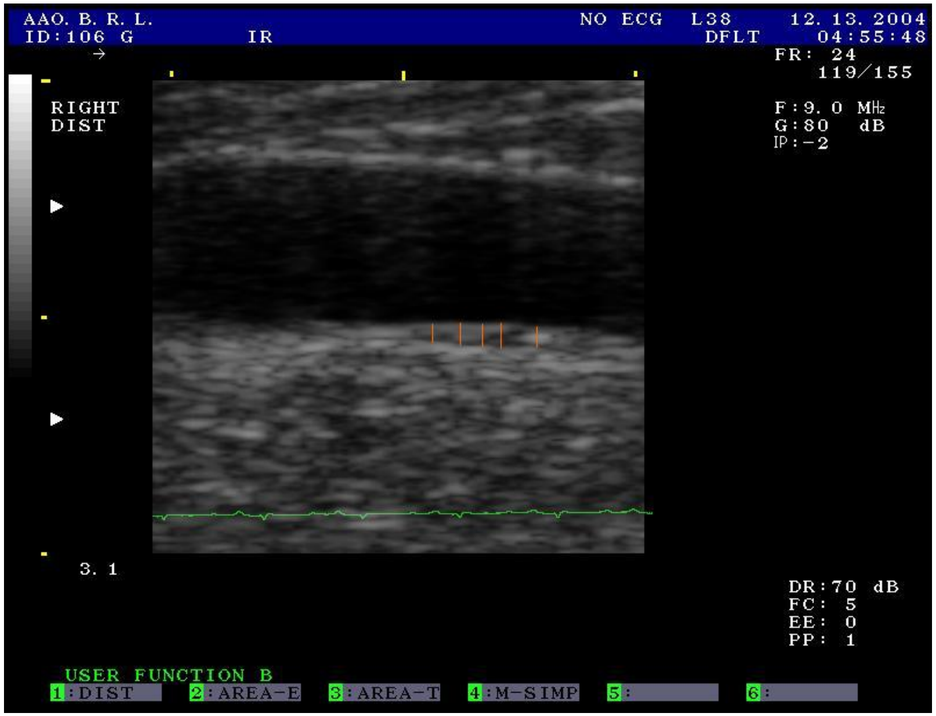
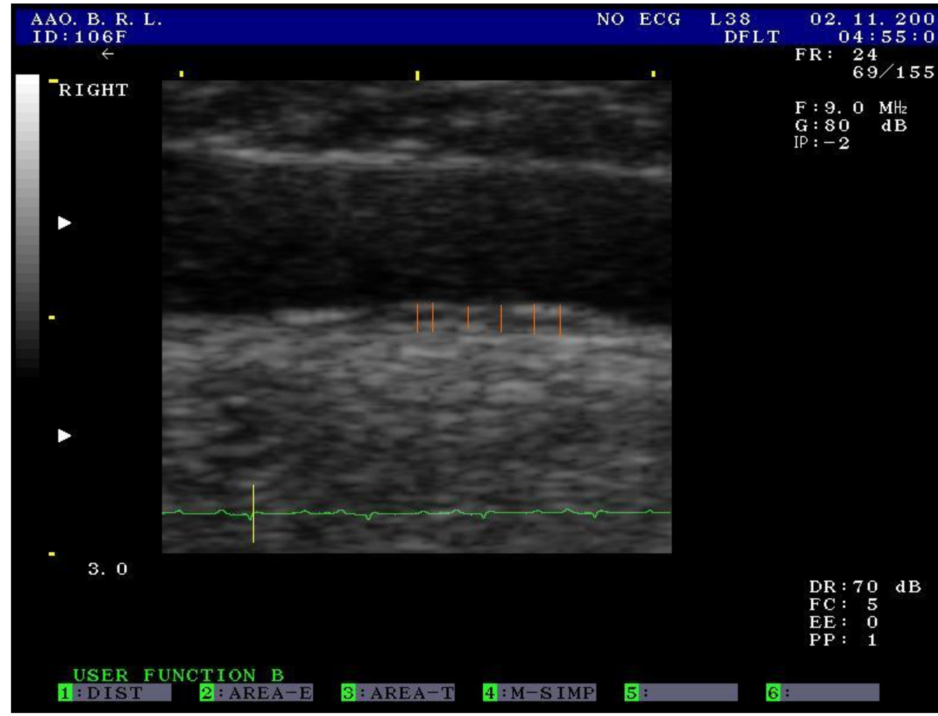
<u>Rate</u> of Femoral Atherosclerosis Progression by Percutaneous Ultrasound-
<u>1. Plaque number & 2. Plaque volume</u>





Rate of Femoral Atherosclerosis Progression by Percutaneous Ultrasound (cont'd) - Intima Media Thickness



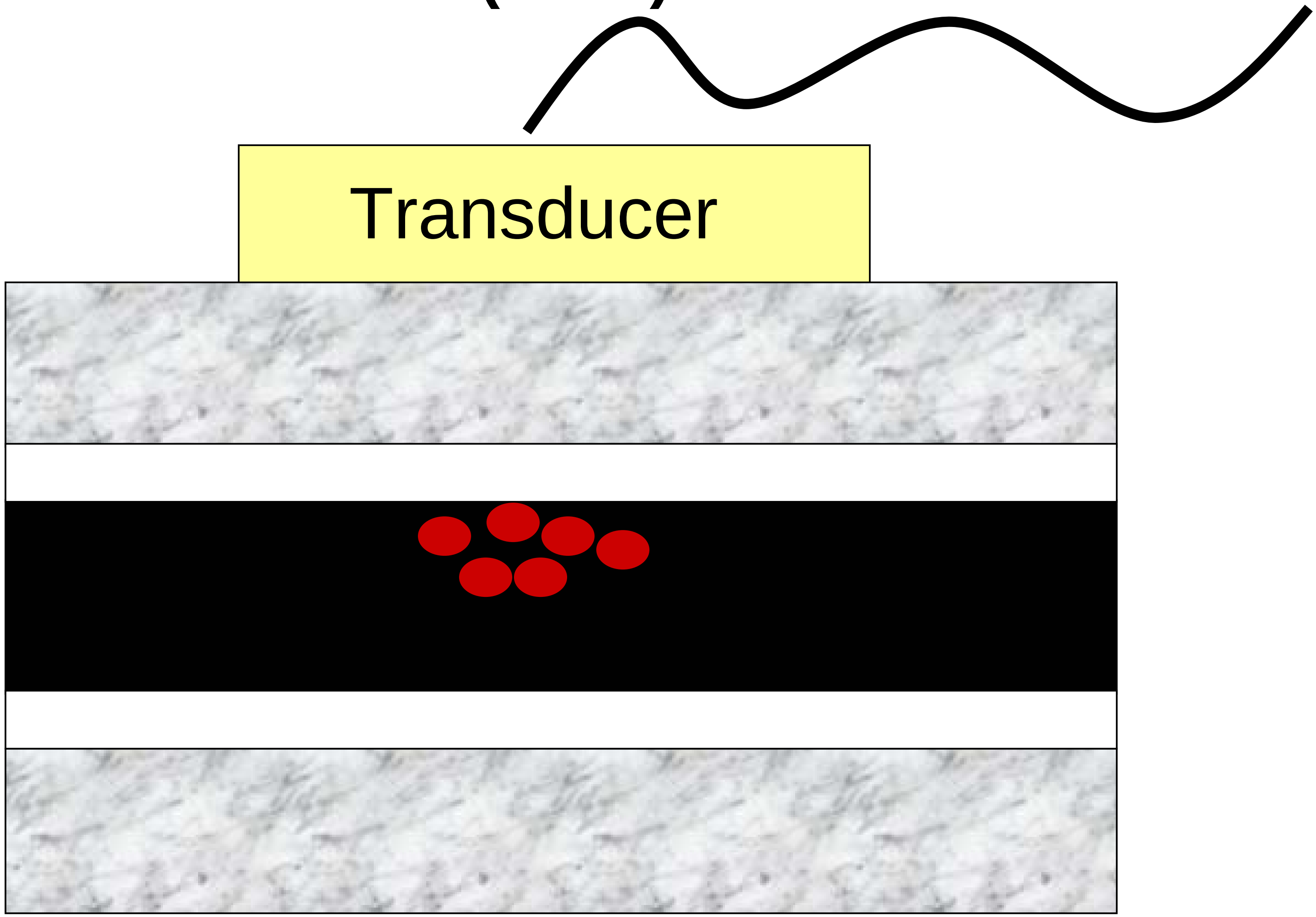


Baseline NL/IS **0.024 ± 0.001 mm²**
Baseline FH/IS **0.076 ± 0.01 mm²**

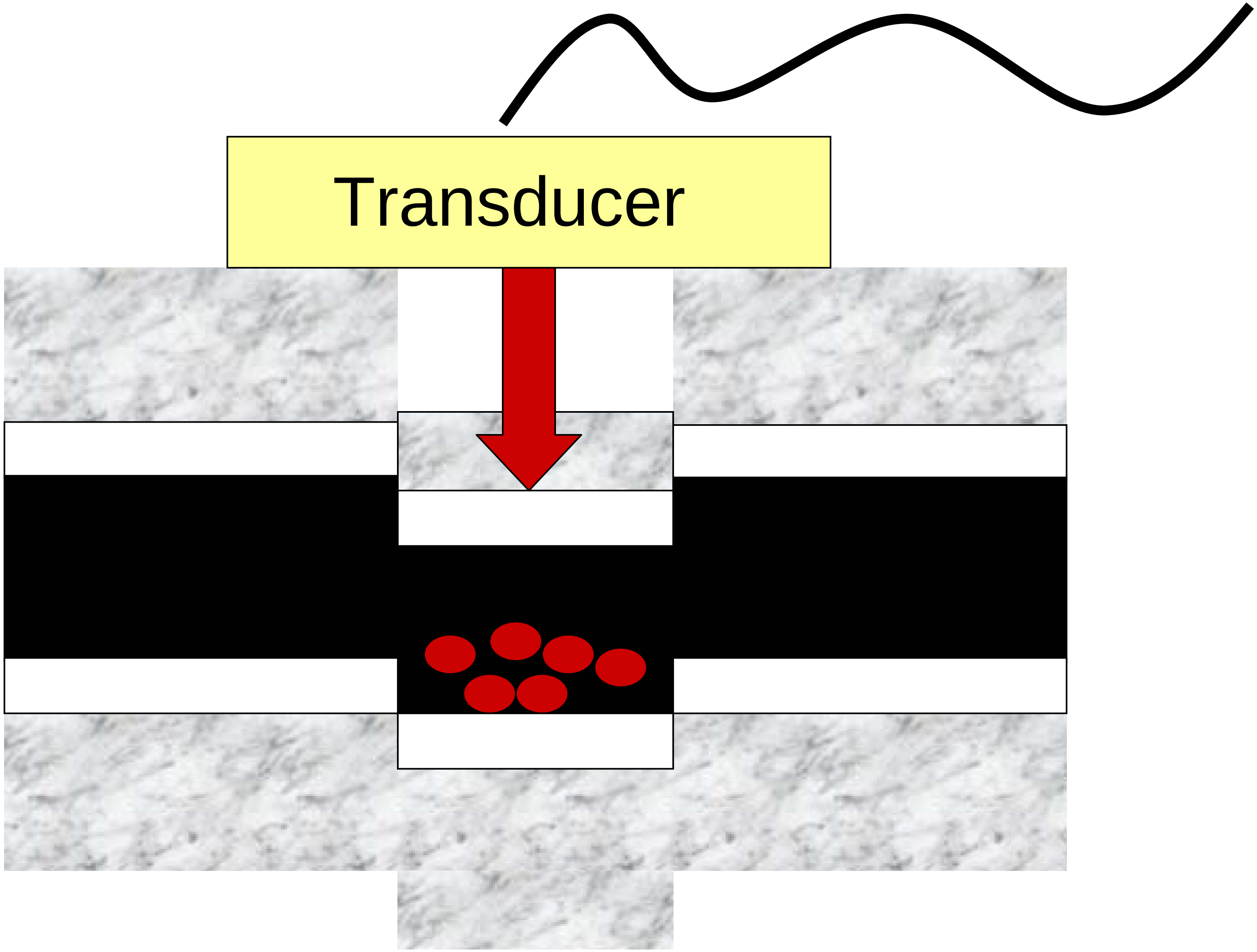
7 month NL/IS **0.033 ± 0.006 mm²**
11 month FH/IS **0.129 ± 0.015 mm²**

Van der Meer, et al. Stroke 2003 34(10): 2374-9

Changes in Arterial Mechanical Properties measured by Acoustic Radiation Force Imaging (ARFI)



ARFI Imaging in the Vasculature



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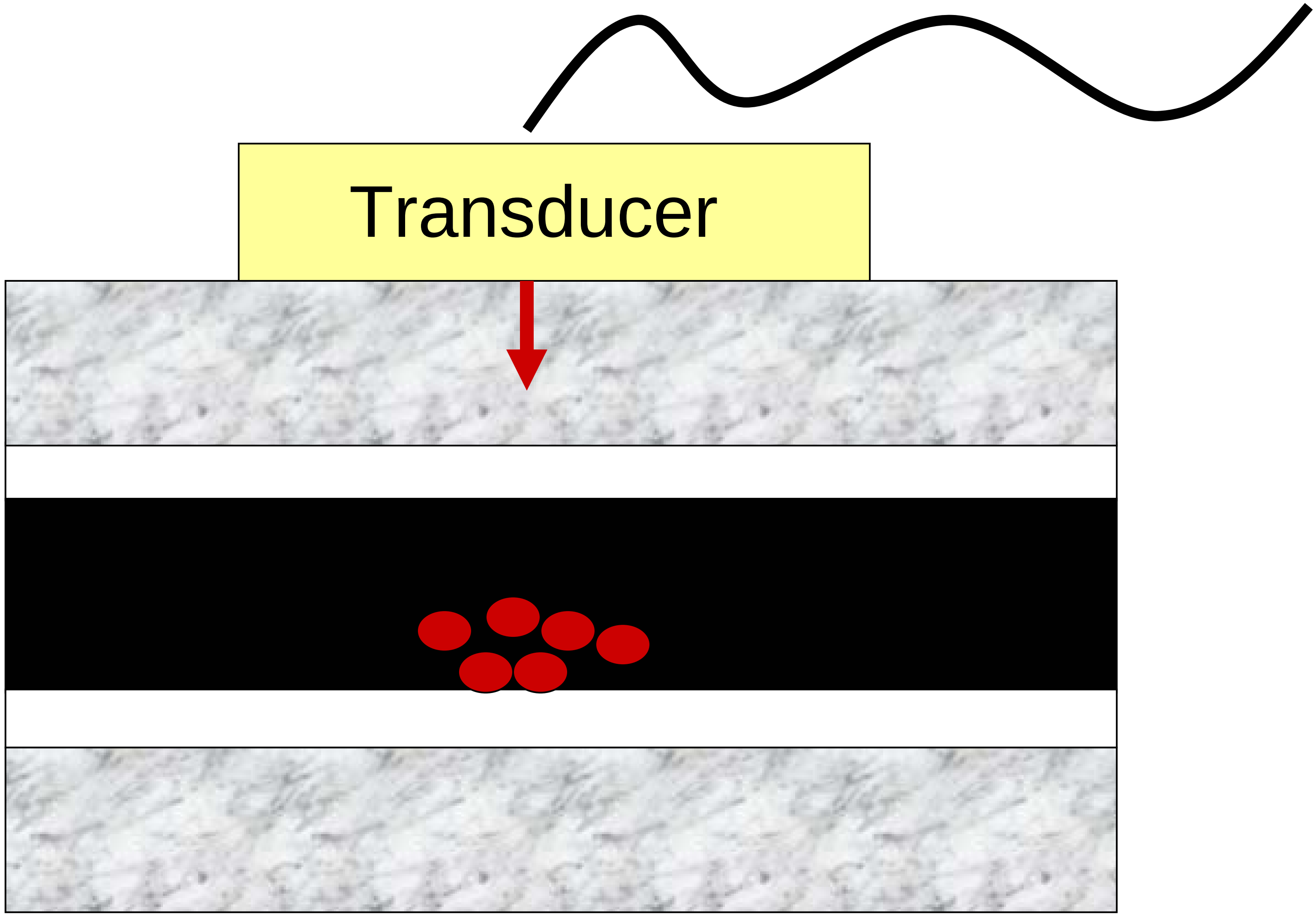
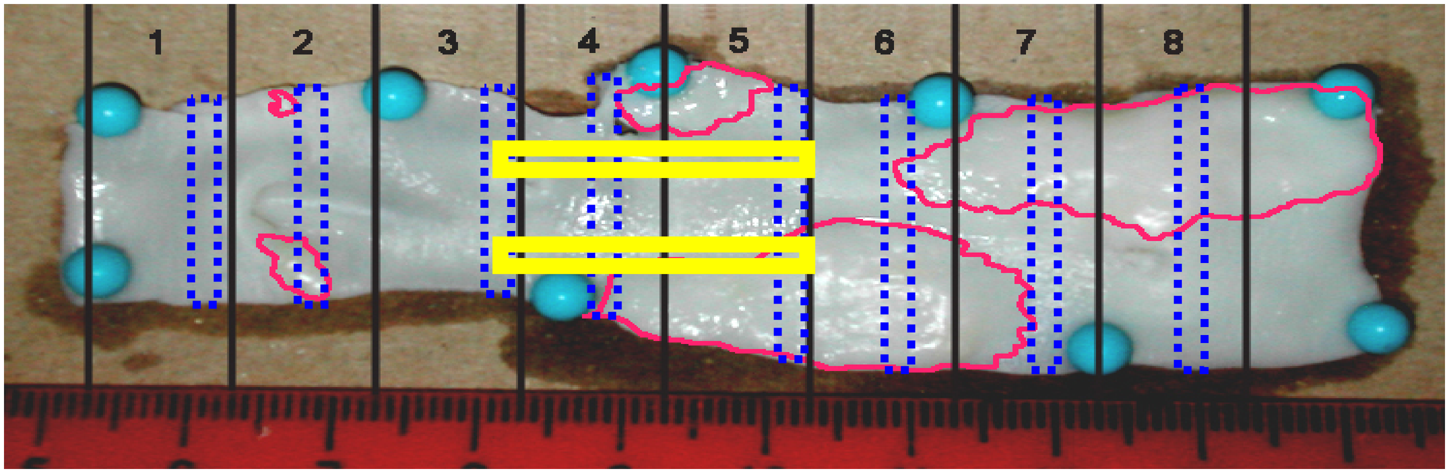
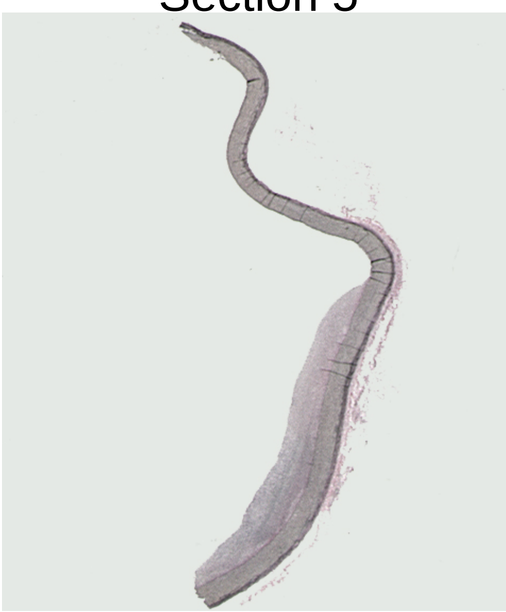
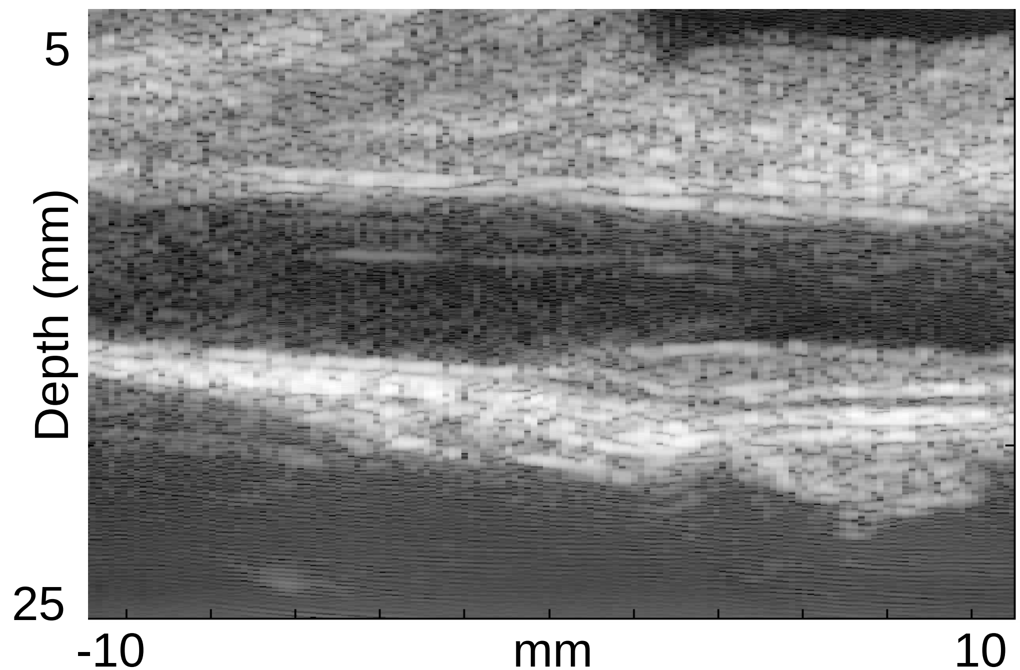


Atherosclerotic Pig Femoral Artery *Ex Vivo* Gross Anatomy & Plaque Histology, Matched *In Vivo* B-Mode Ultrasound

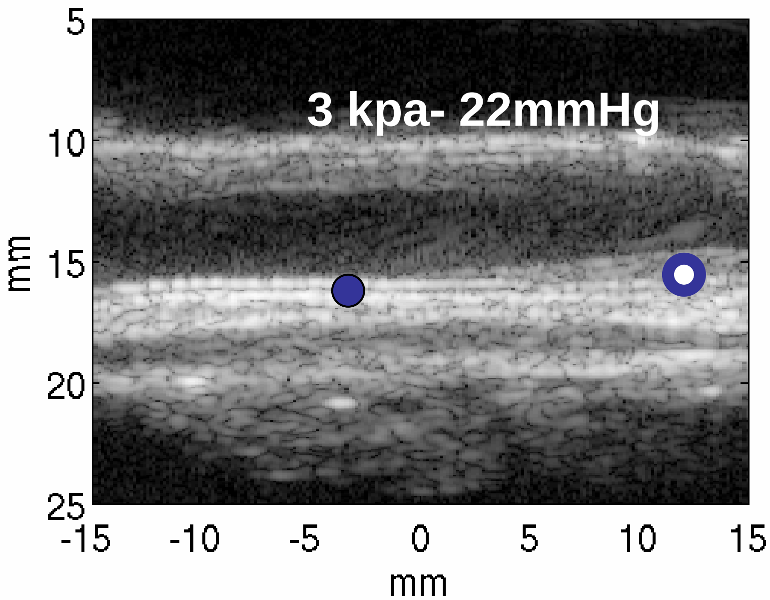


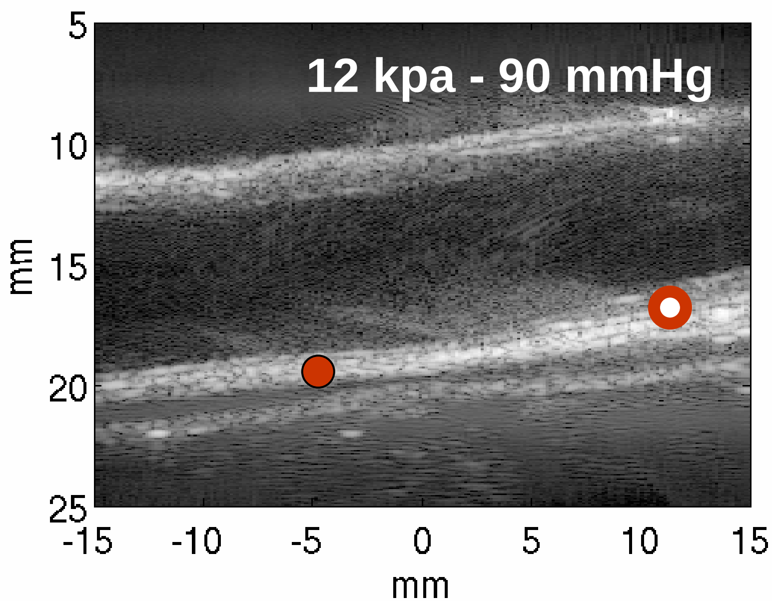
Section 5

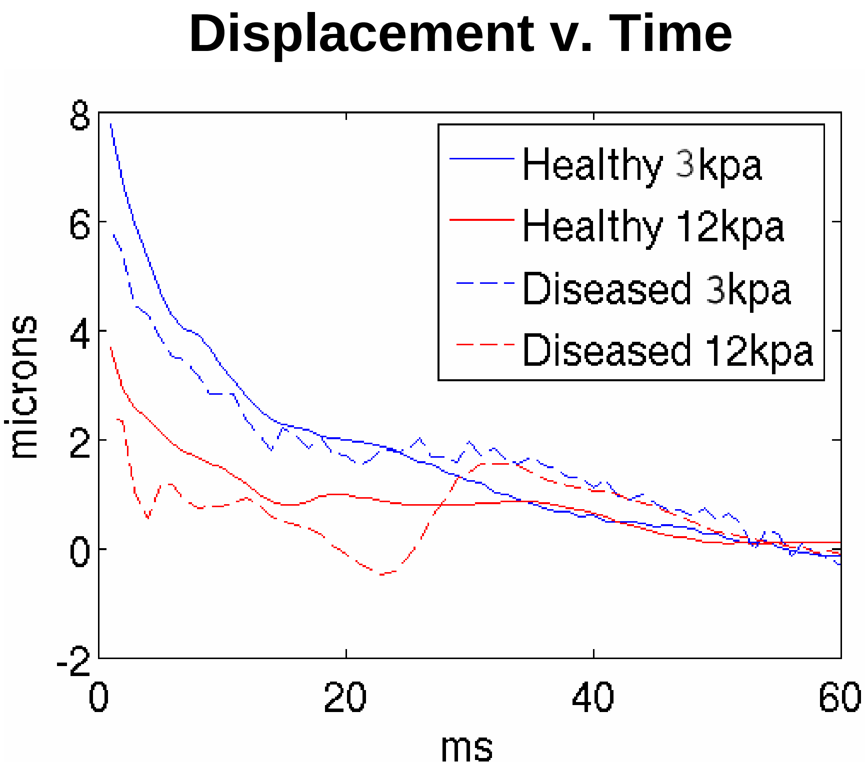




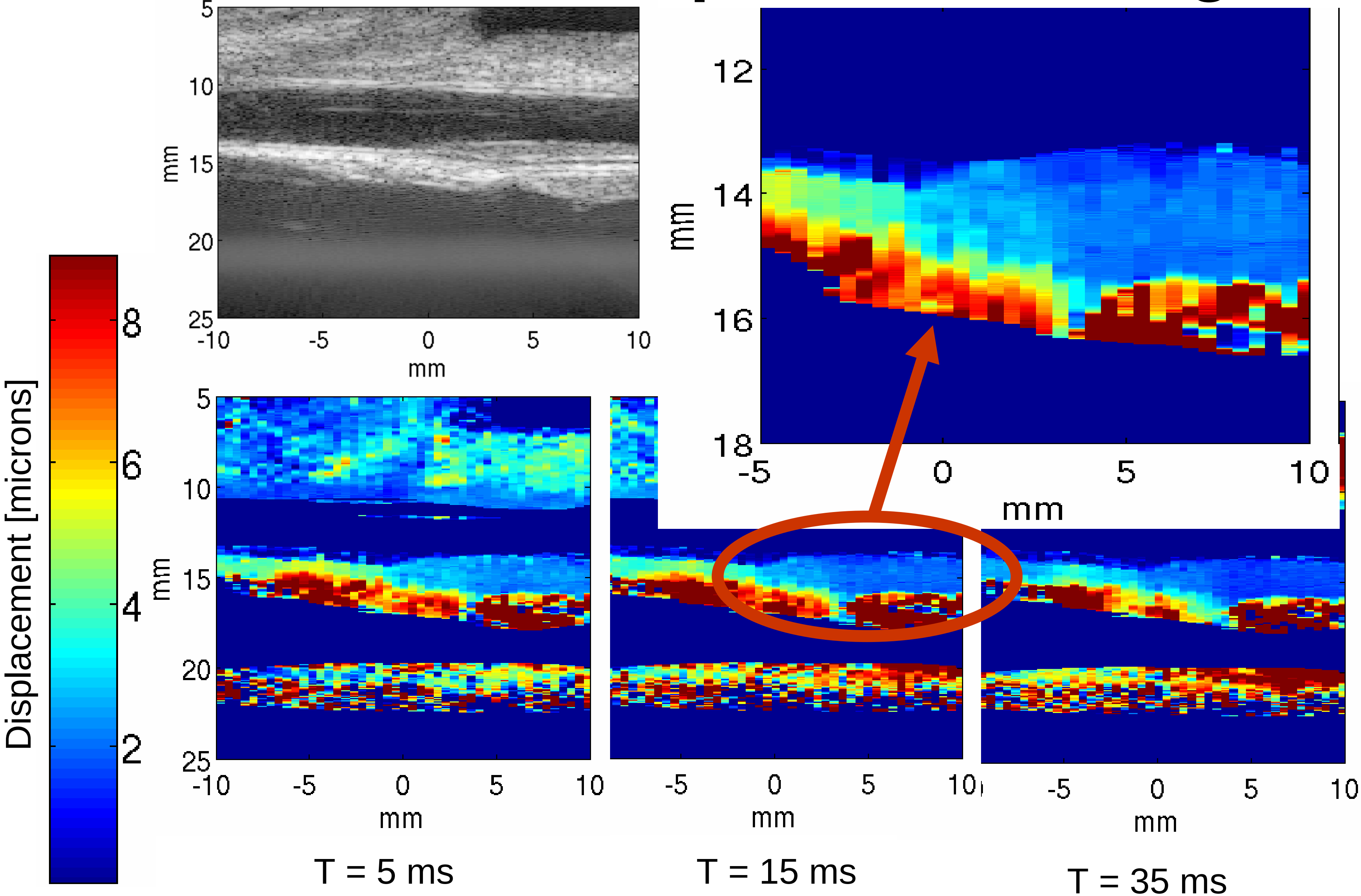
Results: *Ex Vivo* Displacements v. Time

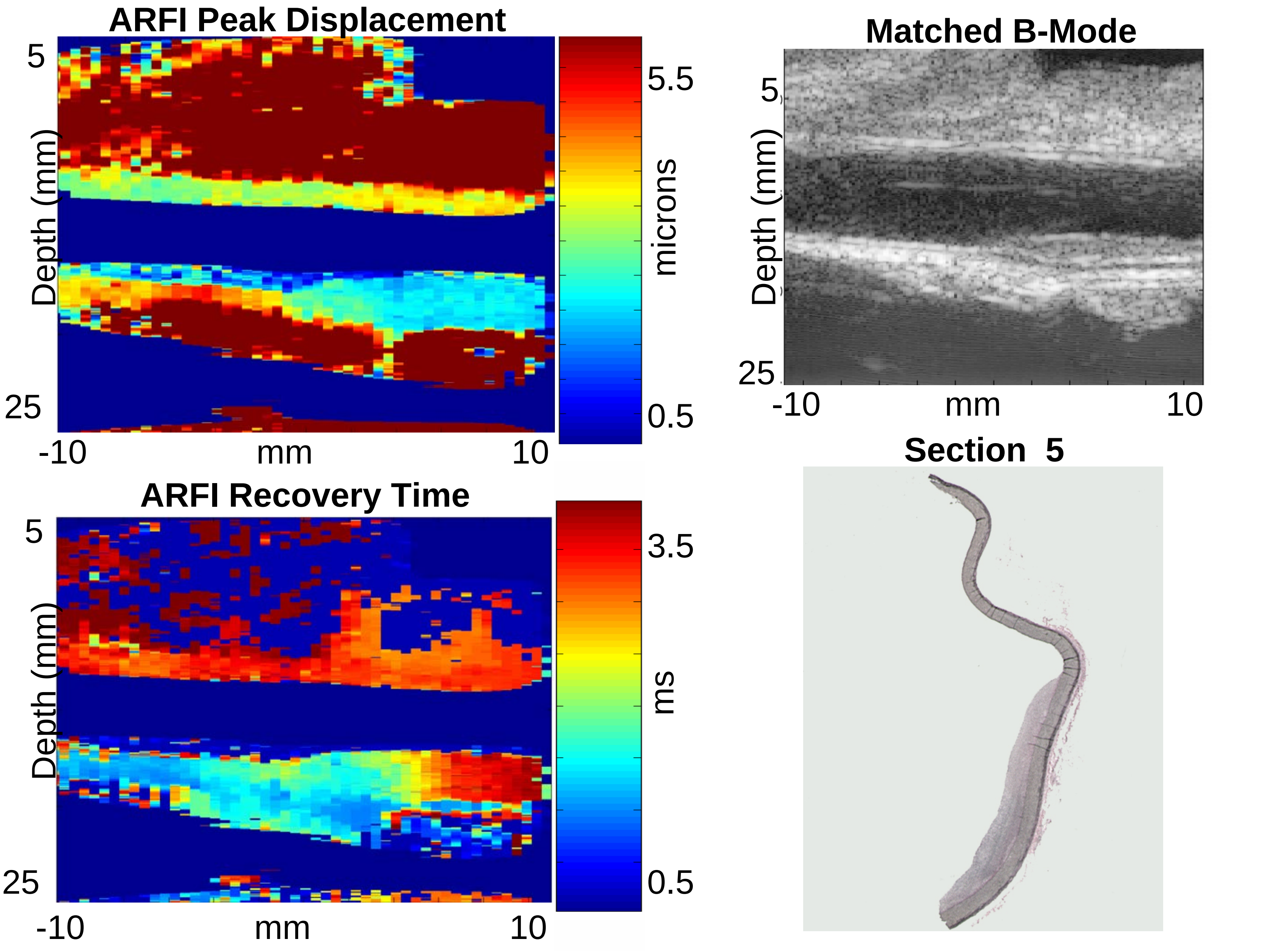






Results: *Ex vivo* Displacement Images





ARFI Peak Displacement
5
25
Depth (mm)
-10
mm
10
5.5
microns
0.5
Matched B-Mode
5
25
Depth (mm)
-10
mm
10
ARFI Recovery Time
5
25
Depth (mm)
-10
mm
10
3.5
ms
0.5
Section 5

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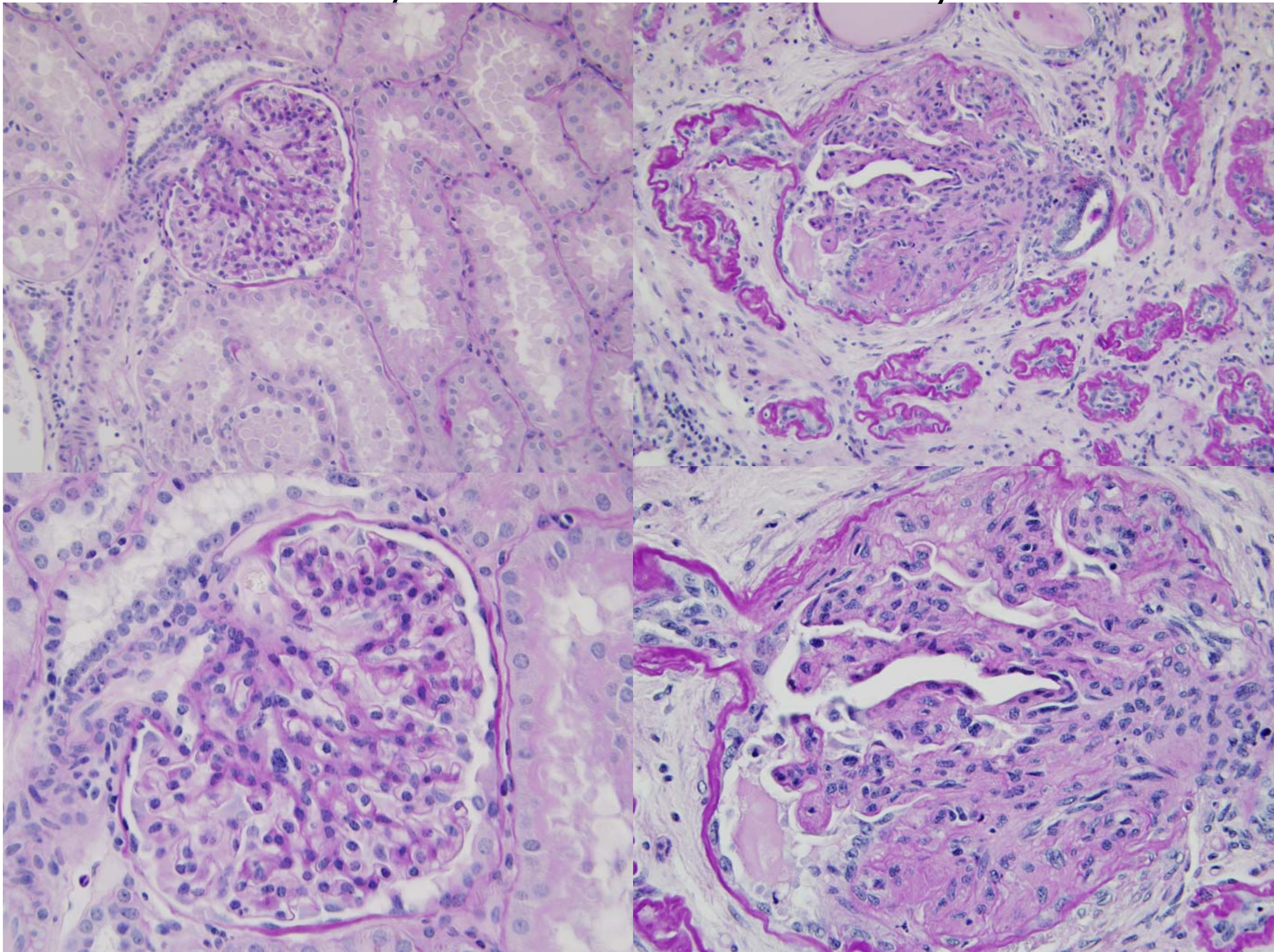
Banking and sharing relevant tissues for current screening and future study

AMDCC Pig Tissue Distribution Plan

INVESTIGATOR	INSTITUTION	SAMPLE	GOAL
Dale Able Don McClean	Utah	Myocardium	Biochemistry and EM mitochondrial analyses
Tim Kern	Case Western	Eyes	Retinal vessel analyses
Firouz Daneshgari	Cleveland Clinic	Bladder	Physiological analyses
Eva Feldman	Univ of Michigan	Neurological tissues (skin bx x 2, sciatic nerve, dorsal root ganglia, other CNS)	Biochemical and microscopic analyses
Eva Feldman	Univ of Michigan	EDTA plasma	Screen for ROS
Charles Jeannette	UNC	Urine and kidneys	Urine protein, glomeruli & other vessel analyses

02F NL/IR, creat 2.1 — 76G NL/IR, creat 9.6



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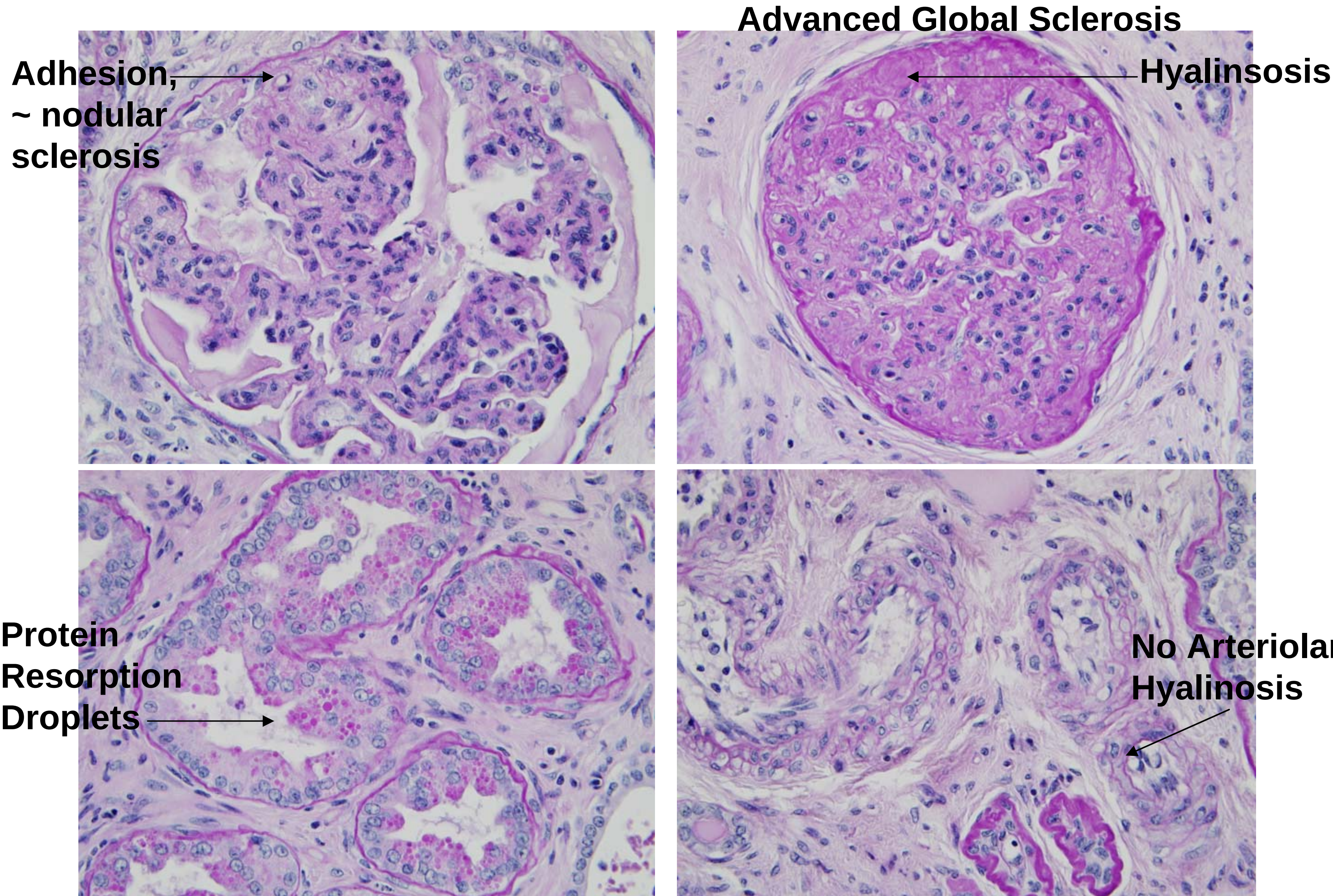
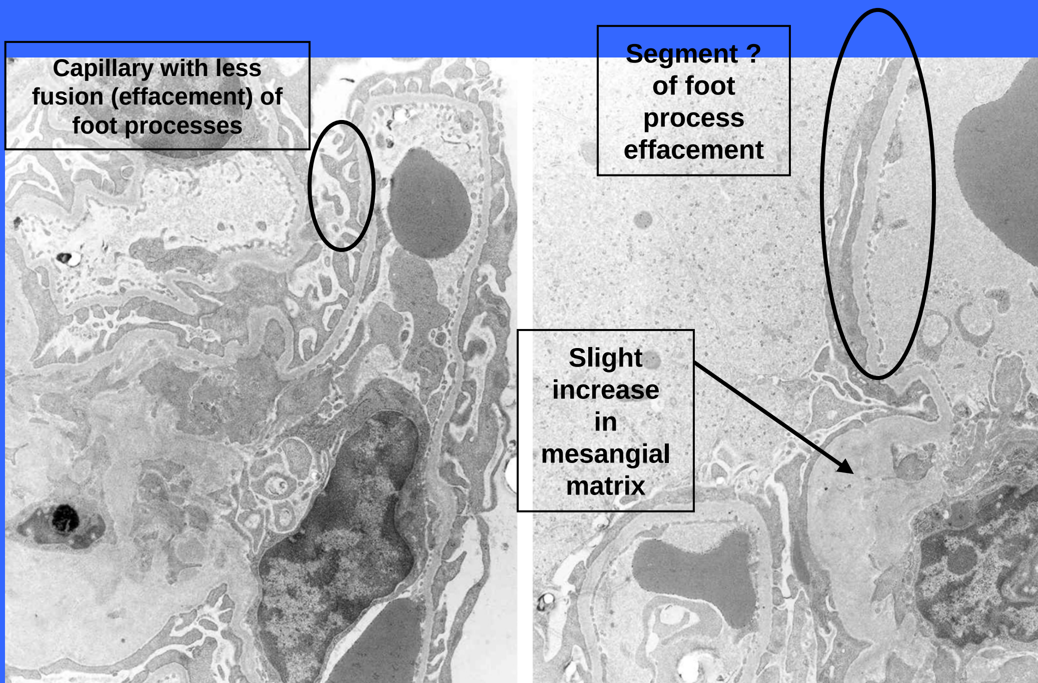
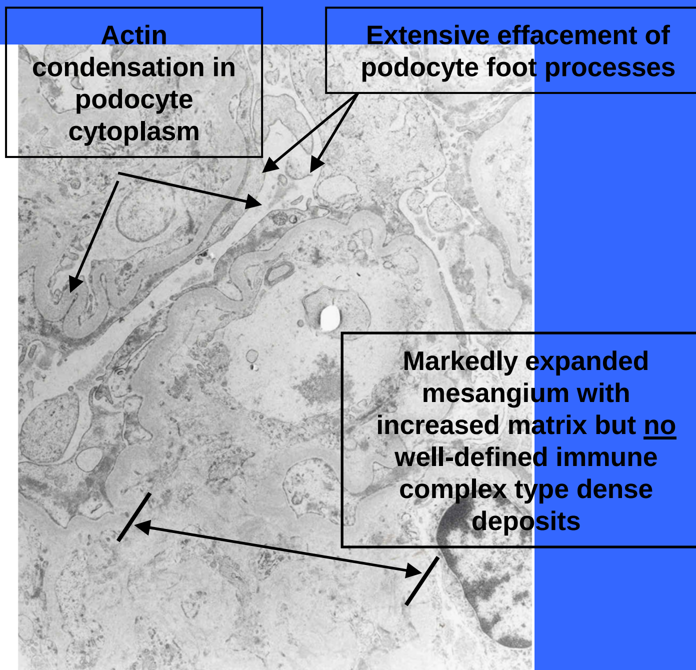


Scanning EM of Kidneys from NL/IR Pigs

06F NL/IR, creat 1.8



76G NL/IR, creat 9.6



Validation Criteria - Human-like Insulin Resistant Type 2 Diabetes Mellitus & ↑ Atherosclerosis in Pigs

1. Insulin Resistance - oral and iv GTT
2. Hyperglycemia - present or absent
3. Body Fat -
4. Dyslipidemia - increased LDL, reduced HDL, ↑TG
5. Arterial Blood Pressure -
6. Serum Markers -
7. Atherosclerosis - Coronary, aortic, carotid, iliac and femoral- a) severity, b) diffuseness, c) rate of progression, and d) changes in mechanical properties
8. Other complications - renal, myocardial, nerve, opthalmic,
9. Reduced complications with successful treatment -
   Metabolic (↓ IR or glucose), devices (e.g., stents), other new rx

Future Model Validation

1. Determine rate of atherosclerosis progression in IR vs IS pigs by percutaneous ultrasound.
2. Determine rate of change in mechanical properties of arteries in IR vs IS pigs by ARFI.
3. Determine if NMR profiling of lipoprotein particle size (small, dense LDL; large HDLs) correlates with IR & IS pig phenotype.
4. Measure 24 hour blood pressure with radiofrequency telemetry monitors in IR vs IS pigs.
5. Establish frequency of combined IR and hyperglycemia.
6. Determine the natural history of atherosclerosis in IR with or without hyperglycemia (ultrasound).
7. Determine the natural history of atherosclerosis in IR with or without hyperlipidemia using NL/IR pigs on low fat diet.

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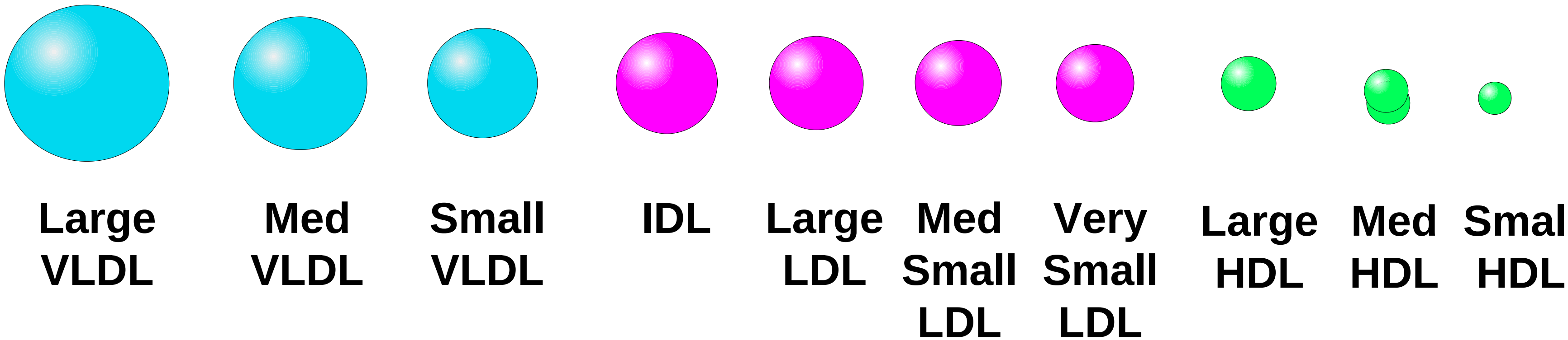
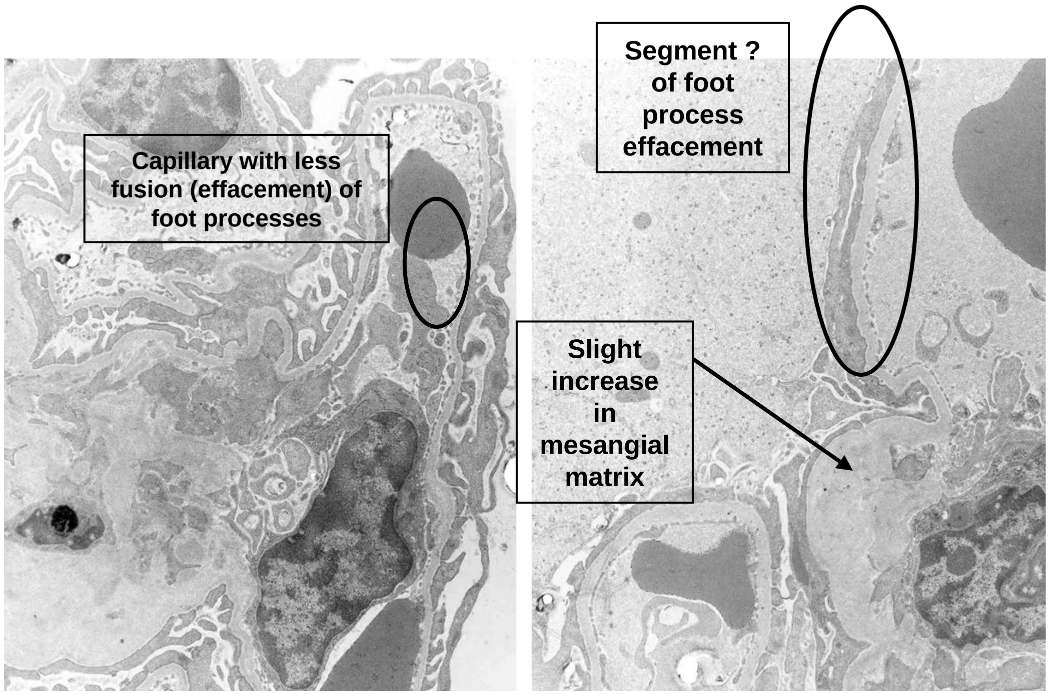
NMR LipoProfile



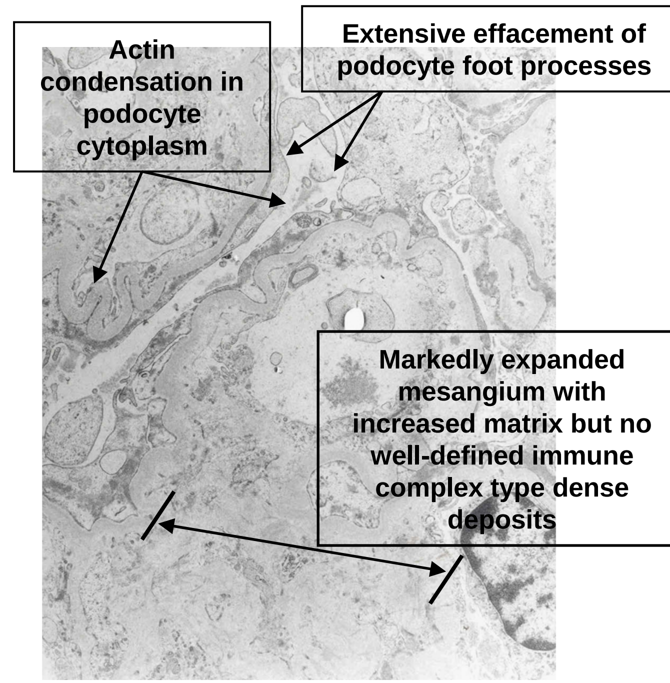
NMR LipoProfile **quantifies lipoprotein subclass particles of different size simultaneously, without requiring a physical separation step (i.e, ultracentrifugation, gel electrophoresis):**

1. **Subclass particle concentrations (nmol/L, μmol/L)**
2. **Mean VLDL, LDL, and HDL particle sizes (nm)**
3. **Calculated lipid mass concentrations (mg/dL Chol or TG)**

06F


Capillary with less fusion (effacement) of foot processes
Segment ? of foot process effacement
Slight increase in mesangial matrix

76G


Actin condensation in podocyte cytoplasm
Extensive effacement of podocyte foot processes
Markedly expanded mesangium with increased matrix but no well-defined immune complex type dense deposits

Dietary composition of cholesterol, fat, and cholate in normal and high fat pig diets

	Normal pig chow	High fat diet
Cholesterol	0.004% (65 mg/day)	1.0% by weight
Corn oil	3%	3%
Lard	3%	3%
Beef tallow	0%	20%
Cholate	0%	0.75%
Cost	$1/day	$7/day