Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs

R01 HL 069364

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AMDCC Presentation

1. Review Research Goals and Progress

   A. Increased coronary and aortic atherosclerosis in IR (Insulin Resistance) pigs

   B. Emergence of a hyperglycemic IR phenotype

   C. Progress with NMR lipoprotein profiling

2. Response to EAC recommendations

   A. Preliminary pig tail blood pressure (BP) data

      - IR pigs may have higher BP than IS pigs.

3. Collaborations within the AMDCC

Program Goals

1. Create NL (i.e. Normal Lipid Chapel Hill pigs with diet-inducible atherosclerosis) and FH (Familial Hypercholesterolemic) pigs with and without IR (Insulin Resistance).
2. Document the rate and extent of development of atherosclerosis in both strains of IR pigs and compare to IS (Insulin Sensitive) controls.
3. Characterize biochemical changes that occur with disease markers in serum, plasma, or lesions:
   - Indices of insulin sensitivity, lipoproteins,
   - Proinflammatory cytokines, growth regulatory proteins, etc.
4. Establish a colony of well-characterized animals for dissemination to the research community

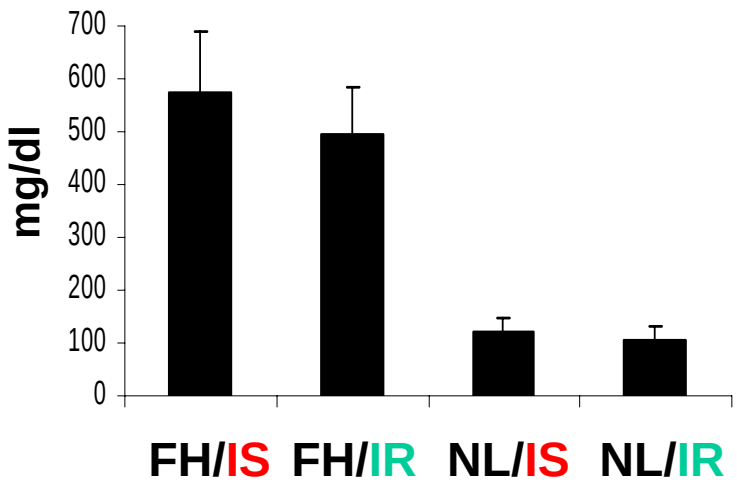
Goal 1 - Create 4 pig phenotypes:

Familial Hypercholesterolemic (FH) & Normal Lipid (NL) Pigs

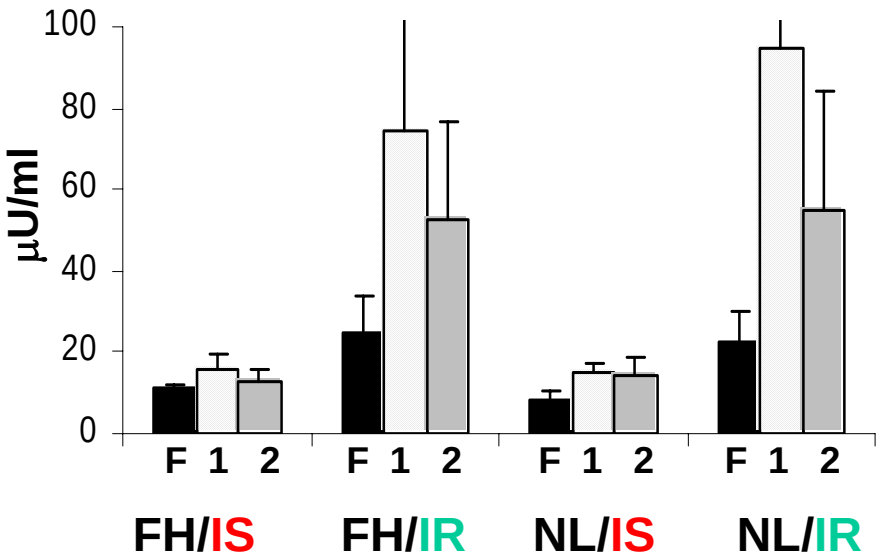
IS – insulin sensitive

IR – insulin resistant

Cholesterol



Serum insulin levels



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Goal 2 - Document the rate and extent of development of atherosclerosis in both IR strains of pigs & compare to IS controls

NL/IR- 2

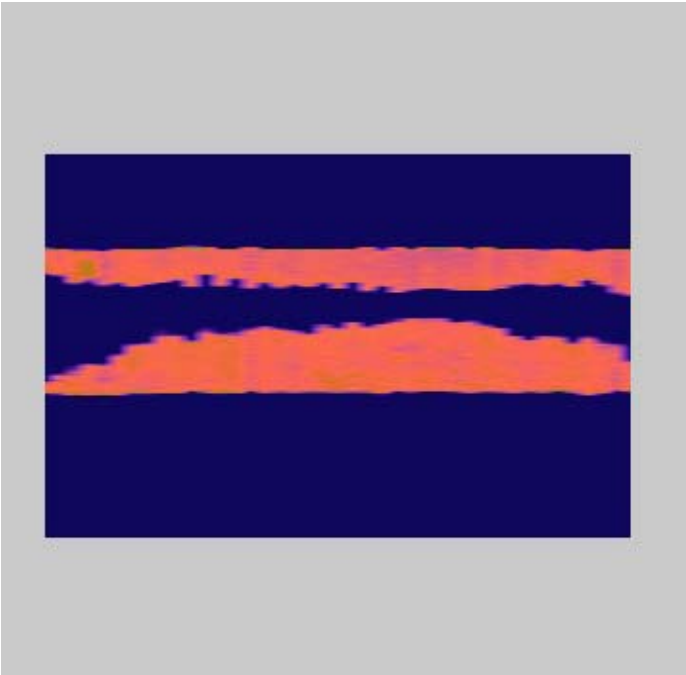
NL/IS - 3

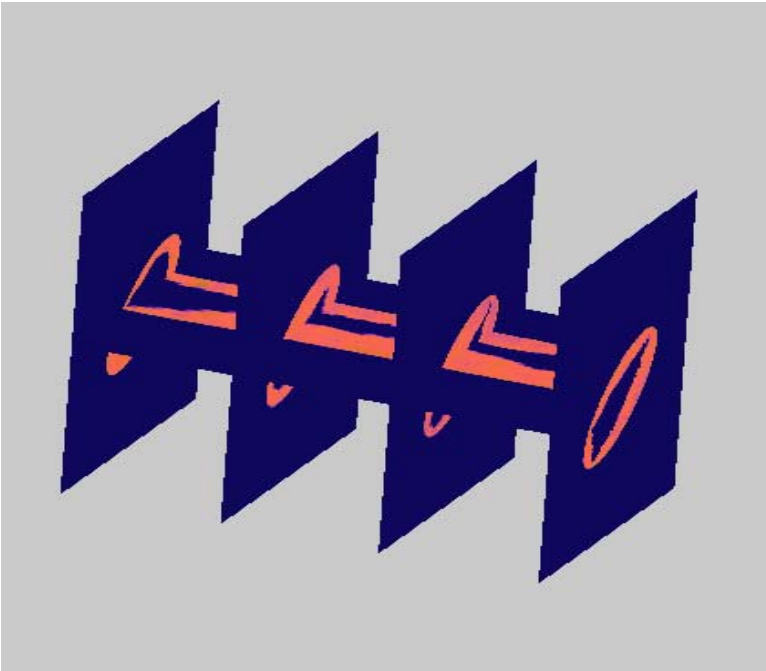
FH/IR - 5

FH /IS - 5

Goal 2- Atherosclerosis in FH/IR & FH/IS pigs

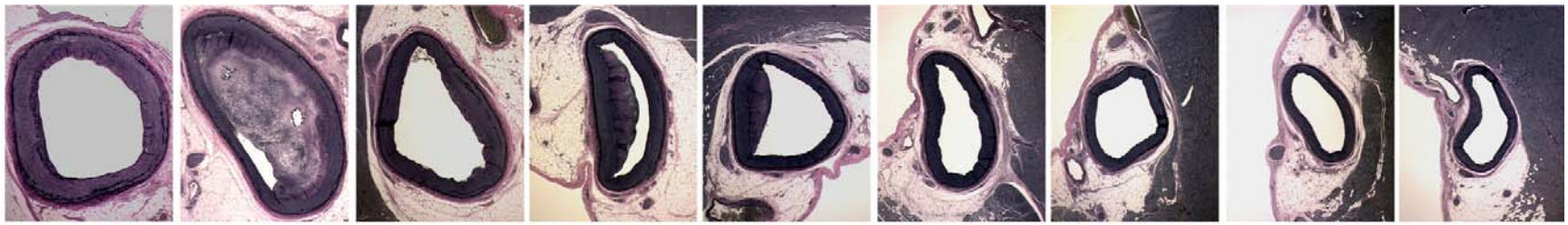
Computer-Assisted Morphometry of
Coronary and Aortic Atherosclerosis
(Image J/ NIH)



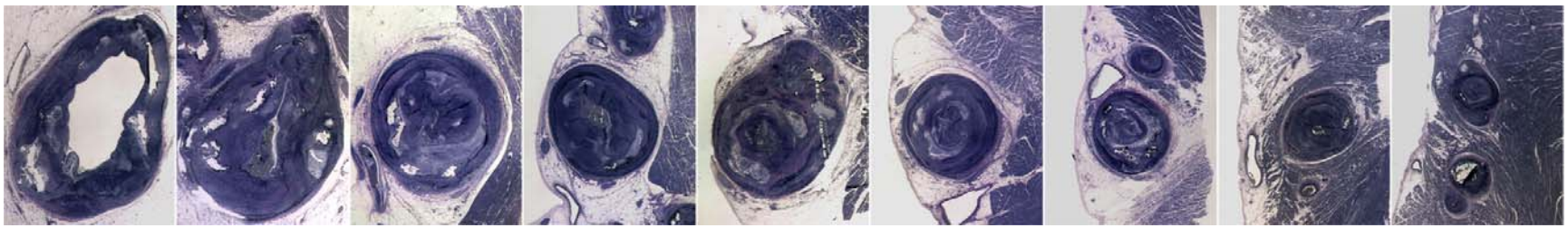


Extent of Coronary Atherosclerosis in FH/IR and FH/IS pigs

49F FH/IS LAD **% stenosis = 17.6 ± 5.8 (n=4 pigs)**



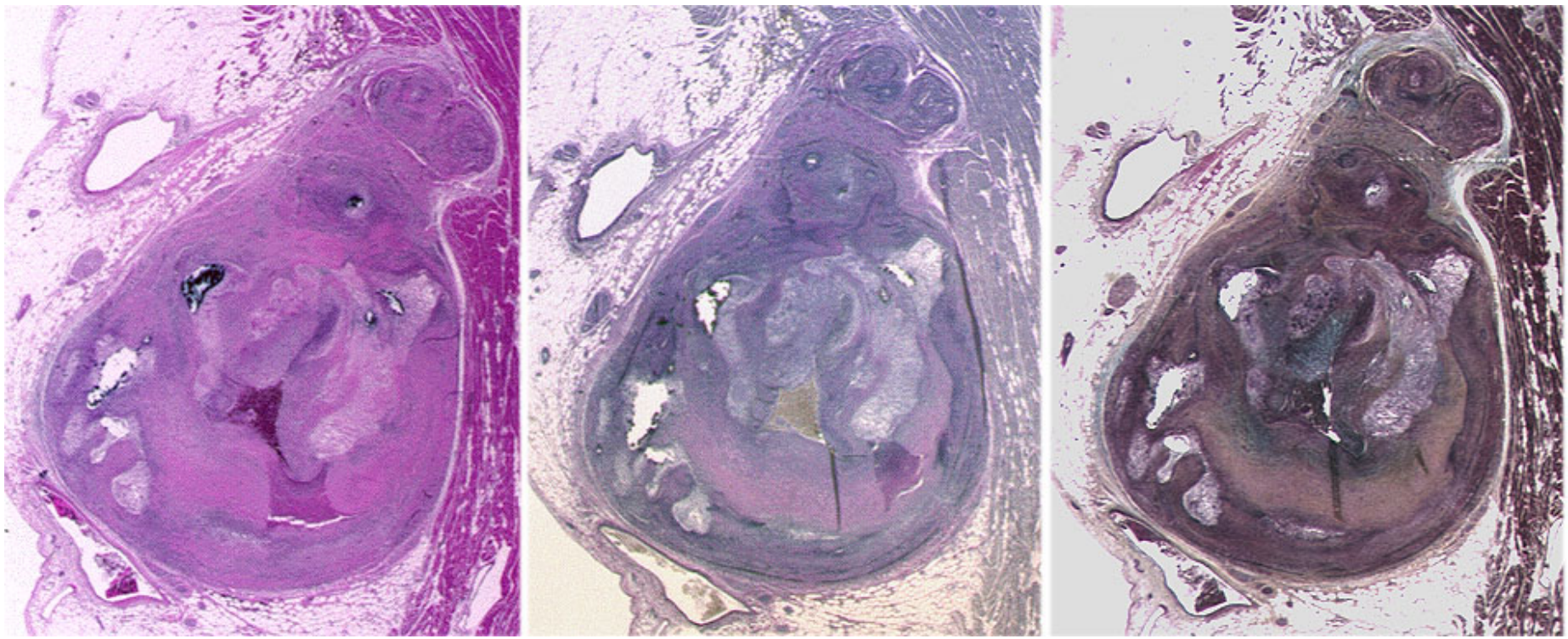
31 FH/IR LAD **% stenosis = 86.9 ± 10.0 (n=4 pigs)**



~1cm

Goal 2- Coronary Atherosclerosis in FH/IR pig # 31

H & E VVG Movats



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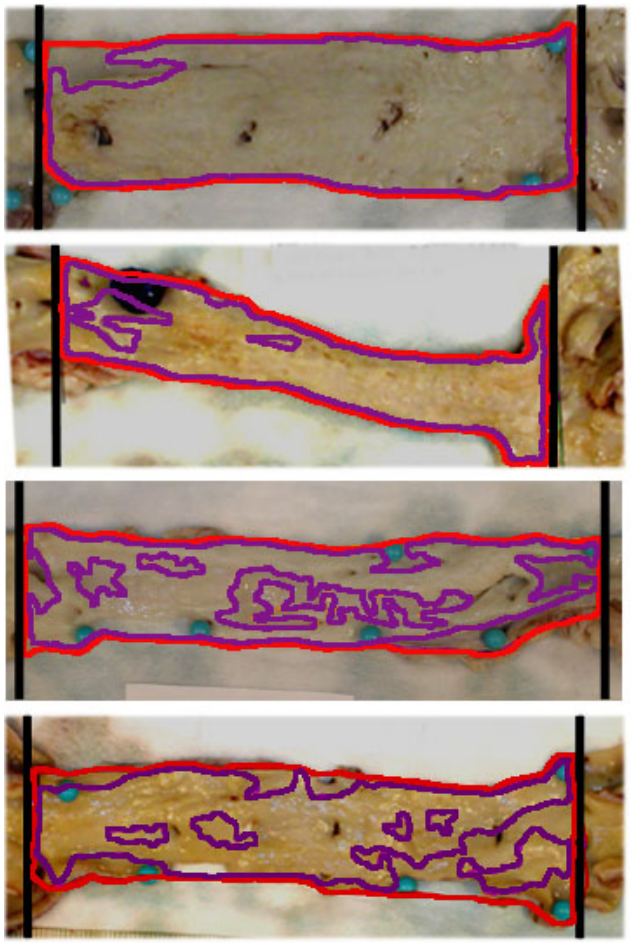
Goal 2- Aortic Atherosclerosis in FH/IR & FH/IS pigs

FH/IR



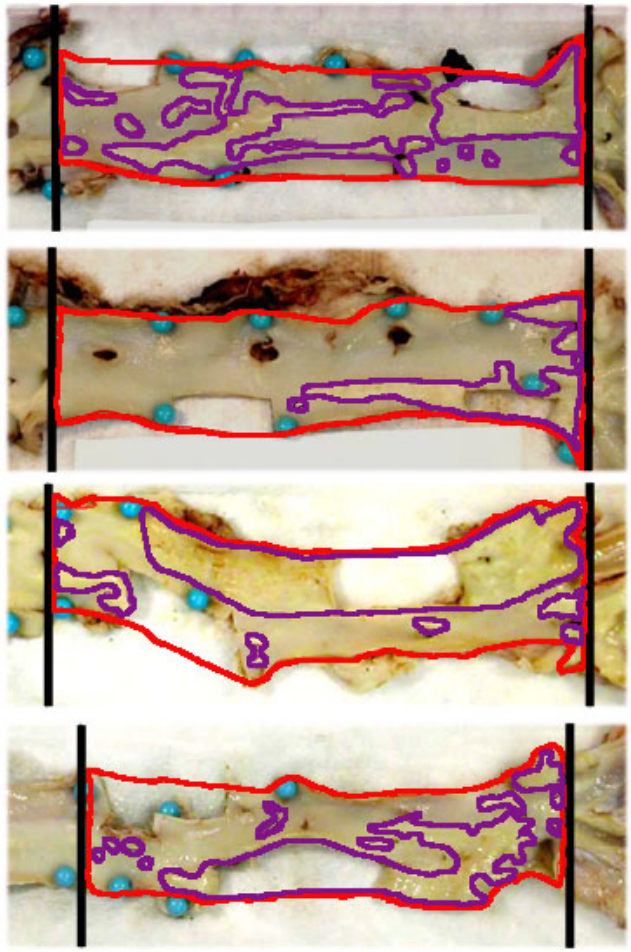
31: 92%

37: 95%

9046: 71%

28G: 68%

FH/IS



47F: 38%

49F: 19%

51F: 50%

52F: 33%

Mean ± SD 82 ± 14 % — 35 ± 13 %

~1cm

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Goal 3 - Characterize biochemical changes that occur with disease markers in serum, plasma, or lesions

Study sample flow chart

Pig ID, age, gender, phenotype

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	**Timepoint (months)**	**Baseline**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
2	**DNA sample**	**x**												**x**
3	**weight**	**x**			**x**			**x**						**x**
4	**% body fat**	**x**			**x**			**x**						**x**
5	**IVUS/FIMT**				**x**			**x**						**x**
6	**Insulin sensitivity, Si (Bergman FSIGT)**	**x**			**x**			**x**						**x**
7	**Lipoprotein and lipid levels**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
8														
9	**Plasma & Serum Markers**	**x**	**x**		**x**			**x**						**x**
10														
11	**Coronary, carotid, aorta, iliac and femoral atherosclerosis morphometry**													**x**
12	**Tissue markers (fat, skeletal muscle, etc.) & AMDCC distribution of tissues**													**x**

Goal 3 - Characterize biochemical changes that occur with disease markers in serum, plasma, or lesions

EMERGENCE OF HYPERGLYCEMIC PHENOTYPE IN FH/IR PIGS

Phenotype	Pig ID	Fasting Insulin	Fasting glucose	Serum creatinine	S_i	Other
FH/IR						
	32	3.8	128	2.5	2.6	
	37	4.6	213	2.1	ND	
	31	21	90	1.4	ND	Sudden death
	34	22.3	84	1.5	ND	Sudden death
FH/IS						
	47F	5.9	76	2.1	4.2	
	49F	8.0	74	2.4	5.4	
	51F	8.4	76	2.9	4.4	
	52F	8.0	78	2.2	4.5	

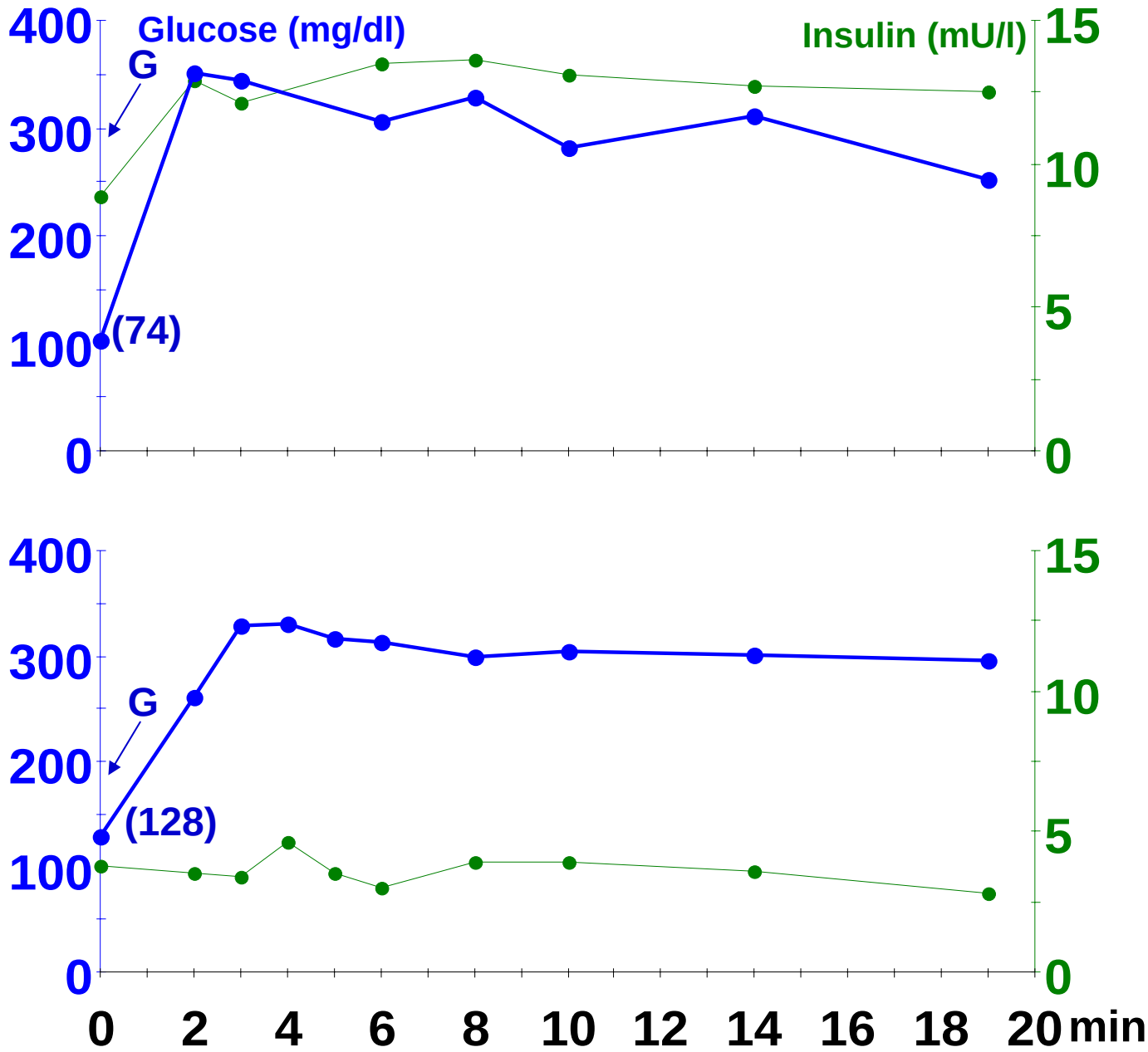
Goal 3 - Bergman FSIGT in FH/IS and FH/IR Pigs

49F FH/IS
(S_i 5.4)

32 FH/IR
(S_i 2.6)

G = glucose,

0.3g/kg iv.

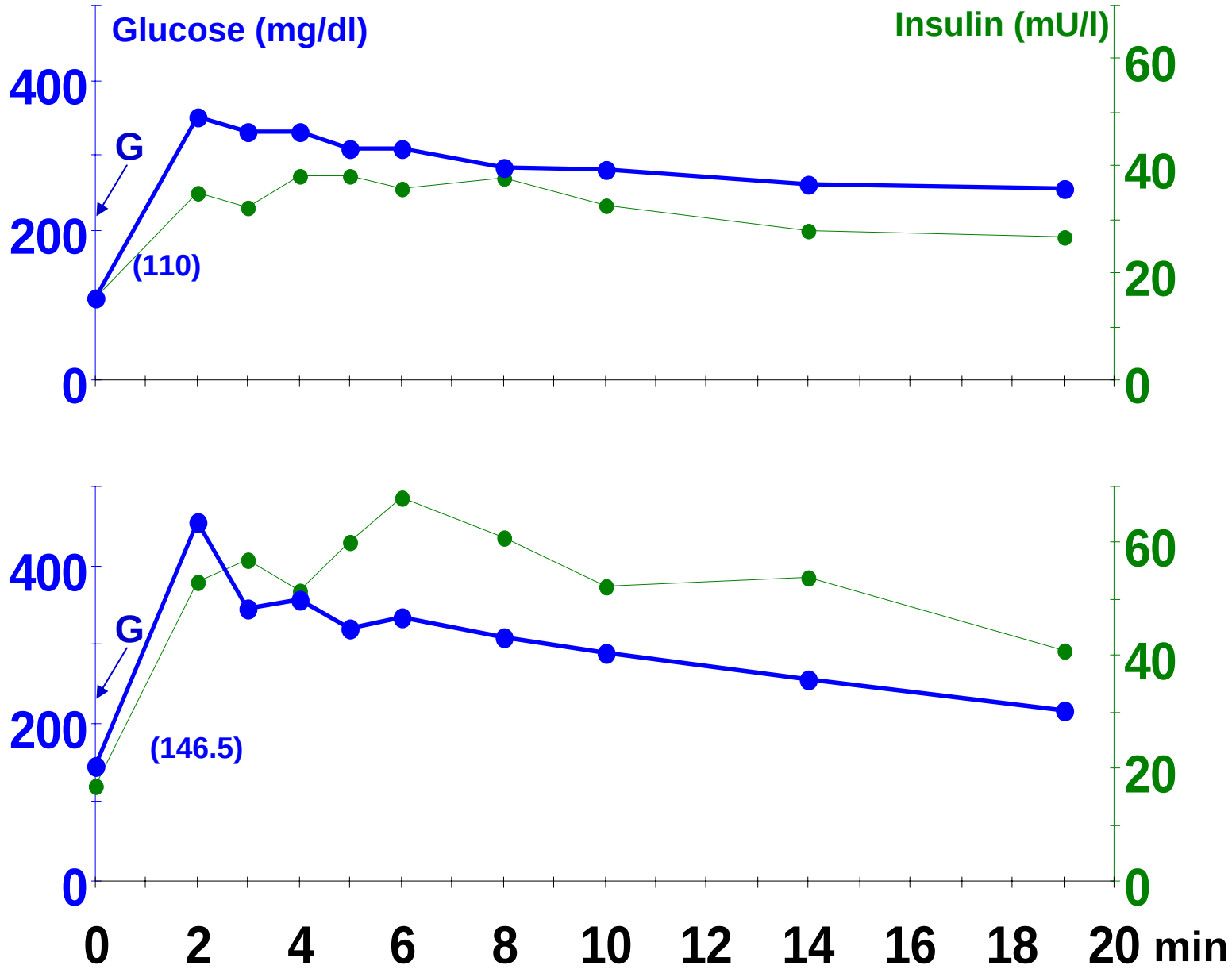


Goal 3 - Characterize biochemical changes that occur with disease markers in serum, plasma, or lesions

EMERGENCE OF HYPERGLYCEMIC PHENOTYPE IN NL/IR PIGS

Phenotype	Pig ID	Fasting Insulin	Fasting Glucose	Serum Creatinine	S_i	Other
NL/IR						
	69G	26.8	147	2.5	3.1	
	76G	27.7	112	13.8	2.9	Renal failure
	02F	12.8	144	2.1	3.8	
	06F	21.1	165	1.7	2.8	
NL/IS						
	69F	19.6	106	2.0	3.9	

Goal 3 - Bergman FSIGT in NL/IS and NL/IR Pigs

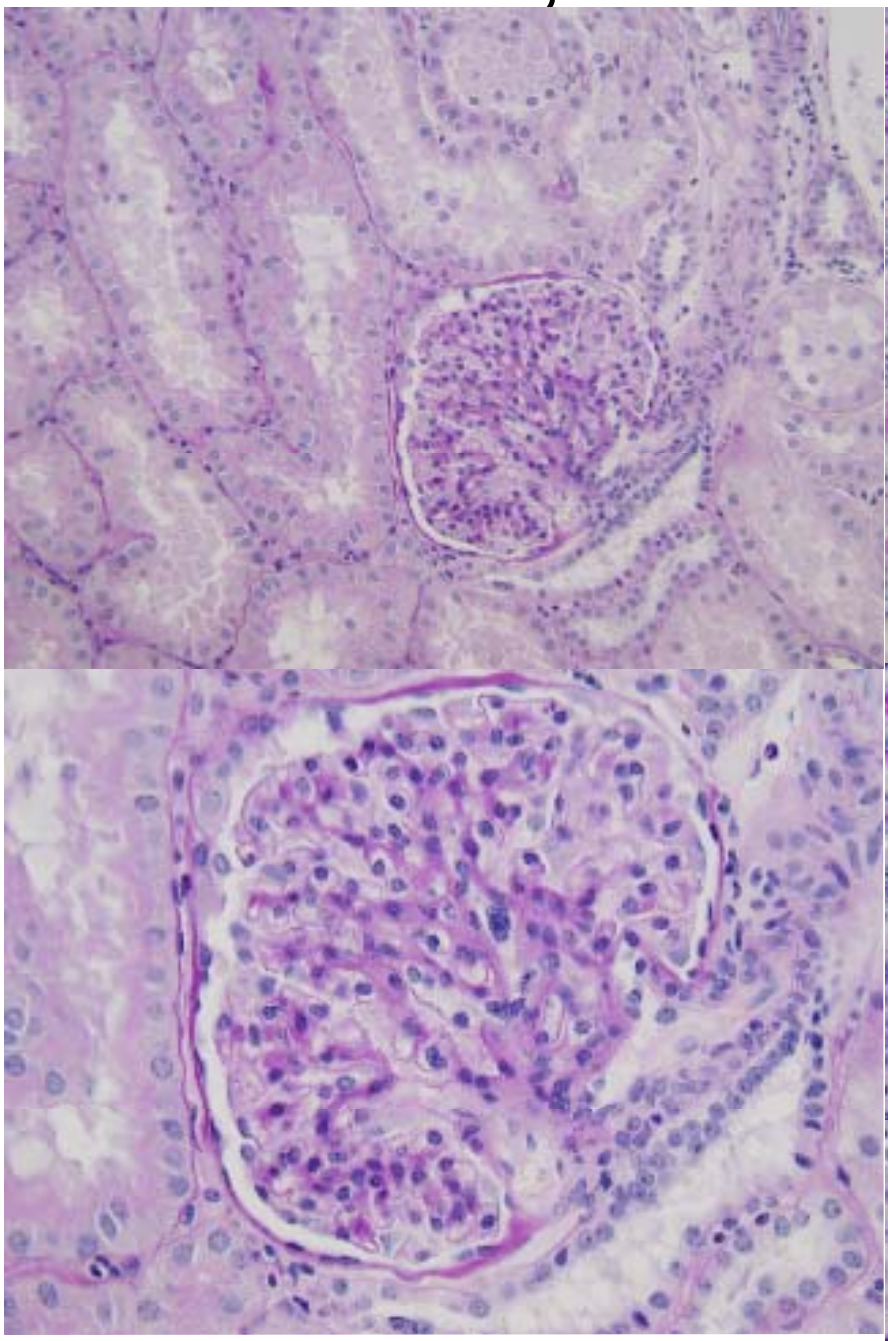


69F NL/IS
(S_i 3.9)

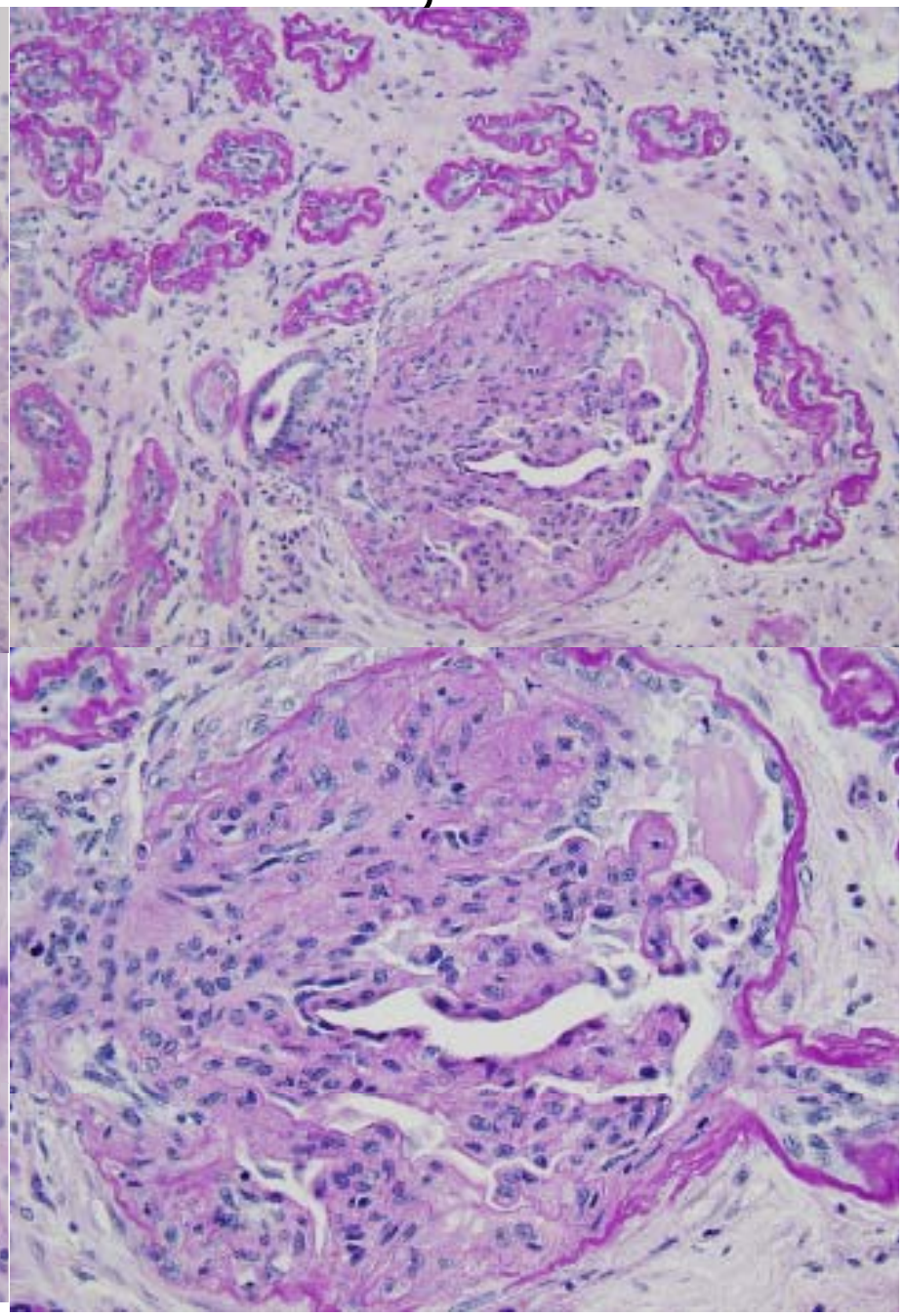
69G NL/IR
(S_i 3.1)

G = glucose,
0.3g/kg iv.

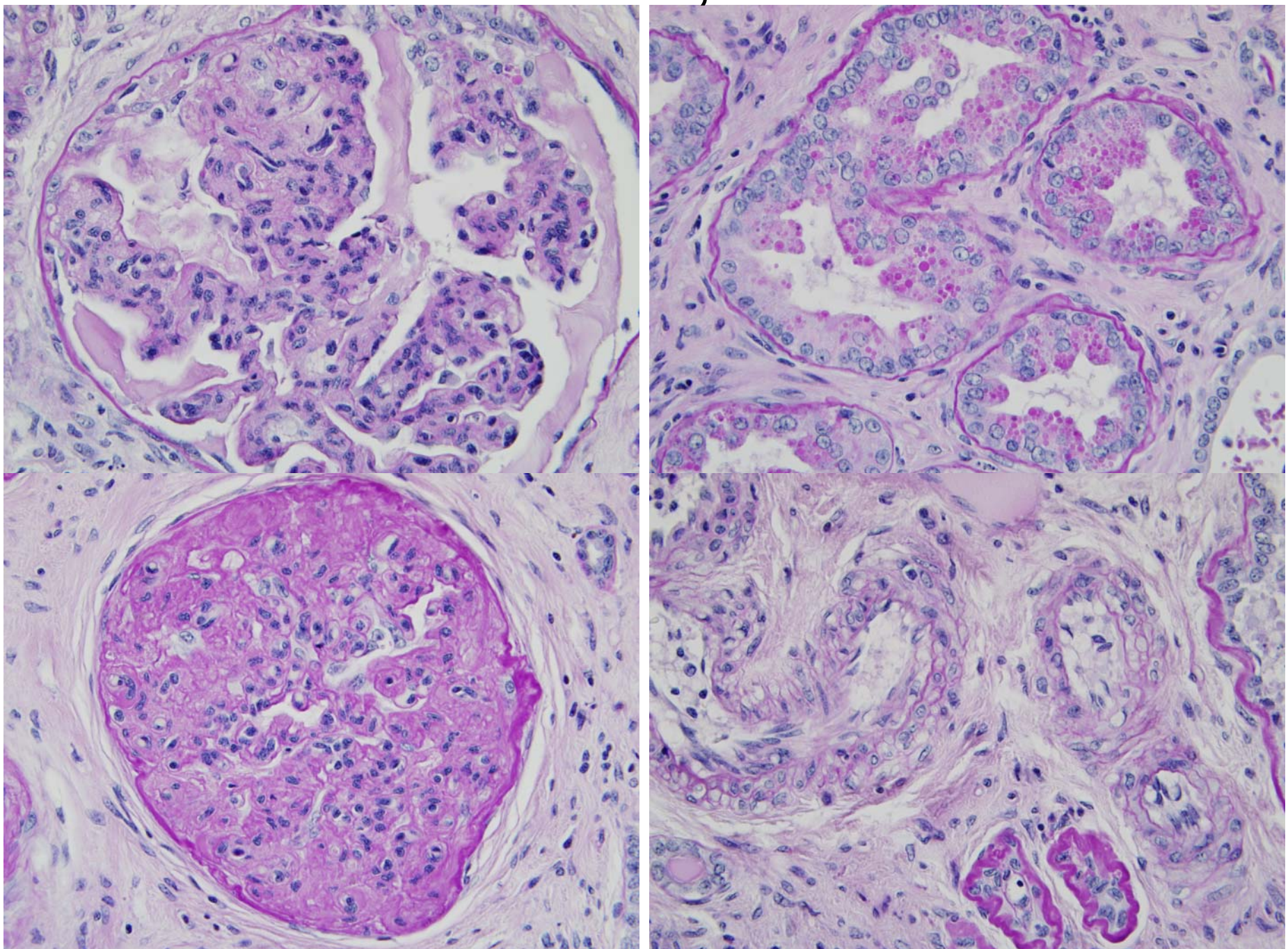
02F NL/IR, creat 2.1



76G NL/IR, creat 13.8



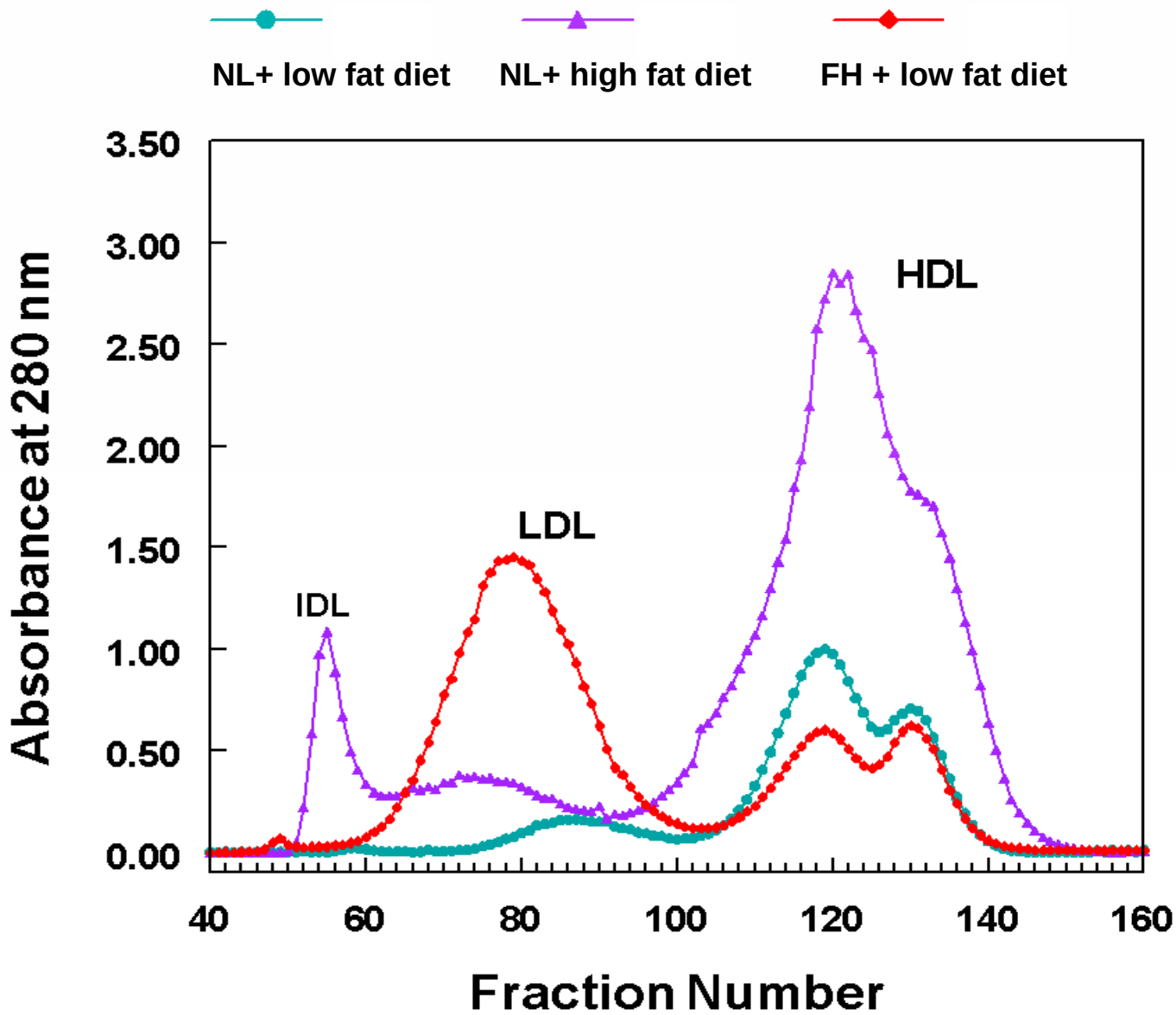
76G NL/IR, creat 13.8



Goal 3 - Validation of NMR LipoProfile for Pig Lipoproteins

A-15 Agarose Column Profiles for UNC Pigs

Density fraction 1.006 - 1.220 kg/L



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   C. Progress with NMR lipoprotein profiling

2. Response to EAC recommendations

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3. Collaborations within the AMDCC

EAC Rec # 1 - Establish plan for banking and sharing relevant tissues for future study

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	A	B	C	D	E	F	G	H	I	J	K	L	M	N
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8														
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11	**Coronary, carotid, aorta, iliac and femoral atherosclerosis morphometry**													**x**
12	**Tissue markers (fat, skeletal muscle, etc.) & AMDCC distribution of tissues**													**x**

EAC Rec # 1 (cont'd)- Establish plan for banking and sharing relevant tissues for future study

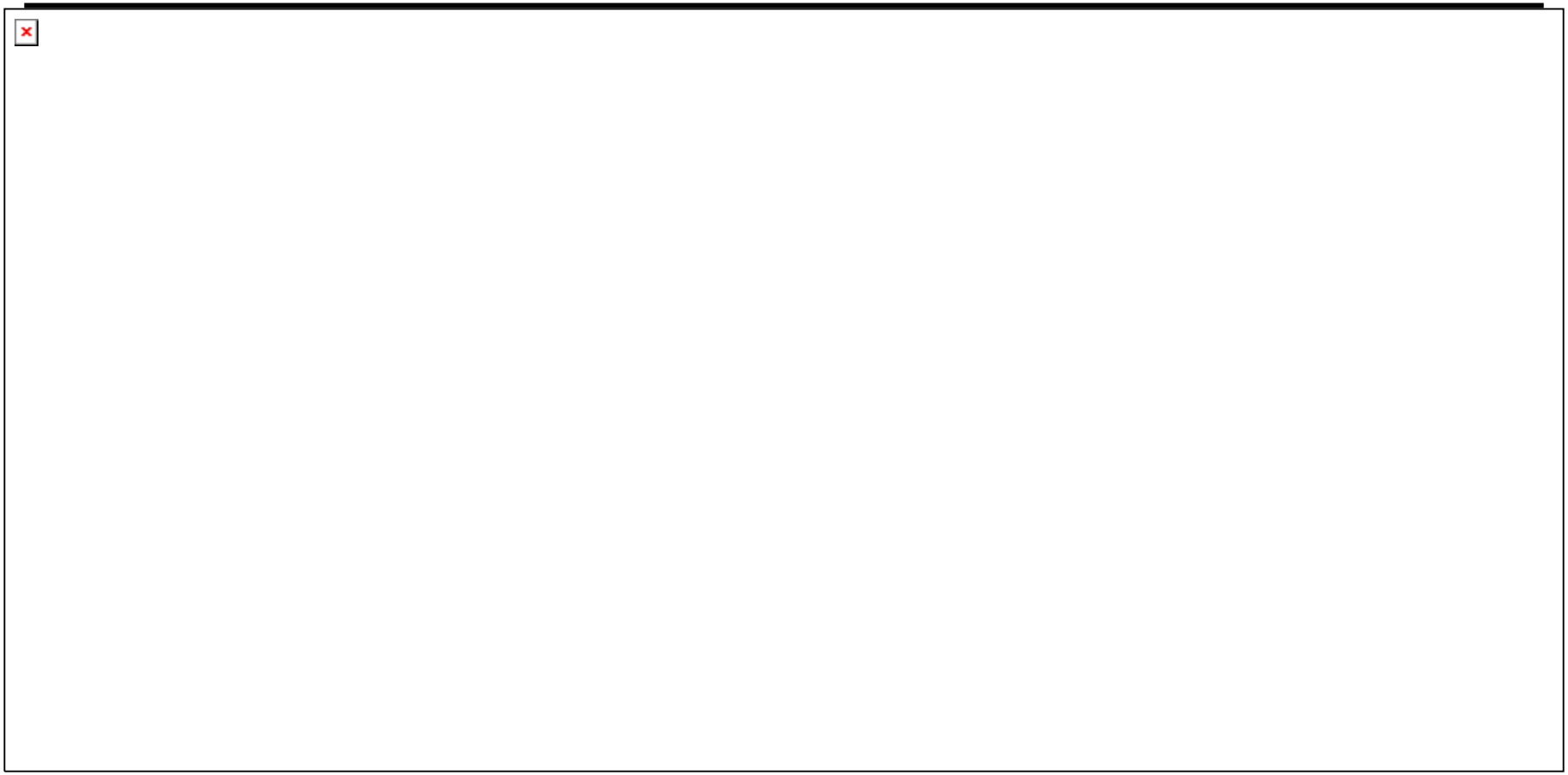
AMDCC Pig Tissue Distribution Plan

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

EAC Rec # 2 - Please upload all relevant assays and protocols to the website. The website should be a destination for pig researchers as well as mouse.

1. **All relevant methods and assays uploaded to AMDCC website with the guidance of Rick McIndoe and his team.**
2. **Data from completed experiments are being uploaded.**

EAC Rec # 3 - Since interventional studies (imaging, etc.) are challenging in such a model, looking at several surrogate markers may be a good approach. These may include markers of insulin resistance as well as inflammation/atherogenesis and could be monitored over time with insulin sensitivity measurements.



EAC Rec # 4- Time course analyses of skin biopsies may be insightful and should be sent to the cores.

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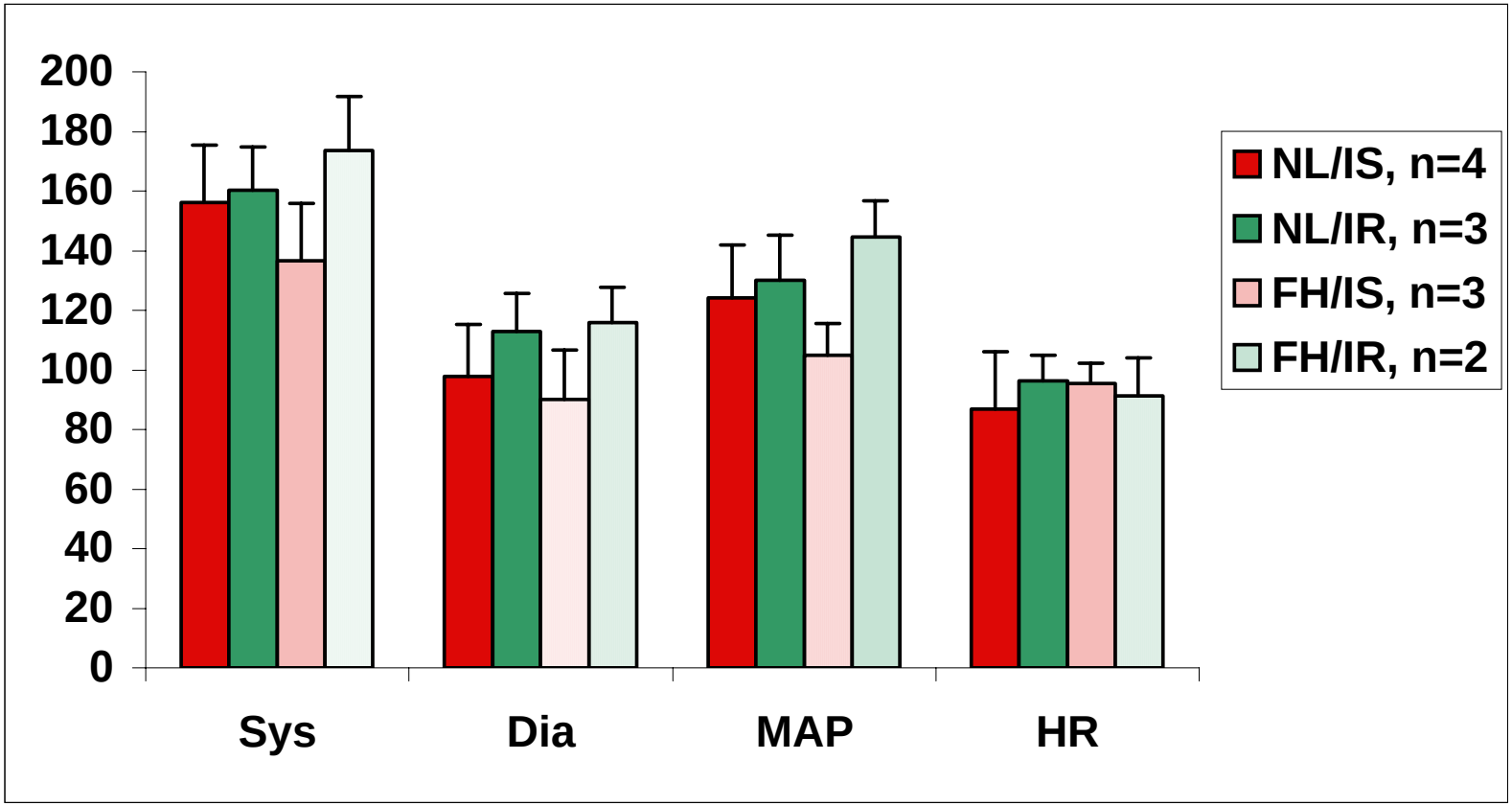
1. This protocol is being developed with Dr. Eva Feldman

EAC Rec #5 Blood Pressure - Pig Tail-Cuff



1. Krege JH, Hodgin JB, Hagaman JR, Smithies O. A noninvasive computerized tail-cuff system for measuring blood pressure in mice. Hypertension. 1995;25:1111-1115.

2.Goodrich JA, Lackland DT, Del Signore MJ, Swindle MM. Non-invasive measurement of blood pressures in the Yucatan micropig (Sus scrofa domestica), with and without midazolam-induced sedation. Comp Med. 2001;51:13-15.

3.Mesangeau D, Laude D, Elghozi JL. Early detection of cardiovascular autonomic neuropathy in diabetic pigs using blood pressure and heart rate variability. Cardiovasc Res. 2000;45:889-899

EAC Rec #6 - Pig work remains exciting, and development of the Ossabaw pigs should be encouraged. The EAC continues to be excited about the progress of the large animal models and every effort should be taken to keep the colony going.

First Ossabaw Litter

Born July 4, 2004

	Insulin, µU/ml		
	Fast,	1h,	2hr
Dam:	15.0,	53.8,	45.3
	X		
Sire:	12.9,	140,	36.8

Produced 5 males.

Second pregnancy in progress.

New Pig Housing Unit - 2005

March 2004

March 21, 2005

Plans for Year 4

1. Enter methods and data on AMDCC web site.
2. Continued selective breeding for increased IR and monitor fructosamine over time in IR and IS pigs.
3. Complete new pig housing.
4. Continue atherosclerosis study with ultrasound monitoring of disease progression.
5. Monitoring insulin sensitivity, glucose levels, blood pressure, creatinine, and characterize proteinuria and alb/creat in pigs.
6. Validate NMR lipoprotein analyses in all phenotypes
7. Begin validation of pig gene microarray analyses for monitoring originally proposed genes and potentially identification of new genes of interest.

Francis Owen Blood Research Laboratory

- Timothy C. Nichols, M.D.
- David Clemmons, M.D.
- Dwight Bellinger, D.V.M., Ph.D.
- Robin Raymer, B.S.
- Elizabeth Merricks, B.S.
- Kent Passingham, B.S.
- Mark Kloos, B.S.
- FOBRL Animal Care Staff

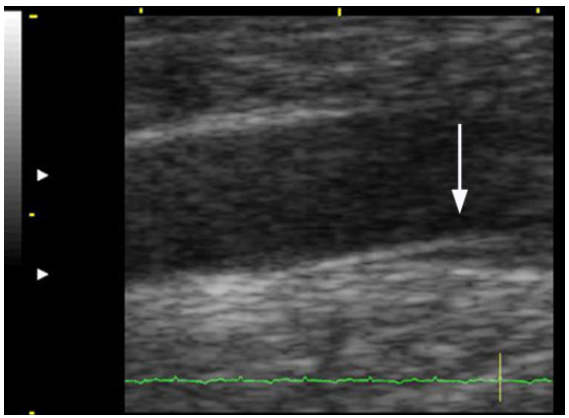
- AMDCC

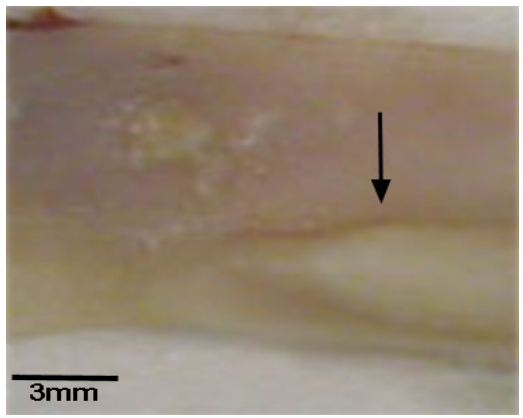
Pig Dietary Fat, Protein, CHO

	Low Fat (15% Pig & Sow Pellets)	High Fat (21% Tail Curler Jr Pig Starter Feed)*
Crude Fat (%)	3.58	24.57
Total Protein (%)	15.42	15.8
Total CHO (%)	56.93	48.83
Estimated kcal/day (6600 kcal/day for 180 to 250 kg pig)	5,841	8,347

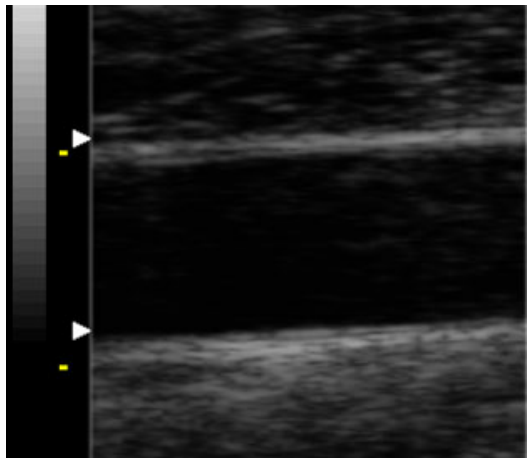
*1% cholesterol, 0.75% cholate, 20% beef tallow by weight added.

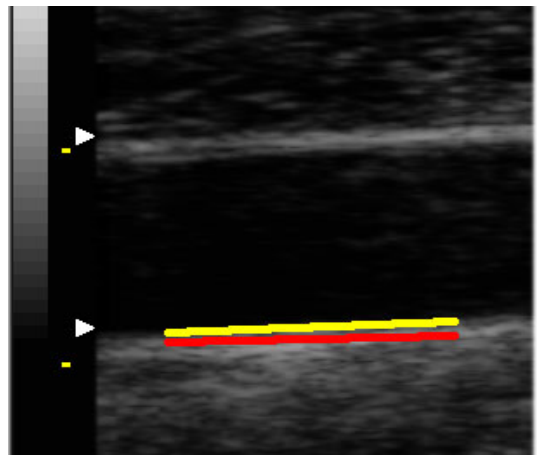
Goal 2 - Rate of Femoral Atherosclerosis Progression by Percutaneous Ultrasound-
1. Plaque number & 2. Plaque volume





Goal 2 - Rate of Femoral Atherosclerosis Progression by Percutaneous Ultrasound (cont'd) - 3. Intima Media Thickness



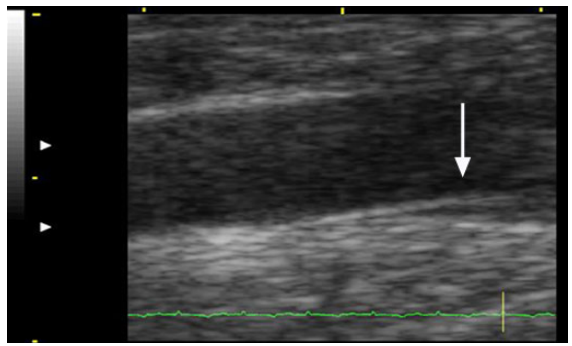


0.5 mm^2
NIH image J

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

EAC Rec # 3.1 - It would be of great interest to evaluate these models for functional (e.g., endothelial function) as well as imaging studies (e.g., ultrasound, CT, MRI).

1. **Flow Mediated Dilation - FMD**
2. **Ultrasound -**



EAC Rec # 3.2 - Also important would be establishing collaborations to investigate the effects upon stent restenosis since DM remains the single most important risk factor for this problem

1. **Future studies**