Animal Models of Diabetic Complications Consortium
(R01 HL 069364)

Annual Report
(2004)

"Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs"
UNC

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Part A:

Principal Investigator's Summary

Animal Models of Diabetic Complications Consortium

Title of Project – R01 HL 069364
Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs

UPDATE REPORT
(September 1, 2001 – February 28, 2005)

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1. Program Accomplishments:

The overall goal of our grant is to produce pigs that have an atherogenic risk factor profile resembling patients with insulin resistance (IR) and hyperlipidemia that will develop coronary, abdominal aortic, and carotid atherosclerosis. Our main strategy is to utilize two strains of pigs available at the University of North Carolina. One strain has traits that predispose it to insulin resistance (IR) and the other has familial hypercholesterolemia (FH). Control strains with normal lipids (NL) and normal insulin sensitivity (IS) are also available. Our breeding program utilizes animals from these groups to develop two new phenotypes: one with more severe IR and one with FH plus IR. As we originally estimated, at least 3 generations of animals have been produced and successfully phenotyped during the first 3 years of this project. All offspring that survived to puberty (~9 months old in pigs) underwent characterization of their degree of insulin resistance and abnormalities in lipid metabolism.

Our major achievement to date has been to produce pigs with one of the following four phenotypes: FH/IS, FH/IR, NL/IS, and NL/IR. The FH/IR pig resembles the human metabolic syndrome exhibiting IR in combination with both elevated triglycerides and LDL cholesterol, and depressed HDL cholesterol. Pigs with all phenotypes are being entered into our proposed studies to validate their usefulness to investigators who are attempting to identify genes that predispose to the development of insulin resistance and to determine the pathophysiological factors that link insulin resistance and atherosclerosis in coronary and carotid arteries and the abdominal aorta. Accordingly, our long-range goal is to exploit new insights into the mechanism(s) by which IR alters atherogenesis and thereby develop and test novel treatments for the growing epidemic of type II diabetes in children and adults and the associated cardiovascular disease.

2. Collaboration within your group:

Not applicable.

3. Collaboration with other AMDCC groups:

Tissues from the pigs with the four phenotypes are being distributed to the investigators listed on the table.

INVESTIGATOR	INSTITUTION	SAMPLE	GOAL
Dale Able Don McClean	Utah	Myocardium	Biochemistry and EM mitochondrial analyses
Tim Kern	Case Western	Eyes	Retinal vessel analyses
Firousz Daneshgari	Cleveland Clinic	Bladder	Physiological analyses
Eva Feldman	Univ of Michigan	Neurological tissues (skin biopsies, sciatic nerve, CNS)	Biochemical and microscopic analyses
Eva Feldman	Univ of Michigan	EDTA plasma	Screen for ROS

4. Pertinent non-AMDCC Collaboration:

Charles Jeannette	UNC	Urine and kidneys	Urine protein, glomeruli & other vessel analyses

5. Address previous EAC comments:

- Do you have an established plan for banking and sharing relevant tissues for future study? Have you courted investigators who might be interested in looking at other tissues such as liver, skeletal muscle, fat, bone, etc.?

We are snap freezing liver, skeletal muscle, and fat in quantities that could be shared with a limited number other investigators as well as to complete our proposed work. At present, bone changes are associated with type 1 or insulin-deficient diabetes. Since our pigs have hypersinsulinemia or insulin resistance, we have not been saving bone.

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

Since the October 2004 AMDCC meeting, all relevant assays and protocols have been uploaded to the AMDCC web site. In addition, Preliminary Data from the atherosclerosis studies also have been uploaded.

- 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.

This work is a major focus of the Nichols' and Clemmons' laboratories.

- 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.

C-reactive protein is being analyzed as a surrogate marker in the experimental pigs.

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

We contacted Eva L. Feldman, M.D., Ph.D., Department of Neurology, University of Michigan, to develop this protocol. This will occur after she completes her NIH grant submissions for March 1, 2005.

- Can you measure BP directly in these animals (perhaps in the ear similar to rabbits)? Can you measure BP in these animals when they are put down?

Blood pressure measurements from the pig's tail have recently been validated in other populations as has been reported for mice.[1-3] We are in the process of adapting these techniques to our pigs and validating measurements.

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.

- Pig work remains exciting, and development of the Ossabaw pigs should be encouraged.

Insulin resistant Ossabaw pigs have been successfully bred. The degree of insulin resistance in the progeny will be characterized after puberty (estimated April 2005).

Animal Models of Diabetic Complications Consortium
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Part B:

Update by Project Leaders

Project 1: “R01 HL 069364 Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs”

Responsible Investigators: David Clemmons and Timothy C. Nichols

1. Rationale and Relevance:

This grant was written in response to RFA HL-01-010 entitled "Non-mouse models of diabetes complications in cardiovascular and microvascular diseases." The RFA stated: "The purpose of this solicitation is to support efforts to develop non-mouse animal models of diabetic complications. The animal models are expected to mimic vascular diseases in patients with type 1 or type 2 diabetes mellitus with an emphasis on, but not limited to, cardiovascular disorders of coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy, and congestive heart failure. Improved animal models of microvascular complications are also needed. The goal of this initiative is to obtain these non-mouse animal models, through the use of selective breeding, dietary manipulation, or molecular genetic approaches. Applicants to this initiative are also expected to characterize and validate the models for use in various aspects of basic, developmental, or translational research including testing prevention, early detection, therapeutic, or diagnostic imaging strategies. Applicants should also propose plans to make these models available to other research investigators for studies to advance our understanding of the etiology, pathobiology, clinical progression, management and prevention of diabetic vascular diseases."

In response to the RFA, the major purpose of our grant is to produce pigs that have an atherogenic risk factor profile resembling patients with insulin resistance (IR) and hyperlipidemia that will develop coronary, abdominal aortic, and carotid atherosclerosis. We proposed to utilize two strains of pigs available at the University of North Carolina. One strain has traits that predispose it to insulin resistance (IR) and the other has familial hypercholesterolemia (FH). Control strains with normal lipids (NL) and normal insulin sensitivity (IS) are also available. Our breeding program utilized animals from these groups to develop two new phenotypes: one with more severe IR and one with FH plus IR. As we originally estimated, at least 3 generations of animals have been produced and successfully phenotyped during the first 3 years of this project. All offspring that survived to puberty (~9 months old in pigs) underwent characterization of their degree of insulin resistance and abnormalities in lipid metabolism. The end result has been to produce pigs with one of the following four phenotypes: FH/IS, FH/IR, NL/IS, and NL/IR. The FH/IR pig resembles the human metabolic syndrome exhibiting IR in combination with both elevated triglycerides and LDL cholesterol, and depressed HDL cholesterol. Pigs with all phenotypes are being entered into our proposed studies to validate their usefulness to investigators who are attempting to identify genes that predispose to the development of insulin resistance and to determine the pathophysiological factors that link insulin resistance and atherosclerosis in coronary and carotid arteries and the abdominal aorta. Accordingly, our long-range goal is to exploit new insights into the mechanism(s) by which IR alters atherogenesis and thereby develop and test novel treatments for the growing epidemic of type II diabetes in children and adults and the associated cardiovascular disease.[2,3]

2. Summary of Accomplishments:

Aim I. **Develop two strains of pigs by selective breeding: Strain #1 will possess insulin resistance, strain #2 will be a cross between insulin resistant pigs and animals with familial hypercholesterolemia.**

Selective breeding has created IR pigs with support from this grant. IS and IR pigs were identified based on insulin (RIA, ICN) and glucose (2300 STAT PLUS, YSI, Yellow Springs, Ohio) levels measured after an overnight fast and 1 and 2 hours after a normal meal (35 kcal/kg/day via once daily feeding), and insulin to glucose ratios (Table 1). The mean fasting insulin levels in the 5 FH and 4 NL pigs classified IS were 11.1 and 7.9 μU/ml respectively, all of whom had mean one and two hour postprandial insulin levels less than 16 μU/ml. In contrast, the 6 FH and 6 NL pigs classified as IR had a mean fasting insulin of 24.8 and 22.2, respectively, and their one and two hour postprandial insulin levels ranged from 44 to 80 μU/ml. Importantly, when the

fasting insulin levels are compared, the difference is significant ($p < 0.05$), and when the postprandial insulin levels are compared, the difference is highly significant ($p<0.001$). IS pigs had a mean fasting glucose of 71 ± 4.4 mg/dl and two-hour postprandial of 110 ± 11 mg/dl. IR pigs had a mean fasting glucose of 76 ± 5.5 mg/dl and postprandial glucose of 125 ± 13 mg/dl ($p=0.06$). Most (>80%) of the pigs born to IS parents are also IS. The remaining offspring usually have an IR phenotype of intermediate severity. At present, we do not know the inheritance pattern of IR. Based on our experience to date, we estimate conservatively that approximately 1/4 of a given litter from IR parents will have the IR trait as adults. Our breeding strategy accounts for this level of IR phenotype expression. It is also worth noting that even among the 12 IR animals, there is heterogeneity and 3 of these animals have an even more severe phenotype with mean two-hour postprandial insulin levels greater than 100 μU/ml. Thus our selective breeding may further increase IR.

Of note, very recently some IR pigs have exhibited fasting hyperglycemia with blood sugars ranging from 115 to 165. If these potentially very important findings are confirmed, these pigs will be back crossed to parents and bred to sibs.

Summary of Aim 1 Accomplishments:

1. Our breeding program has shown that we can create pigs with progressive increases in IR phenotype by selective breeding in the NL and FH backgrounds.
2. Selective breeding can preserve the IS phenotype in both the NL and FH backgrounds.
3. Proven breeders for all 4 phenotypes have been identified.
4. Very recently, some IR pigs have exhibited fasting fasting hyperglycemia with blood sugars ranging from 115 to 165, suggesting that a diabetes mellitus phenotype is emerging in the IR background.

Table 1. Phenotype of Proven Breeder FH & NL & Ossabaw pigs with or without IR at UNC

Pig Phenotype	Gender + n	Cholesterol (mg/dl)	Serum Insulin level (μU/ml)		
			fasting	1hr	2hr
1. FH/IS	3M/2F	572.5 ± 99.7	11.1 ± 2.9	15.9 ± 2.4	13.0 ± 2.4
2. FH/IR	3M/2F	494 ± 78.5	24.8 ± 9.2	74.5 ± 30.4	52.3 ± 24.4
3. NL/IS	2M/2F	119.5 ± 28.8	7.9 ± 2.6	15.1 ± 2.2	14.5 ± 4.4
4. NL/IR	5M/2F	109.7 ± 25.8	22.2 ± 8.2	80 ± 68.4	44.8 ± 30.2
5. Ossabaw*	2M/3F	65.6 ± 4.9	14.2 ± 2.6	75.7 ± 46.2	32.6 ± 15.3

*The Ossabaw pigs exhibit an NL/IR phenotype but are 75 to 100 kg vs. 200+ kg.

Aim II. To characterize the time course of lesion development over one year and relate these changes to changes in insulin sensitivity and lipoproteins in 4 groups of animals.

The studies in this specific aim will be the most important in the grant because they will validate the usefulness of the FH/IR and NL/IR pigs and the feasibility of using these animal models to analyze the relationship between the presence of insulin resistance and the development of atherosclerosis. The primary objective is to determine if IR pigs have differences in the development of atherosclerotic lesions and changes in markers of atherosclerosis compared to IS animals. Although the number of animals is too few for formal statistical analyses at present, the preliminary results suggest that the IR trait exacerbates coronary artery and abdominal aortic atherogenesis in both the FH and NL backgrounds (Tables 2 and 3). In addition, complicated lesions with hemorrhage in coronary atherosclerotic plaques have been noted in an FH/IR pig (Fig 1).

With encouragement from the AMDCC Advisory Committee, an additional objective was added that utilized downsized pigs to produce smaller animals with insulin resistance and familial hypercholesterolemia. The added objective has been approached in two ways. First, 29 downsized FH pigs from the Rapacz colony in Wisconsin were screened and 5 were identified as having combined FH/IR. These five have been moved to Chapel Hill, completed quarantine, and bred to produce pigs for this grant. This downsized FH strain is in a “pot-bellied” background and weighs around 250 lbs as an adult. This size has proven to be too large for reliable intravascular ultrasound (IVUS) due to the limitations of the existing animal fluoroscopy at UNC. We are dealing with this

limitation in three ways. First, producing even smaller pigs; second, using transcutaneous ultrasound imaging of the femoral arteries, and third seeking to upgrade the animal fluoroscopy unit.

First, to produce an even smaller pig, we have recently acquired a new strain of Ossabaw pigs that weigh between 100 and 150 lbs as adults.[4] These pigs were not available when this grant was written due to their limited availability and herd health issues. This strain is descended from Spanish pigs either shipwrecked or purposefully left on Ossabaw Island, Georgia, in the 16th-17th century, where they have lived for the past 450 - 500 years in genetic isolation. Both hyperinsulinemia and increased percent body fat have been reported in this strain and we have confirmed that our Ossabaw pigs also exhibit IR (see Table 1. Item 5. Ossabaw).[5-7] We will breed these Ossabaw pigs with the downsized FH pigs with IR to produce a smaller pig with combined FH/IR that would be a more suitable size for investigations such as monitoring atherogenesis by IVUS.

Second, transcutaneous ultrasound has been utilized to monitor femoral atherogenesis *in vivo*. Femoral atherosclerosis has been shown to correlate with atherosclerosis in other vasculature in human diabetics (van der Meer IM et al., Risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree: the Rotterdam Study. Stroke. 2003;34:2374-2379). The pig lies in lateral recumbency and three ECG electrodes (pos., neg., ground) are attached. Using grayscale 2D echo (Fukuda Denshi, model UF750XT), a 9 Mhz linear array probe is positioned over each femoral artery sequentially and the scale marker is added to the screen. The artery is scanned in a transverse view starting at the level of the inguinal fold until it is no longer visible (~ 5 cm) noting any plaque or areas of thickening. In our experience, the image disappears as the vessel bifurcates into the distal muscle groups. In the longitudinal view, one-centimeter segments are scanned (2 to 3 seconds of data acquisition), taking care to define adventitia, media and intima clearly. The near and far wall may not be visible in the same projection. Care is taken to define all plaque areas on near and far walls and to address acoustical shadowing (Fig 2). Color Doppler is used to outline plaque if necessary. Arterial images containing ECG tracing are saved in jpeg and dicom formats. All measurements are made at the peak of the R wave on ECG. There are 4 potential variables that can be measured or derived by ultrasound: (1) intima-media thickness, (2) number of plaques, (3) plaque volume and (4) percent stenosis caused by the plaques. Intima-media thickness (mean maximal FIMT), is measured by two blinded observers using NIH Image with Image J and manual edge detection techniques.[8] The number of plaques is noted on the transverse scan. The estimated lesion volume is determined as follows. First, the lumen area is measured; second, the area within the internal elastic lamina, representing the combined (lesion + lumen) areas is measured; third, the lumen area is subtracted from the [lesion + lumen] area providing a measure of the lesion area (mm^2); fourth, the lesion area is multiplied by the length of the lesion to derive an estimate of individual plaque volumes; and finally, the lumen area is divided by the [lesion + lumen] area giving the percent stenosis. Summation of all plaque volumes over the length measured will be used as the estimate of total plaque volume in mm^3. Ultrasound measurements will be made every 2 months and compared for lesion progression. In addition, the 12-month ultrasound results for the four variables will be compared with the femoral atherosclerosis histomorphometry after euthanasia (see below). Euthanasia will occur immediately after the 12-month ultrasound.

Third, an upgraded animal fluoroscopy suite is available to be installed in the Division of Laboratory Animal Medicine at UNC pending identification of funds.

Summary of Aim 2. Accomplishments:

1. Atherosclerosis study has been initiated and preliminary results suggest that the IR trait exacerbates coronary artery and abdominal aortic atherogenesis in both the FH and NL backgrounds (Tables 2 and 3).

2. Echo measurements of femoral atherosclerosis progression appear feasible.
3. Bergman methodology established for testing S_i.
4. Atherogenic diet does not appear to alter IS in the NL pigs.
5. FH pigs appear to have stable IS.
6. Porcine NMR lipoprotein validation is in progress

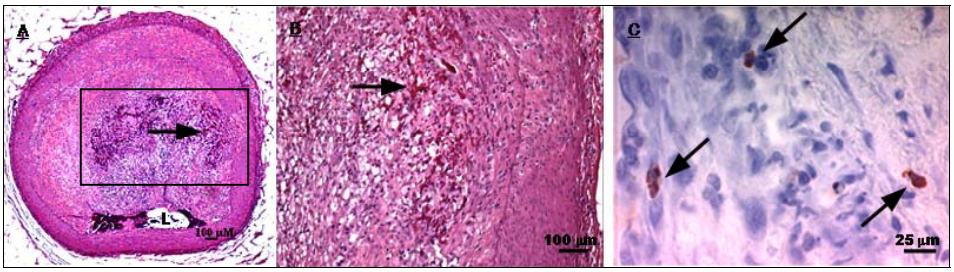

Fig. 1. Panel A. Hemorrhage into coronary atherosclerotic plaque of a 2-year FH/IR pig (total cholesterol 561 mg/dl; insulin: fasting, 4.6; 1 hr, 93.6; 2 hr, 27.1 µU/ml). Box indicates region of hemorrhage. Panel B shows higher magnification of region indicated by arrow from Panel A. (L = lumen.) Panel C. Macrophages in atherosclerotic plaque in FH pig coronary artery (monoclonal antibody MAC387, NeoMarkers, Fremont, CA).

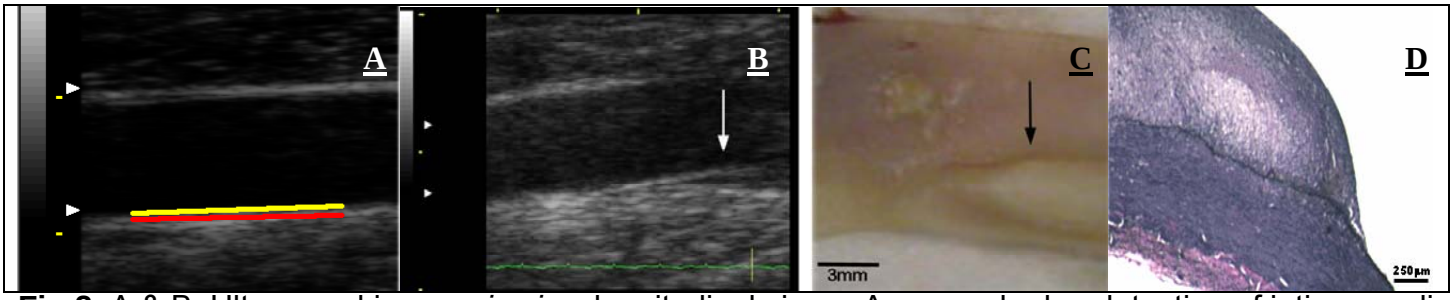

Fig 2. A & B. Ultrasound images *in vivo,* longitudinal views. A. manual edge detection of intima media thickness measurement of 0.05 cm^2 on far wall of FH/IS pig femoral artery (color lines bolded for emphasis). B. Atherosclerotic plaque from an FH/IS pig (total cholesterol 412 mg/dl; insulin: fasting, 11; 1 hr, 22; 2 hr, 13 µU/ml). Green line is ECG tracing allowing gating to R wave. C. En face image of opened artery from Panel B (arrow indicates raised plaque). D. Micrograph of plaque from section taken at arrow in panels B and C. (Verhoff van Gieson).

Table 2. AMDCC - Familial Hypercholesterolemia/Insulin Sensitive (FH/IS) vs. Familial Hypercholesterolemia/Insulin Resistant (FH/IR)
Nichols/Clemmons R01 HL 069364 Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs

Animal Model/Background strain	Swine (*Sus scrofa*)/mixed breed	
Phenotype	FH/IS vs FH/IR	
Protocol (age at start and sacrifice, diet, etc)	1.5-2 years age at start. Basic swine maintenance chow, 12 months with monthly sampling. Bergman Insulin Sensitivity testing at 4 pts	
Primary Screening	**FH/IS**	**FH/IR**
Insulin Resistance	no	yes
Type 2 Diabetes mellitus	no	no
Type 1 Diabetes mellitus	no	no
Fasting Glucose (mg/dl)	71.5 ± 3.8	80.2±12.5
Fasting Insulin (μU/ml)	11.0 ± 1.9	15.2±8.4
1 Hour Insulin (μU/ml)	19.0 ± 9.1	68.9±22.4
2 Hour Insulin (μU/ml)	12.1 ± 2.4	36.2±8.6
Abdominal Aortic atherosclerosis 1. % surface area with raised lesions 2. intimal area (mm^2) 3. intimal area as a % medial area	 34.9±12.8 n = 4 2.8±1.4 n = 4 9.1±4.6 n = 4	 81.6±13.8, n=4 14.5±3.9, n=2 73.4±16.8, n=2
Coronary artery atherosclerosis 1. intimal area (mm^2) 2. % stenosis 3. intimal area as a % medial area	 0.7±0.3 n = 4 17.6±5.8 n = 4 17.1±2.0 n = 4	 6.9±3.3, n=3 86.9±10.0, n=3 211.2±63.3, n=3
Secondary Screening	Monthly serum and plasma	
Total Serum Cholesterol (mg/dl)	379.2±69.4 n = 4	448.2±151.2, n=6
Serum Triglycerides (mg/dl)	42.8±19.1	56.3±24.1, n=6
Serum HDL-C (mg/dl)	Pending	Pending
Body Weight end of study (lb)	481±55	Pending
Back Fat (inches)	Pending	3 inches, n=1
Blood Pressure (mmHg)	Pending	Pending
Evidence of inflammation in the lesions 1. IL-1β IL-6 2. MCP-1, CRP, P-selectin, E-selectin	Pending	Pending
Serum markers of inflammation 1. Primary marker - CRP 2. Other potential markers - TNF-αIL-1β IL-6, MCP-1, P-selectin, E-selectin	Pending	Pending
Gene Expression (include specific genes of interest) **Growth regulatory proteins**: IGF-1, IGFBP-5, PDGF, IGF-1 receptor phosphorylated forms Insulin sensitivity and action: **IGFBP-1 and PEPCK (Hepatic mRNA), PI-3 kinase, phosphorylated form, Insulin receptor, phosphorylated form, Insulin receptor substrate-(PO4)** **Extracellular matrix and ECM related proteins**: Vitronectin, Thrombospondin, Osteopontin, Matrix metalloproteinase 2 and 9	Pending	pending
Other comments	Study in progress n=4 at present	Study in progress n=6 at present
Collaborators	Tissue distribution to AMDCC, see table.	

Table 3. AMDCC - Normal Lipid/Insulin Sensitive (NL/IS) Pigs vs. Normal Lipid/Insulin Resistant (NL/IR)

Nichols/Clemmons R01 HL 069364 Atherosclerosis in Insulin Resistant Hyperlipidemic Pigs

Animal Model/Background Strain	Swine (*Sus scrofa*)/Poland China mixed	
Phenotype	NL/IS vs NL/IR	
Protocol (age at start and sacrifice, diet, etc)	2-2.5 yrs at start of 12 month study. High fat, high cholesterol (1%) diet, 12 months with monthly sampling. Bergman Insulin Sensitivity testing at 4 pts	
Primary Screening	**NL/IS**	**NL/IR**
Insulin Resistance	no	yes
Type 2 Diabetes mellitus	no	no
Type 1 Diabetes mellitus	no	no
Fasting Glucose (mg/dl)	75	72
Fasting Insulin (μU/ml)	12.1	12.4
1 Hour Insulin (μU/ml)	15.9	106.2
2 Hour Insulin (μU/ml)	25.3	41.9
Abdominal Aortic atherosclerosis 1. % surface area with raised lesions 2. intimal area (mm^2) 3. intimal area as a % medial area	 32.2 0.8±1.0 2.0±2.6	 89.9 17.5±7.3 28.4±13.1
Coronary artery atherosclerosis 1. intimal area (mm^2) 2. % stenosis 3. intimal area as a % medial area	 0.6±0.8 19.0±21.5 14.7±19.4	 4.7±2.3 72.3±23.3 93.2±61.4
Secondary Screening	**Monthly serum and plasma**	
Total Serum Cholesterol (mg/dl)	319.1±101.7	531.9±244.8
Serum Triglycerides (mg/dl)	28.6±13.4	58.3±71.6
Serum HDL-C (mg/dl)	Pending	Pending
Body Weight end of study (lb)	540	580
Back Fat end of study (inches)	15/16 (0.94)	12/16 (0.75)
Blood Pressure (mmHg)	Pending	Pending
Evidence of inflammation in the lesions 1. IL-1β IL-6 2. MCP-1, CRP, P-selectin, E-selectin	Pending	Pending
erum markers of inflammation 1. Primary marker - CRP 2. Other markers - TNF-αIL-1β IL-6, MCP-1, P-selectin, E-selectin	Pending	Pending
Gene Expression (include specific genes of interest) **Growth regulatory proteins**: IGF-1, IGFBP-5, PDGF, IGF-1 receptor phosphorylated forms Insulin sensitivity and action**: IGFBP-1 and PEPCK (Hepatic mRNA), PI-3 kinase, phosphorylated form, Insulin receptor, phosphorylated form, Insulin receptor substrate-(PO4)** **Extracellular matrix and ECM related proteins**: Vitronectin, Thrombospondin, Osteopontin, Matrix metalloproteinase 2 and 9	Pending	pending
Other comments	Study in progress n=1 at present	Study in progress n=1 at present
Collaborators	Tissue distribution to AMDCC, see table.	

Completion of new pig housing unit
An additional accomplishment that cannot be over emphasized is the January 2005 completion of a new pig housing unit. In the original grant application, we requested support for this facility to maintain the number of pigs required for this study. The grant and UNC have generously provided the support that made this facility feasible.

3. Plans for the coming year:

During the remainder of Year 4 and beginning of Year 5, our emphasis will be on the following 4 items:

1. Continued evaluation of pigs entered into Exp 1 and 2 of Aim II and breeding additional pigs for these studies. In addition to the previously described studies, we are now using ultrasound to monitor femoral artery intima media thickness and to detect femoral lesions. This approach should allow us to achieve our goal of documenting the extent and rate of development of atherosclerosis in both strains of IR pigs and comparing to IS (Insulin Sensitive) controls.
2. Characterizion of biochemical changes that occur with disease markers in serum (e.g. CRP), plasma, or lesions. The Bergman frequently sampled insulin glucose tolerance test will be used to measure insulin sensitivity in experimental pigs. In addition, we are working with LipoScience, Inc, Raleigh NC to validate NMR lipoprotein analyses in all pig phenotypes.
3. Selective breeding to obtain increased insulin resistance will continue. The breeders listed on Table 1 have been and will be used to produce the remaining pigs needed to complete Exp 1 and 2. We will continue to re-evaluate the breeding strategies with each data set to maintain the goal of producing pigs with an increased severity of IR as proposed (> 30 to 50 □U/ml fasting insulin or elevated mean fasting insulin concentrations ≥ 2.1 times greater than age and weight matched control animals and 1 and/or 2 hour postprandial insulin levels that are elevated ≥ 4-fold times that of controls).
4. Production of smaller pigs with FH and IR. Both the down-sized FH pigs from the Rapacz colony and the recently acquired Ossabaw strains that exhibit insulin resistance will be used to produce smaller pigs for current and future studies.
5. Begin validation of pig gene microarray analyses for monitoring originally proposed genes and potential identification of new genes of interest.

4. Most significant achievement:

Our most significant achievement is the significant amount of progress made towards achieving our 4 program goals.

Goal 1. To Create NL (i.e. Chapel Hill pigs with diet-inducible atherosclerosis) and FH pigs with and without IR. Selective breeding strategies implemented in Years 1 to 3 were designed to increase the severity of IR in the NL and FH pigs. Pigs have a 4-month gestation and achieve puberty at 9 months of age. Following puberty, the IR trait should be fully expressed and the FH trait is stable. We had projected producing and phenotyping three generations during the first three years of the grant and we have done that. Our results suggest that breeding strategies consisting of two IR parents, sib crosses, and backcrosses selecting for IR have produced breeder pigs with an increased IR severity while preserving the FH and NL phenotypes. Equally important, the IS trait in the FH and NL backgrounds has been preserved for producing essential control pigs. Breeding strategies remain in place to produce pigs with a more severe insulin resistance phenotype in sufficient numbers for the experiments in Aim II (see Table 1) and to develop further the recent appearance of hyperglycemia in the IR background.

Goal 2. Document the extent and rate of development of atherosclerosis in both strains of pigs and compare to IS controls. Both of the proposed atherosclerosis studies have been initiated. Although the number of animals is too few for formal statistical analyses, the preliminary results suggest that the IR trait exacerbates coronary artery and abdominal aortic atherogenesis in both the FH and NL backgrounds (Tables 2 and 3). Additional pigs are being entered into the study as they are documented to have the desired phenotype.

Goal 3. Characterize biochemical changes that occur with disease markers in serum, plasma, or lesions. We have established Bergman methodology for testing insulin sensitivity, $S_{i,}$ in our pigs. To date, the S_i appears to be stable in the experimental pigs. Lipoprotein analyses, serum and plasma markers and tissue markers will be analyzed as originally described with the addition of NMR analyses by LipoScience, Inc., Raleigh NC.

Goal 4. Establish a colony of well-characterized animals for dissemination to the research community. This long-range goal appears to be within reach by the end of the granting period. The down-sized FH and even smaller Ossabaw pigs have been recently transferred to UNC and offer the possibility of reducing expenses and handling difficulties without loss of the inherited human-type FH phenotype. This background has the greatest potential for providing a scientifically useful animal model of the human metabolic syndrome.

Publications: none.