

Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs

R01 HL 069364

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AMDCC Presentation

- 1. Review Research Goals and Progress**
- 2. Response to EAC recommendations**
- 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: NL/**IS**, NL/**IR**, FH/**IS**, FH/**IR**

Method - Selective breeding based on screening post pubertal pigs for **IR** and **IS** phenotype.

Goal - postpubertal pigs, insulin ($\mu\text{U/ml}$) & chol:

	<u>Fast</u>	<u>1 hr</u>	<u>2 hr</u>	<u>Cholesterol</u>
<u>IR</u>	> 25	(1 or 2 hr >80)		NL or FH
<u>IS</u>	< 10	< 20	<20	NL or FH

Goal 1 - Breeders for 4 pig phenotypes

**FH/IS, FH/IR,
NL/IS, NL/IR,**

Phenotype	Gender + n	Cholesterol (mg/dl)	Serum Insulin level (μ U/ml)		
			fasting	1hr	2hr
1. FH/IS	3M/2F	572.5 \pm 115.1	11.1 \pm 1.3	15.9 \pm 3.4	13.0 \pm 2.8
2. FH/IR	3M/2F	494 \pm 87.7	24.8 \pm 9.2	74.5 \pm 30.4	52.3 \pm 24.4
3. NL/IS	2M/2F	119.5 \pm 28.8	7.9 \pm 2.6	15.1 \pm 2.2	14.5 \pm 4.4
4. NL/IR	5M/2F	106.8 \pm 27.1	22.2 \pm 8.2	95.1 \pm 61.6	54.6 \pm 29.9

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3. Characterize biochemical changes that occur with disease markers in serum, plasma, or lesions:
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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

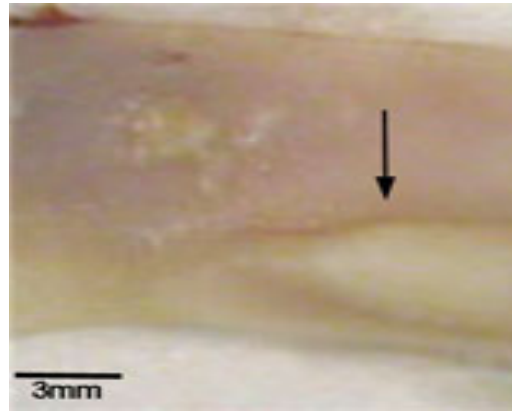
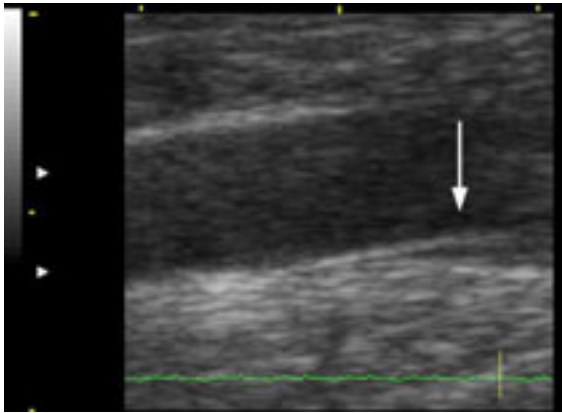
NL/IS - 3

FH/IR - 2

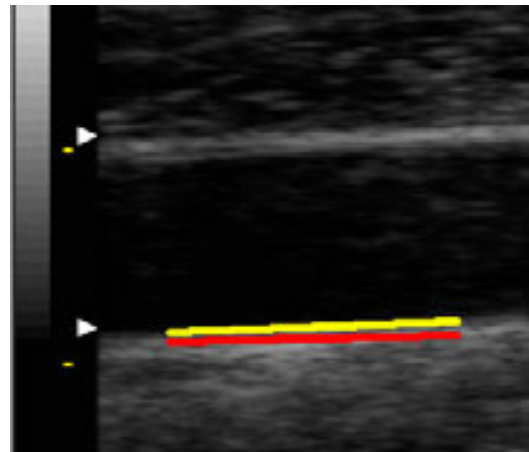
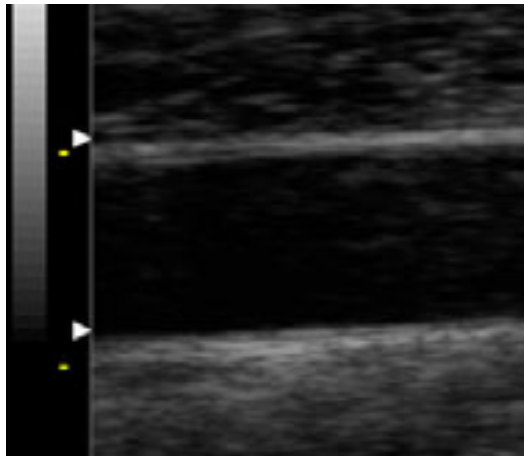
FH/IS - 5

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 mm²

NIH image J

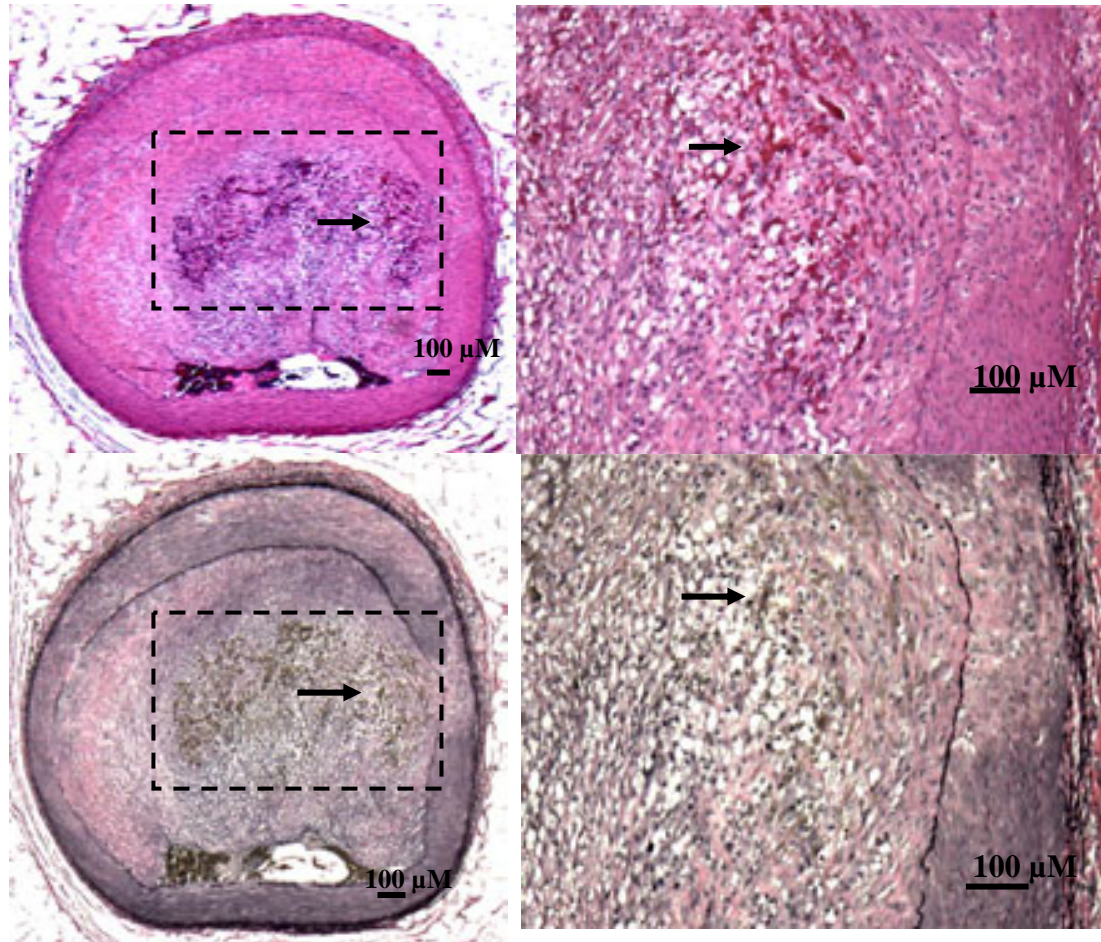
**Van der Meer, et al. Stroke
2003 34(10): 2374-9**

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 coronary and aortic 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,
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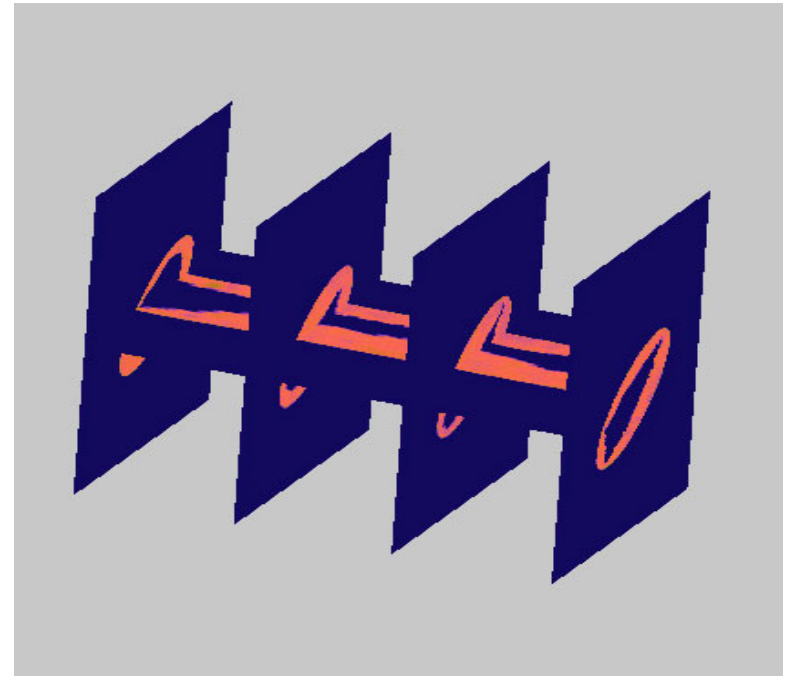
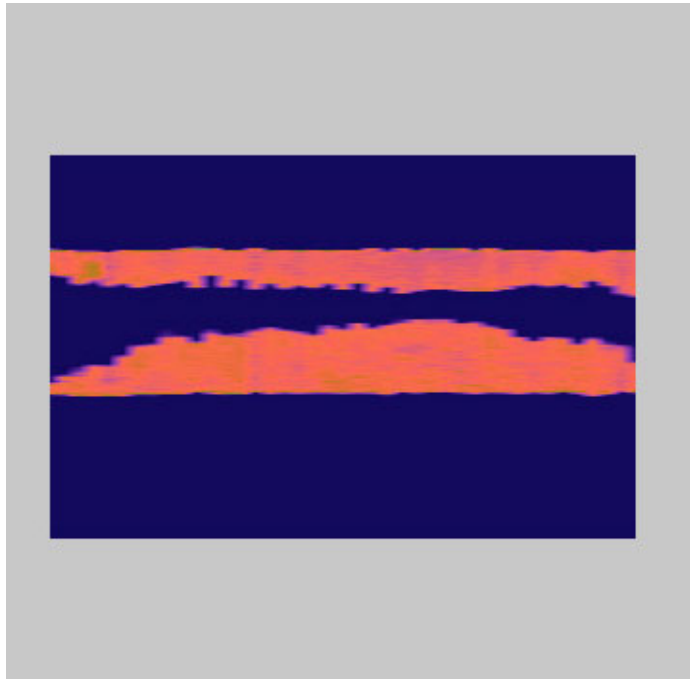
Goal 2- Coronary Atherosclerosis in FH/IR & FH/IS pigs

Hemorrhage in Coronary Atherosclerotic Plaque from 2 yr-old FH pig:
Total chol - 561 mg/dl; insulin - fasting 4.6, 1 hr 93.6, 2 hr 27.1 μ U/ml



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

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



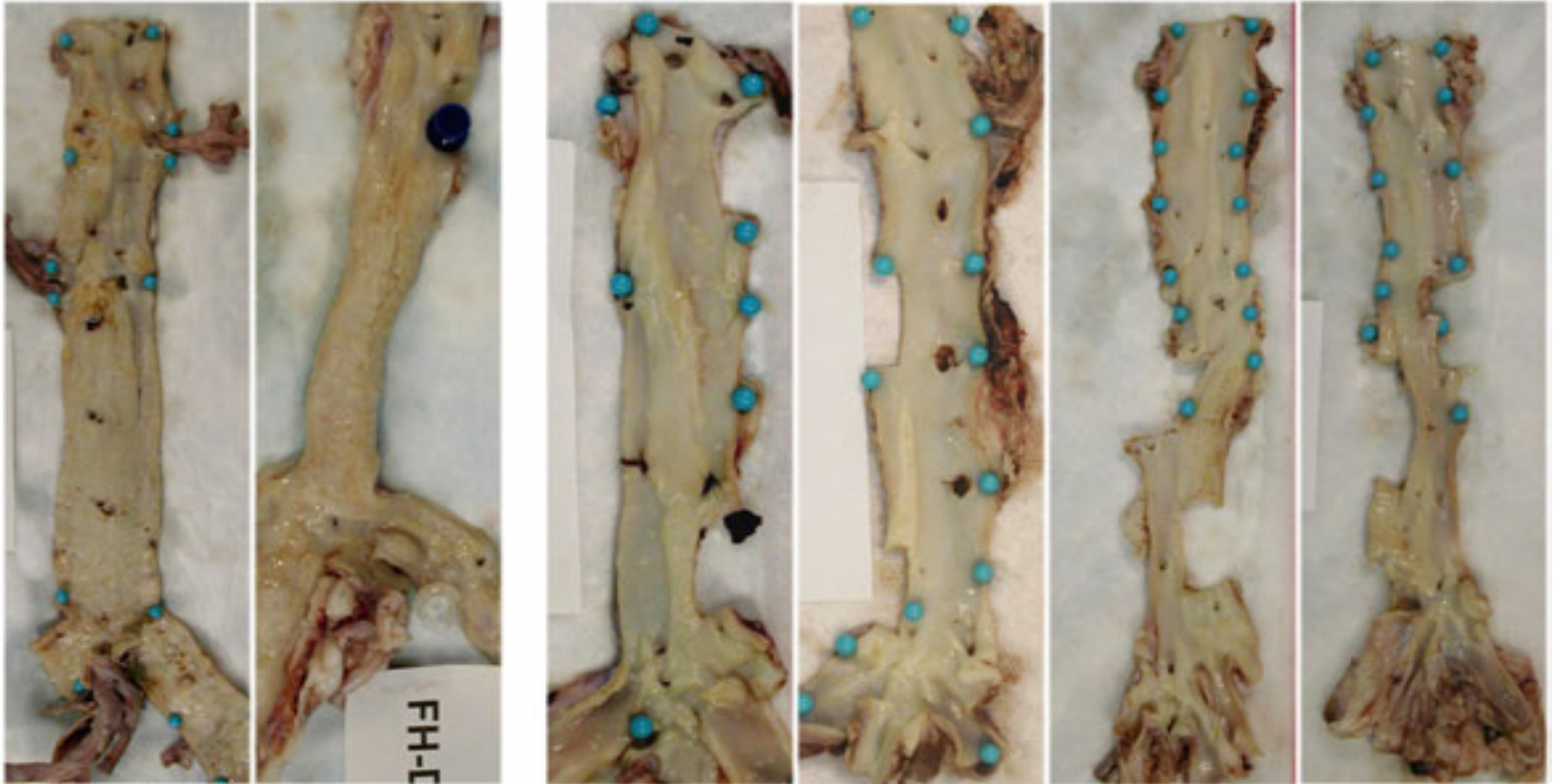
Goal 2- Coronary Atherosclerosis in FH/IR & FH/IS pigs



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

FH/IR

FH/IS



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

FH/IR

FH/IS

92.4%

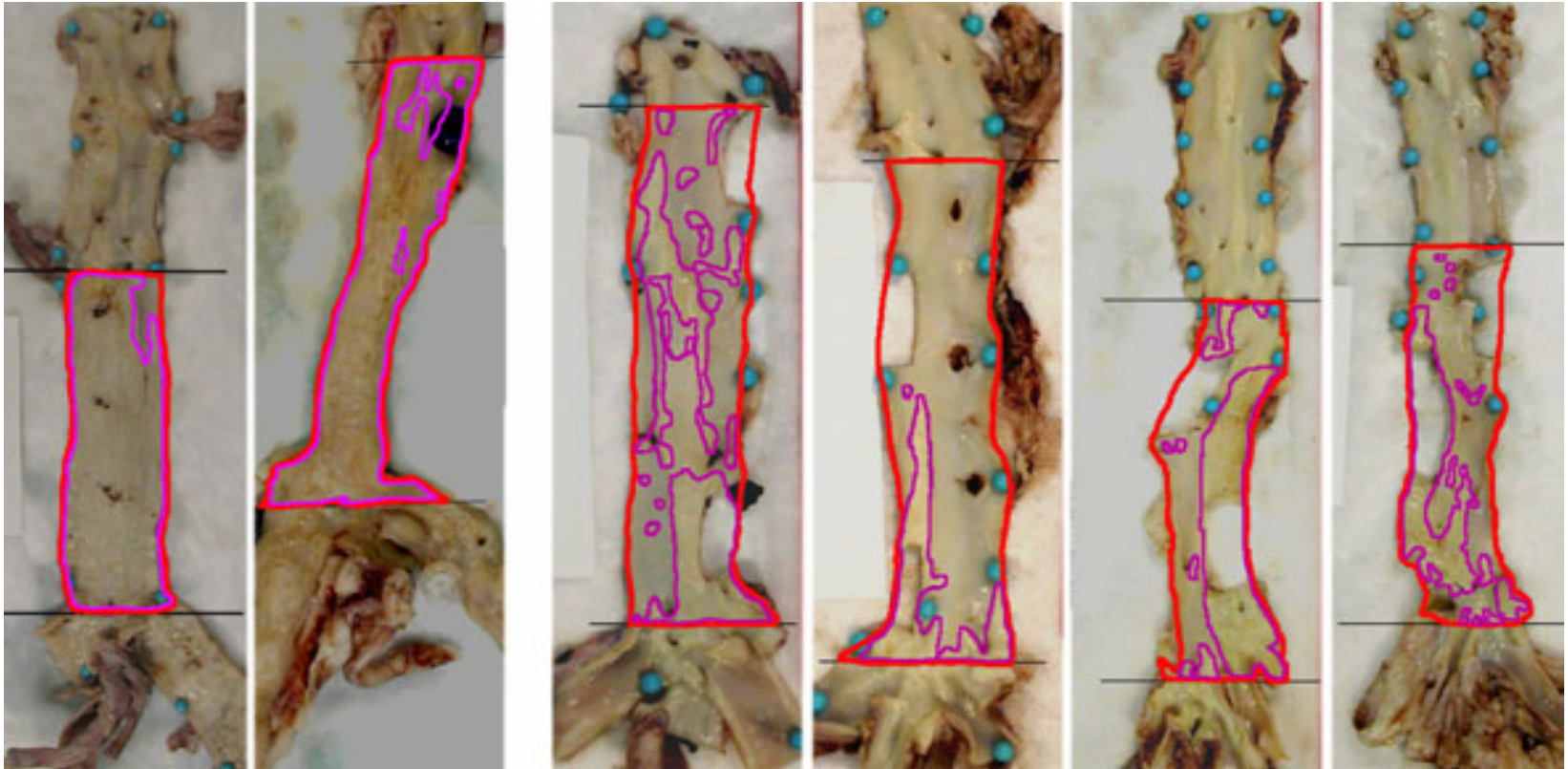
94.7%

38.2%



18.9%


49.7%

32.9%



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

	
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Program Goals

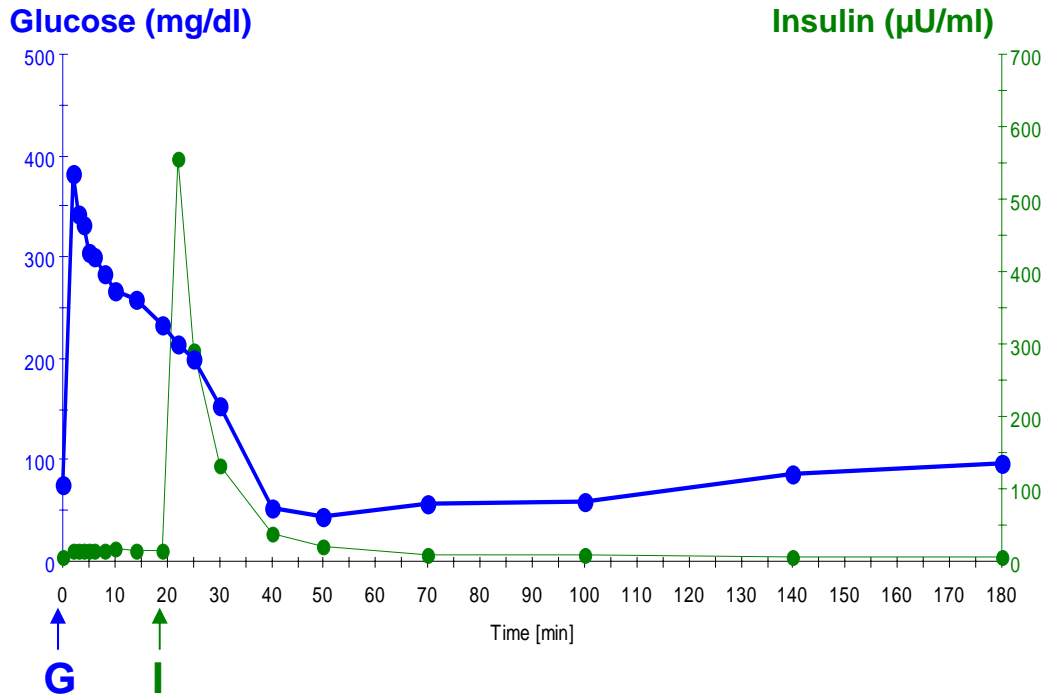
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Goal 3 - Characterize biochemical changes that occur with disease markers in serum, plasma, or lesions

Bergman FSIGT

Individual Bergman FSIGT

52F FH/IS
12 month $S_i = 4.2$
Wt 488 lbs.
Chol 359 mg/dl



Average Bergman FSIGT

FH/IS	NL/IS
(5)	(3)
4.5 ± 0.6	4.2 ± 0.1

**Goal 3 - Characterize biochemical changes that
occur with disease markers in serum, plasma, or lesions:
Serum Lipids and Lipoproteins in NL and FH Pigs**

Lipid or Lipoprotein[§]	<u>NL +normal chow</u> †	<u>NL + high fat</u> †	<u>FH+normal chow</u> †
Total cholesterol	80.0 ± 9.3	774.3 ± 54.5 *	316.5 ± 36.1 *
HDL-C	33.5 ± 1.9	137.0 ± 9.9 *	22.3 ± 2.2*
Triglycerides	33.0 ± 16.3	40.3 ± 11.7	56.8 ± 7.2
LDL(ApoB)	35.7 ± 3.1	142.0 ± 4.8*	169.3 ± 13.9*
ApoA-1	62.4 ± 9.3	170.9 ± 6.9*	42.6 ± 4.8

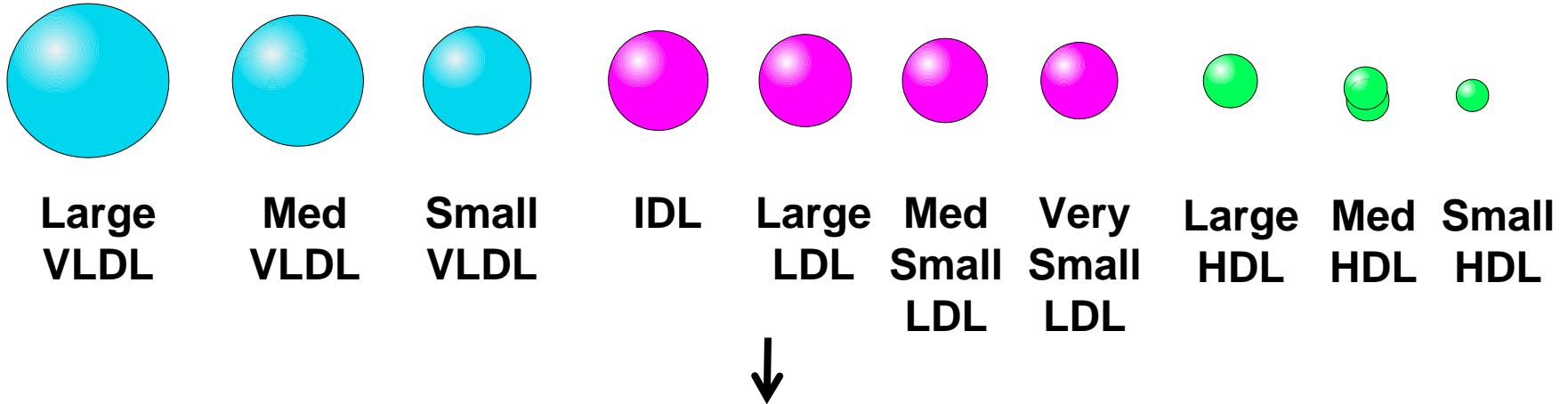
§ mg/ dl

***p < 0.05 (except triglycerides)**

† n = 5 pigs in each group

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

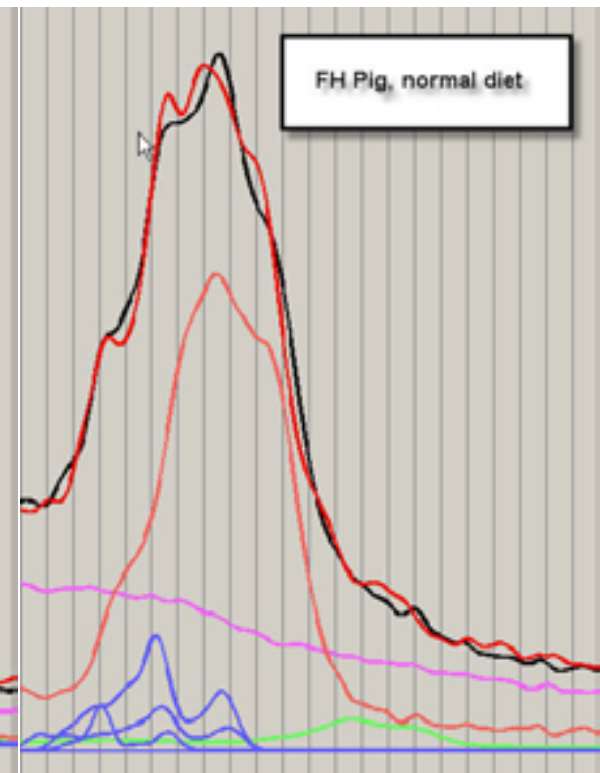
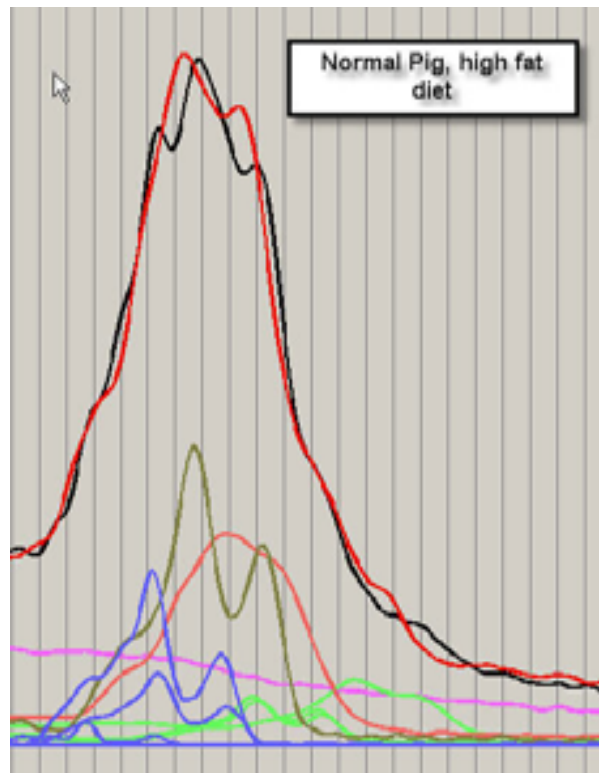
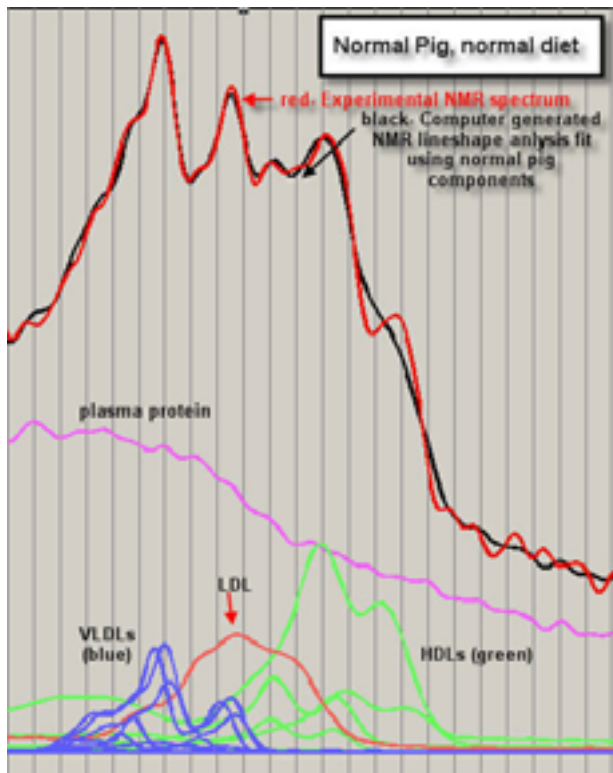
NMR LipoProfile



***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, μ mol/L)**
- 2. Mean VLDL, LDL, and HDL particle sizes (nm)**
- 3. Calculated lipid mass concentrations (mg/dL Chol & TG)**

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



Plans for Year 4

- 1. Enter methods and data on AMDCC web site**
- 2. Continued selective breeding for increased IR.**
- 3. Complete new pig housing - Nov 2004.**
- 4. Continue atherosclerosis study with ultrasound monitoring of disease progression.**
- 5. Monitoring insulin sensitivity in experimental 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.**

New Pig Housing Unit - 2004



March 2004



October 15, 2004

AMDCC Presentation

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- 2. Response to EAC recommendations**
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EAC Rec # 1 - Encouraged to share tissues with other AMDCC members for phenotypic analysis

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 - Strong Support for Ossabaw Model

First Ossabaw Litter Born July 4, 2004

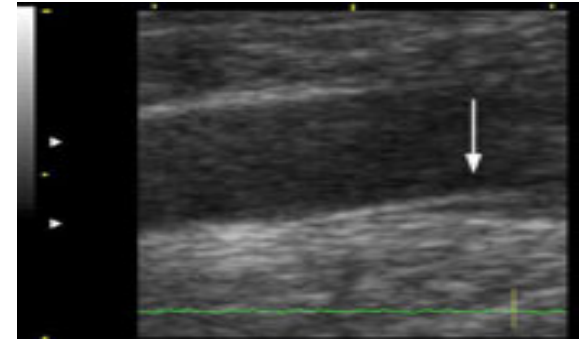
	Insulin, $\mu\text{U/ml}$		
	Fast,	1h,	2hr
Dam:	15.0,	53.8,	45.3
	X		
Sire:	12.9,	140,	36.8

Produced 5 males.



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**

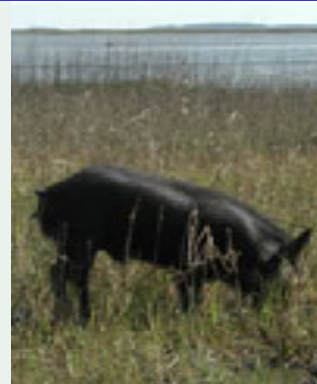
Francis Owen Blood Research Laboratory



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

• AMDCC

Ossabaw Island pigs



Size ~50 kg

Hypersinsulinemia -

$14.2 \pm 2.6, 75.7 \pm 46.2, 32.6 \pm 15.3 \mu\text{U/ml}$

Cholesterol $65.6 \pm 4.9 \text{ mg/dl}$

Obesity

Whitfield J, Fat pigs ape obese humans

Nature News Aug 6, 2003