ANIMAL MODELS OF DIABETIC COMPLICATIONS CONSORTIUM (U01 HL70525)

UPDATE REPORT (January 2005 –February 2006)

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PART A:

PRINCIPAL INVESTIGATOR'S SUMMARY

Program Accomplishments:

The University of Utah site of the Animal Models of Diabetic Complications Consortium has the aim of developing models for diabetic cardiomyopathy and other forms of diabetic cardiovascular disease. Within this theme we have focused on refining and phenotyping various mouse models of diabetic cardiovascular complications and defining the standards for diabetic cardiomyopathy using several different animal models of diabetes:

- Models of type 2 diabetes. We have extensively characterized the cardiac phenotypes of mouse models of severe insulin resistance, obesity and type 2 diabetes, namely the ob/ob and the db/db mouse at multiple levels including *in vivo* cardiac function, isolated perfused heart studies and characterization of mitochondrial function. Much of this work was published within the last year and will included in the bibliography that accompanies this report. We have now proceeded to model the cardiovascular consequences of a more physiologically relevant model of type 2 diabetes that is induced by high-fat feeding in a susceptible background strain, the C57BL6/J mouse.
- Models of type 1 diabetes. The Akita mouse has emerged as an important platform upon which many of the other complications of diabetes have been modeled. To this end we have embarked upon extensive phenotyping of the myocardial phenotype of Akita mice and our progress in this area will be summarized.
- Models with impaired insulin signaling specifically targeted to the heart. This approach is based on the well accepted idea that insulin resistance is central to the development of type 2 diabetes, and that insulin resistance, even before the onset of overt diabetes, is a significant risk factor for cardiovascular disease. These specific models include: the CIRKO mouse with targeted deletion of the insulin receptor in the heart; and a mouse with a dominant negative PI3-kinase targeted to the heart, PI3-kinase being a central mediator of many aspects of downstream insulin signaling. We have extensively characterized the CIRKO and PI3-Kinase mutant animals, and have presented these data in prior reports. Moreover, we have published two manuscripts that describe the mechanisms by which impaired myocardial insulin signaling may contribute to cardiac maladaptation in the face of ischemia and pressure overload hypertrophy. The CIRKO mouse exhibited mitochondrial dysfunction (similar to observations noted in ob/ob and db/db mouse hearts), but did not have a sustained increase in myocardial FA utilization, despite an initial increase. Given the important role of lipotoxicity in pathogenesis of diabetic cardiomyopathy we have chosen to model the combined effect of myocardial insulin resistance and increased myocardial lipid delivery by crossing CIRKO mice with a transgenic mouse with low-level overexpression of acyl-CoA synthetase (MHC-ACS). Phenotyping of CIRKO-ACS mice will be summarized in this report.

Our approach has been to perform detailed phenotypic analysis of these models at the level of cardiac function in the intact animal, in the isolated heart, and in isolated cells and tissues to examine cell signaling, mitochondrial function, and gene expression

profiling. These studies have determined that like the human disorder, the cardiac phenotypes of the diabetic heart may be subtle. However, the response of the heart to stress is usually impaired. Our observations to date support the paradigm that in the hearts of animals with insulin resistance, obesity and type 2 diabetes there is downregulation of insulin signaling and progressive mitochondrial dysfunction that develops in the heart. Increased myocardial FA utilization is associated with mitochondrial uncoupling, increased myocardial oxygen consumption and reduced myocardial energy reserves. Moreover, there is evidence of oxidative stress that likely occurs on the basis of increased mitochondrial superoxide generation.

SUMMARY OF ACHIEVEMENTS IN THE PAST 12 MONTHS

- Completion of the phenotyping of ob/ob and db/db mice and publication of three peer-reviewed reports.
- Completion and publication of a study that demonstrates mechanisms by which impaired myocardial insulin signaling impairs the vascular adaptation to myocardial ischemia and hypertrophy.
- Development of a model of type 2 diabetes induced by high-fat feeding of C57BL6J mice which also leads to obesity and insulin resistance, and characterization of these animals in terms of glucose homeostasis, cardiac and vascular function.
- Expansion of our colony of Akita mice, which is a common type-1 diabetes platform that has been used throughout the consortium, and characterization of this model in terms of cardiac function *in vivo*.
- Generation and characterization of CIRKO-ACS mice to determine if a combination of impaired insulin signaling and increased myocardial FA delivery will accelerate myocardial dysfunction and mitochondrial dysfunction.

Interrelationships of projects:

All of the projects that are described in this report were conducted at the University of Utah in the laboratories of the PI, Dr. McClain and the co-investigators, Drs Abel and Litwin. Thus although the projects are described as separate projects in reality they represent a truly collaborative effort of all of the investigators involved.

Collaborations with other Groups (Including Core Facilities):

CURRENT: (1) Phenotyping mice with altered expression (over-expression or KO) of lipoprotein lipase in the heart. We specifically determined myocardial substrate utilization and oxygen consumption in these hearts. These animals were generated in the laboratory of Dr. Ira Goldberg (Columbia University), who is a part of the Rockefeller/NYU/Columbia group. A manuscript summarizing some aspects of this work was recently published in the Journal of Biological Chemistry.

Pertinent non-AMDCC Collaborations:

(1) We have collaborated with Daniel Kelly (Washington University in St. Louis) to characterize the mitochondrial phenotype of PGC-1 alpha KO mice. PGC-1 may play a role in the maturation/maintenance of cardiac mitochondria. These studies were published last year in the Journal PLoS Biology. (2) We have collaborated with Robert

Lane MD in the Department of Pediatrics (Neonatology) at the University of Utah to characterize the mitochondrial and myocardial phenotypes of rats with intrauterine growth retardation (IUGR). IUGR might be an important predisposing risk factor for the development of type-2 diabetes and the metabolic syndrome later in life. (3) We have collaborated with the laboratory of Antonio Vidal-Puig (Cambridge University) in characterizing the myocardial phenotype of mice with germ-line deletion of the PGC-1 beta.

Response to EAC Comments:

- The main concerns of the EAC that are directly relevant to our group is the need to phenotype many models across all complications. Particularly the sharing of mice across the consortium, and the shipment of mice to the Utah center for cardiovascular phenotyping.

Response: We are poised and prepared to do this, and will accept animals from other members of the consortium once sufficient numbers of animals have been generated to be sent for further phenotyping.

- Publication of a position paper on cardiovascular complications of diabetes.

Response: A manuscript is in the process of being prepared.

Relevant Publications Since the Last Report

1. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, **Abel ED**, Semenkovich CF, Kelly DP. PGC-1α deficient mice exhibit multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. 2005. *PLOS Biology 3(4)e101.*
2. Buchanan J, Mazumder PK, Hu P, Chakrabarti G, Roberts MW, Yun UJ, Cooksey RC, **Litwin SE**, **Abel ED.** Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity.2005. *Endocrinology146:5341-5349.*
3. McQueen AP, Zhang D, Hu P, Swenson L, Yang Y, Zaha VG, Hoffman JL, Yun UJ, Chakrabarti G, Wang Z, Albertine KH, **Abel ED*, Litwin SE***. Contractile dysfunction in hypertrophied hearts with deficient insulin receptor signaling: possible role of reduced capillary density. 2005. *J. Molecular and Cellular Cardiology.39:882-892* (* Corresponding authors)
4. Boudina S, Sena S, O'Neill BT, Tathireddy P, Young ME, **Abel ED.** Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impairs myocardial energetics in obesity. 2005. *Circulation*.112*:2686-2695.*
5. Augustus AS, Buchanan J, Park TS, Hirata K, Noh HL, Sun J, Homma S, D'armiento J, **Abel ED**, Goldberg IJ. Loss of lipoprotein lipase-derived fatty acids leads to increased cardiac glucose metabolism and heart dysfunction. 2006. *J Biol Chem. Epub in press.*
6. Durgan DJ, Smith JK, Hotze MA, Egbejimi O, Cuthbert KD, Zaha VG, Dyck JR, **Abel ED**, Young ME. Distinct Transcriptional Regulation of Long-Chain Acyl-CoA Synthetase Isoforms and Cytosolic Thioesterase 1 in the Rodent Heart by Fatty Acids and Insulin. 2006. *Am J Physiol Heart Circ Physiol. Epub in press.*
7. **Abel ED**. Myocardial insulin resistance and cardiac complications of diabetes*. Curr. Drug Targets –Immune, Endocrine and Metabolic Disorders.* 2005: 5; 219-226
8. **Abel ED**. Metabolic perturbations in the diabetic heart: Mechanisms and molecular targets. *Drug Discovery Today: Disease Mechanisms*. 2005: 2; 115-122.

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PART B:

UPDATE BY PROJECT LEADERS

Responsible Investigators: **Donald A. McClain, M.D., Ph.D.**

Project 1: Myocardial Adaptation to Diet-Induced Obesity

A. Rationale and Relevance:

Type-2 diabetes represents the major clinical burden in terms of cardiovascular complications of diabetes. Therefore relevant animal models need to be developed and or characterized that address the pathogenesis of heart disease in type 2 diabetes and insulin resistant states. Therefore, establishing standards for determining the presence of insulin resistance, diabetes and glucose intolerance are important pre-requisites that must precede the detailed analysis of relevant mouse models. We have used this approach to characterize ob/ob and db/db mice. In order to model diabetes and cardiovascular disease pathogenesis in a clinically relevant model system we began a study in which we subjected C57BL6J mice to high-fat feeding. We have now characterized these animals in terms of whole body glucose homeostasis as well as cardiac and vascular function.

B. Summary of Accomplishments

The studies on ob/ob and db/db mice have been completed and manuscripts published (see bibliography). The high-fat feeding study is well advanced and results are summarized below. In order to identify the most appropriate model of diet-induced diabetes we compared C57BL6 and FVB/N mice. Thus some of the data depicted below will show comparisons of these two strains, and reveal that the C57BL6J are susceptible to diet-induced insulin resistance and diabetes, whereas the FVN/N mice are resistant to diet-induced diabetes.

- C57BL6J mice develop glucose intolerance following high-fat feeding (Figs 1-3). Changes in glucose homeostasis are evident as early as 2 –weeks (Fig. 1), and are well established by 5-weeks (Fig 2). By 10-weeks these animals are frankly diabetic (Fig 3). Insulin resistance as evidenced by glucose clamp studies are evident by 10-weeks (Fig 4).
- Changes in myocardial substrate utilization (decreased myocardial glucose utilization, increased myocardial FA utilization, increased myocardial oxygen consumption, and impaired insulin responsiveness) were evident from as early as two-weeks on the high-fat diet (table 1).
- Mitochondrial dysfunction develops in the skeletal muscle of these animals. we are in the process of determining if similar changes also develop in the heart. (Fig. 5)
- High-fat fed C57BL6J mice develop significant hypertension (Fig. 6).

C. Plans for the coming year

We will complete the myocardial mitochondrial phenotyping in the high-fat fed animals.

D. Most significant achievement.

These studies have indicated that alterations in myocardial substrate utilization are a consistent feature in the hearts of mice across many models of obesity and insulin resistance. Moreover, these changes occur very early in the course of diabetes and insulin resistance and therefore represents a critical step in the pathogenesis of cardiac dysfunction that ultimately ensues.

Publications

1. Mazumder PK, O'Neill BT, Roberts MW, Buchanan J, Yun UJ, Cooksey RC, Boudina S, **Abel ED.** Impaired Cardiac Efficiency and Increased Fatty Acid Oxidation in Insulin Resistant *ob/ob* mouse hearts. 2004: *Diabetes*. 53: 2366-2374.
2. Buchanan J, Mazumder PK, Hu P, Chakrabarti G, Roberts MW, Yun UJ, Cooksey RC, **Litwin SE, Abel ED.** Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity.2005. *Endocrinology146:5341-5349.*
3. Boudina S, Sena S, O'Neill BT, Tathireddy P, Young ME, **Abel ED.** Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impairs myocardial energetics in obesity. 2005. *Circulation*.112*:2686-2695.*
4. Augustus AS, Buchanan J, Park TS, Hirata K, Noh HL, Sun J, Homma S, D'armiento J, **Abel ED**, Goldberg IJ. Loss of lipoprotein lipase-derived fatty acids leads to increased cardiac glucose metabolism and heart dysfunction. 2006. *J Biol Chem. Epub in press.*

Aspects of this work have been presented at national meetings.

1. Boudina S, O'Neill BT, **Abel ED**. Mitochondrial uncoupling and decreased oxidative capacity contributes to cardiac dysfunction in db/db mice. Diabetes 2004; 53, Suppl. 1 A335
(Presented at the 64th Scientific sessions of the American Diabetes Association 2004)

2. Boudina S**,** Cooksey RC, **McClain DA**, **Abel ED**. Mitochondrial Dysfunction Precedes Generalized Insulin resistance in Response to High-Fat Feeding.
(Presented at the IXth International Symposium on Insulin Receptors and Insulin Action, Nice, France 2004)

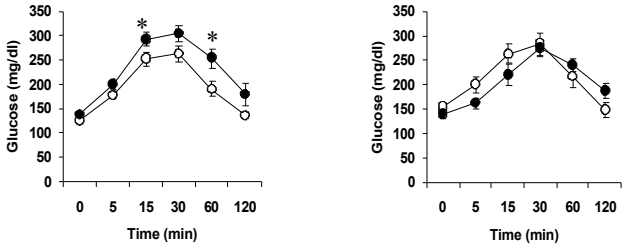
3. Boudina S, Cooksey RC, Jones D, **McClain DA**, **Abel ED**. Insulin resistance, mitochondrial dysfunction and energy expenditure following high fat feeding. Diabetes 2005; 54, Suppl. 1 A381
(Presented at the 65th Annual Meeting and Scientific Session of the American Diabetes Association, San Diego, CA, 2005)

Tables and Figures for Project 1.

Figure 1.

Glucose Tolerance Tests after 2 Weeks of High Fat Feeding

C57BL/6J background **FVB/NJ background**

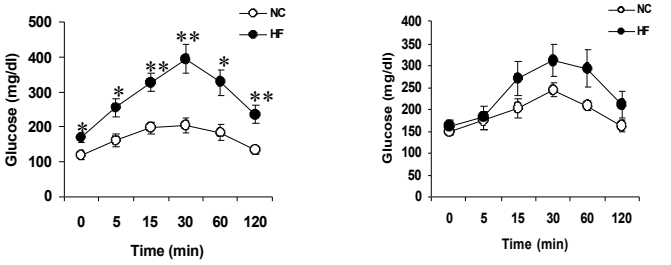


*P<0.05 vs. NC of the same strain. NC (open circle); HF (filled circle).

Figure 2.

Glucose Tolerance Tests after 5 Weeks of High Fat Feeding

C57BL/6J background **FVB/NJ background**



*P<0.05 vs. NC of the same strain.

Figure 3 (data from C57BL6)

Glucose Tolerance Test after 10-weeks of High Fat Feeding

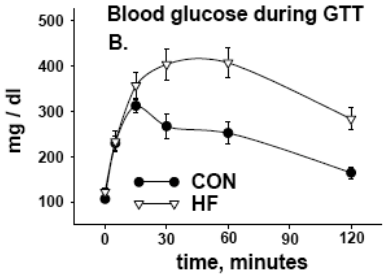
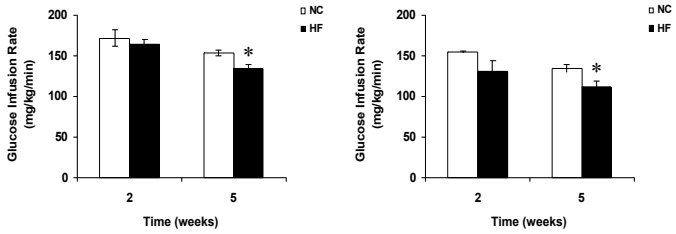


Figure 4.

Insulin Sensitivity: Glucose Infusion Rates

C57BL/6J background **FVB/NJ background**

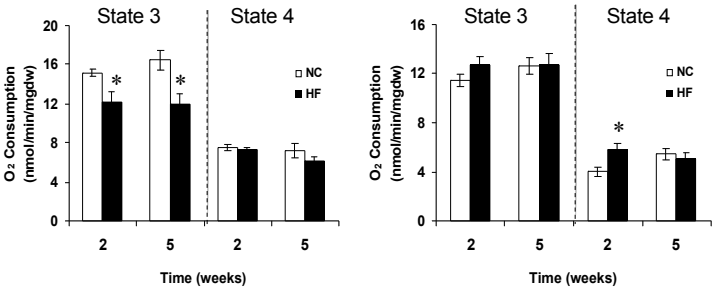


*P<0.05 vs. NC of the same strain. NC (White bars); HF (black bars).

Figure 5.

Changes in Mitochondrial Oxidative Capacity In Skeletal Muscle of Mice Subjected to High Fat Diet

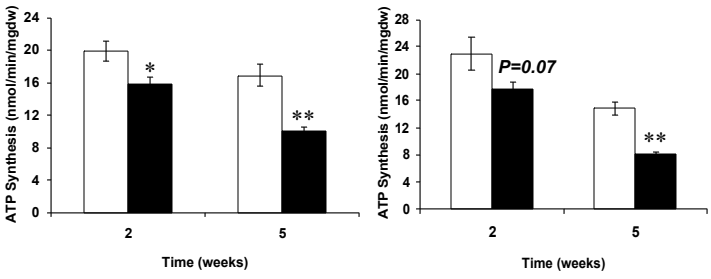
C57BL/6J background **FVB/NJ background**



*P<0.05 vs. NC of the same strain.

Changes in Mitochondrial ATP Synthesis Rates In Skeletal Muscle of Mice Subjected to High Fat Diet

C57BL/6J background **FVB/NJ background**



*P<0.05; **P<0.005 vs. NC of the same strain.

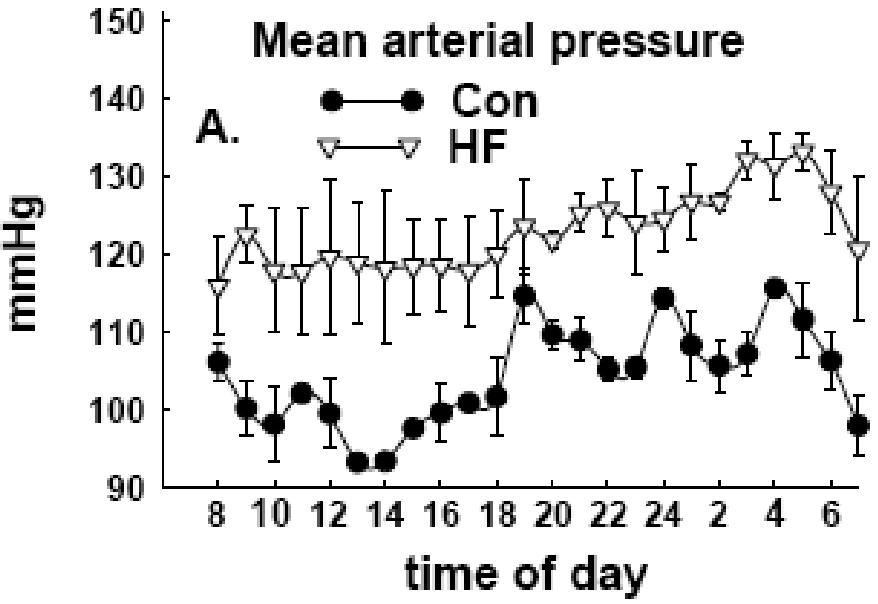
Table 1: Cardiac Substrate Metabolism after 5-weeks of High Fat Feeding in C57BL6 Mice

Substrate Metabolism After 5 Weeks on HFD

	High Fat Diet		Normal Chow	
	0 nM Insulin	1 nM Insulin	0 nM Insulin	1 nM Insulin
Palmitate Oxidation (nmol/min/g DHW)	619.2 ± 33.9 ‡	526.4 ± 20.0 **	416.5 ± 25.1	312.3 ± 8.3 **
Glucose Oxidation (nmol/min/g DHW)	329.5 ± 30.9 ‡	416.9 ± 31.3 *	488.9 ± 16.3	644.2 ± 24.7 **
Glycolysis (nmol/min/g DHW)	2993.2 ± 209.1 ‡	3022.6 ± 259.1	5120.8 ± 178.5	6098.5 ± 194.2 †
MVO2 (nmol/min/g DHW)	193.9 ± 6.1 ‡	187.2 ± 9.4	138.5 ± 8.7	127.0 ± 3.9

* p=0.07 compared to 0 nM insulin.
** p<0.01 compared to 0 nM insulin.
† p<0.05 compared to 0 nM insulin.
‡ p<0.01 compared to Normal Chow with 0 nM insulin.

Figure 6. Hypertension develops in C57BL6 mice after 10-weeks of High fat Feeding. Data were collected using continuous in vivo telemetry.



Responsible Investigators: **Sheldon E. Litwin MD**

Project 2: Characterization of the in vivo cardiac function in Akita mice under basal conditions and in response to hemodynamic stressors.

A. Rationale and relevance

We have previously shown that impaired myocardial insulin signaling (CIRKO mice) impairs that adaptation of the heart to hemodynamic stressors such as hypertrophy and ischemia. We therefore wanted to test the hypothesis that similar mechanisms could be at play in a model of insulin deficiency namely the Akita mouse. These studies are also of particular relevance because the Akita model has emerged as an important platform in which many of the other organ-specific complications within the consortium are being modeled.

B. Summary of Accomplishments

We measured in vivo cardiac function in Akita mice using echocardiography at baseline and following infusion for with isoproterenol via osmotic minipumps, for up to 12 days.

<u>Findings</u>:

The results of these experiments are summarized in **Tables 2-5.**

Table 2: Echocardiographic Analysis after 5-days of Isoproterenol

Genotype	**WILDTYPE**		**AKITA**	
Treatment	Saline	Isoproterenol	Saline	Isoproterenol
LVDd	0.38±0.01	0.31±0.02*	0.37±0.01	0.33±0.01
LVDs	0.26±0.01	0.19±0.02*	0.26±0.01	0.21±0.01
IVSd	0.09±0.00	0.13±0.01*	0.10±0.01	0.11±0.01
LVPWd	0.08±0.00	0.12±0.02	0.09±0.01	0.11±0.01
%FS	31±2	39±4	29±2	36±5
LVEF	0.67±0.03	0.81±0.06	0.67±0.02	0.72±0.07
Mass	0.12±0.01	0.16±0.02	0.12±0.02	0.13±0.02
LVOTVi	3.45±0.16	3.76±0.26	2.83±0.23	2.14±0.52
HR	378±17	599±35*	360±16	509±27*
Calc. CO	10.3±0.9	17.7±1.5*†	7.9±0.3	8.7±2.4

Data are means ±SE. SE are rounded to 2 decimal places thus a SE of <0.005 is entered as 0.00. * $p<0.05$ versus ISO of the same genotype, † $p<0.05$ versus ISO Akita. Number of animals =3. LVDd-LV diastolic diameter (cm), LVDs-LV systolic diameter (cm), IVSd-Interventricular septum thickness in diastole (cm), LVPWd-LV posterior wall thickness in diastole (cm), %FS-percent fractional shortening, LVEF-LV ejection fraction, Mass-LV mass (g), LVOTVi –LV stroke volume index (cm), HR- Heart Rate, Calc. CO – Cardiac Output (ml/min).

Table 3: Echocardiographic Analysis after 11-days of Isoproterenol

Genotype	WILDTYPE		AKITA	
Treatment	Saline	Isoproterenol	Saline	Isoproterenol
LVDd	0.39±0.01	0.32±0.01*	0.37±0.01	0.32±0.02
LVDs	0.27±0.01‡	0.23±0.00*	0.24±0.01	0.22±0.02
IVSd	0.09±0.00	0.14±0.01	0.10±0.01	0.10±0.01
LVPWd	0.08±0.00	0.12±0.02*	0.09±0.01	0.11±0.01
%FS	29±1‡	28±2	34±1	32±3
LVEF	0.70±0.05	0.63±0.03	0.68±0.02	0.68±0.04
Mass	0.12±0.01	0.19±0.01*‡	0.11±0.01	0.13±0.02
LVOTVi	3.71±0.30	3.30±0.7*	2.65±0.25	3.40±0.50*
HR	437±12	668±23*	381±17	617±25*
Calc. CO	12.7±1.1‡	17.4±4.1	7.9±0.5	16.4±2.4

Data are means ±SE. SE are rounded to 2 decimal places thus a SE of <0.005 is entered as 0.00. * p<0.05 versus ISO of the same genotype, ‡ p<0.05 versus Saline Akita. Number of animals =3. LVDd-LV diastolic diameter (cm), LVDs-LV systolic diameter (cm), IVSd-Interventricular septum thickness in diastole (cm), LVPWd-LV posterior wall thickness in diastole (cm), %FS-percent fractional shortening, LVEF-LV ejection fraction, Mass-LV mass (g), LVOTVi –LV stroke volume index (cm), HR- Heart Rate, Calc. CO – Cardiac Output (ml/min).

Table 4: Echocardiographic Analysis after 12-days of Isoproterenol

Genotype	WILDTYPE		AKITA	
Treatment	Saline	Isoproterenol	Saline	Isoproterenol
LVDd	0.39±0.01	0.32±0.03	0.37±0.01	0.36±0.02
LVDs	0.27±0.01‡	0.20±0.03	0.24±0.01	0.26±0.03
IVSd	0.09±0.00	0.13±0.01*	0.09±0.01	0.10±0.01
LVPWd	0.07±0.00	0.12±0.00*	0.09±0.01	0.10±0.01
%FS	29±1‡	40±4	34±1	26±9
LVEF	0.70±0.05	0.77±0.05	0.68±0.02	0.56±0.13
Mass	0.12±0.01	0.17±0.02*‡	0.11±0.01	0.14±0.01
LVOTVi	3.71±0.30‡	3.04±0.02	2.65±0.25	3.28±0.34
HR	437±12	625±33*†	381±17	450±23
Calc. CO	12.7±1.1‡	15.0±1.0	7.9±0.5	11.6±1.4

Data are means ±SE. SE are rounded to 2 decimal places thus a SE of <0.005 is entered as 0.00. * p<0.05 versus ISO of the same genotype, † p<0.05 versus ISO Akita, ‡ p<0.05 versus Saline Akita. Number of animals =3. LVDd-LV diastolic diameter (cm), LVDs-LV systolic diameter (cm), IVSd-Interventricular septum thickness in diastole (cm), LVPWd-LV posterior wall thickness in diastole (cm), %FS-percent fractional shortening, LVEF-LV ejection fraction, Mass-LV mass (g), LVOTVi –LV stroke volume index (cm), HR- Heart Rate, Calc. CO – Cardiac Output (ml/min).

Table 5: Heart Weights and Glucose after 12-days of Isoproterenol

Genotype	WILDTYPE		AKITA	
Treatment	Saline	Isoproterenol	Saline	Isoproterenol
Body Weight (g)	25±1	27±1	23±1	24±0.40
Heart Weight (g)	0.12±0.01	0.16±0.01*	0.12±0.00	0.15±0.01*
HW:BW	4.6±0.05	6.2±0.2*	5.1±0.3	6.1±0.3*
Glucose (mg/dl)	258±19	253±6	600±0†	566±16†

Data are means ±SE, * $p<0.05$ versus saline, † $p<0.05$ versus wildtype with similar treatment.

The key observations from the echocardiographic studies are: (1) Under basal conditions Akita mice have reduced cardiac output that can be accounted in large part by a reduction in ejection fraction. We cannot conclude if this reflects intrinsic myocardial dysfunction, but given the relatively preserved ejection fraction, the greater likelihood is that these changes reflect plasma volume contraction in the hyperglycemic Akita mice. (2) The hypertrophic response of Akita hearts to Isoproterenol were somewhat attenuated and delayed.

Overall the cardiac phenotype in the Akita mouse is subtle and there are important differences with the phenotypes of mouse models that are characterized by insulin resistance and obesity.

C. Plans for the coming year

We plan to extend this study in a number of ways in the coming year. (1) We will be evaluating cardiac function in this model as a function of age. The studies reported above were performed in animals that were 12-weeks of age. It is possible that more significant cardiac dysfunction might be evident as animals age. We will also extend these studies by performing invasive LV hemodynamics as the possibility exists that changes in cardiac function might be present in these animals, but might be subtle and only discernable by using more sensitive phenotyping methods such as LV catheterization. (2) We will subject Akita mice to a more chronic hemodynamic stress, namely LV hypertrophy following transverse aortic banding. (3) We will analyze cardiac function and metabolism in isolated perfused hearts and examine mitochondrial function and bioenergetics.

D. Significant Achievement

These studies will represent the first detailed *in vivo* hemodynamic analysis of cardiac function in Akita mice and will provide important information regarding the evolution of myocardial dysfunction in this model of type 1 diabetes.

Publications:

Two manuscripts have been published by our group that describe the mechanisms responsible for impaired myocardial adaptations to stress in hearts with impaired insulin signaling (CIRKO).

1. Hu P, Zhang D, Swenson L, Chakrabarti G, **Abel ED**, **Litwin SE.** Minimally invasive aortic banding in mice: Effects of altered cardiomyocyte insulin signaling during pressure-overload . *American Journal of Physiology*.2003; 285(3): H1261-1269**.**
2. McQueen AP, Zhang D, Hu P, Swenson L, Yang Y, Zaha VG, Hoffman JL, Yun UJ, Chakrabarti G, Wang Z, Albertine KH, **Abel ED*, Litwin SE***. Contractile dysfunction in hypertrophied hearts with deficient insulin receptor signaling: possible role of reduced capillary density. 2005. *J. Molecular and Cellular Cardiology.39:882-892* (* Corresponding authors)

Responsible Investigators: **E. Dale Abel MD Ph.D.**

Project 3: Characterization of cardiac function, myocardial substrate metabolism and mitochondrial function in a mouse model of myocardial insulin resistance and lipotoxicity – CIRKO-ACS mice.

A. Rationale and Relevance

We have extensively characterized the impact of myocardial insulin resistance on cardiac function (*in vivo*), cardiac function in isolated hearts, substrate metabolism, mitochondrial function, and the response to hemodynamic stressors such as ischemia and hypertrophy, using mice with cardiomyocyte-restricted deletion of insulin receptors (CIRKO) as our model platform. Our studies revealed that in the absence of insulin signaling there is an initial increase in myocardial FA utilization that is associated with increased superoxide generation and mitochondrial oxidative stress. Over time there is progressive mitochondrial dysfunction, which is manifested by a global reduction in mitochondrial oxidative capacity for all substrates including FA and glucose. In one sense, this model diverges from other models of obesity and diabetes that we have examined in that elevated levels of FA utilization do not persist. We believe that this is due in part to fact that in the CIRKO mouse there is no increase in FA delivery to the myocardium. Thus to model the additional consequence of increasing myocardial FA delivery in the insulin resistant mouse heart, we have crossed the CIRKO mouse with a transgenic mouse with relatively low-level overexpression of acyl-CoA synthetase (MHC-ACS), which promotes increased myocardial lipid delivery that is insufficient on its own to lead to cardiac dysfunction. This model was generated to specifically test the hypothesis that the combination of myocardial insulin resistance and a modest increase in myocardial FA delivery would have synergistic effects in terms of precipitating myocardial dysfunction.

B. Summary of accomplishments

We have successfully generated a colony of CIRKO-ACS mice and have performed in vivo phenotyping by echocardiography as well as analysis of mitochondrial function. These data are summarized in figures 7-11 and tables 6 and 7 below. As can be seen, the *in vivo* hemodynamic phenotype in CIRKO-ACS mice is subtle (table 7), but is more evident in isolated hearts (table 8), which is in keeping with many of the other models that we have evaluated under basal conditions. However, the possibility exists that these mice could be sensitized to manifest greater degrees of cardiac dysfunction in the face of hemodynamic stressors such as ischemia and cardiac hypertrophy. The mitochondrial phenotyping has been very informative however. Specifically we were able to confirm that myocardial deficiency of insulin signaling precipitated mitochondrial dysfunction (Figure 10,11). Interestingly we also observed that a modest increase in myocardial FA-CoA delivery to the heart was sufficient to induce mitochondrial dysfunction (Figure 10,11). Finally, we demonstrate that the combination of insulin resistance and lipotoxicity leads to accelerated mitochondrial dysfunction, which is evident in the analysis of the 12-week-old animals (Figures 8,9).

C. Plans for the coming year

These initial studies were performed in mice that are on a mixed genetic background. We will migrate both the CIRKO and the ACS mutation to a common C57BL6J background, in order to determine if the phenotypic changes that we have observed persist. Second, we will continue to evaluate the mitochondrial phenotype in this model at an earlier time point to determine the time-point at which mitochondrial dysfunction is precipitated in the CIRKO-ACS mouse. Finally we will examine substrate metabolism in isolated hearts in a more complete cohort, and examine the response of these animals to hemodynamic stressors such as ischemia and LV hypertrophy.

D. Significant achievement and its importance

These studies are highly significant because they now begin to elucidate the synergistic mechanisms by which insulin resistance and lipotoxicity might precipitate myocardial dysfunction in diabetes.

Publications

A manuscript describing the mitochondrial phenotypes of the CIRKO mouse has been submitted and is currently undergoing revision.

Aspects of this work has been presented at National Meetings and are summarized below.

1. Boudina S, O'Neill B, Belke DD, Rodnick KJ, **Abel ED**. Insulin resistance leads to progressive mitochondrial dysfunction in the mouse heart. Circulation 2003; 108 IV-1404.

2. Mazumder PK, Hu P, Chakrabarti G, Zhang D, Avelar E, **Litwin SE**, **Abel ED**. Insulin signaling is required for the metabolic and functional adaptation of the heart to pressure overload hypertrophy. Circulation 2003; 108 IV-438

(1-2 Presented at the American Heart Association Scientific Sessions 2003)

3. Boudina S, Sena S, Wright J, O'Neill BT, Mazumder PK, **Abel ED.** Postnatal deletion of insulin receptors augments myocardial fatty acid utilization and enhances mitochondrial biogenesis. Circulation 2004; 110: III-324

(Presented at the American Heart Association Scientific Sessions 2004)

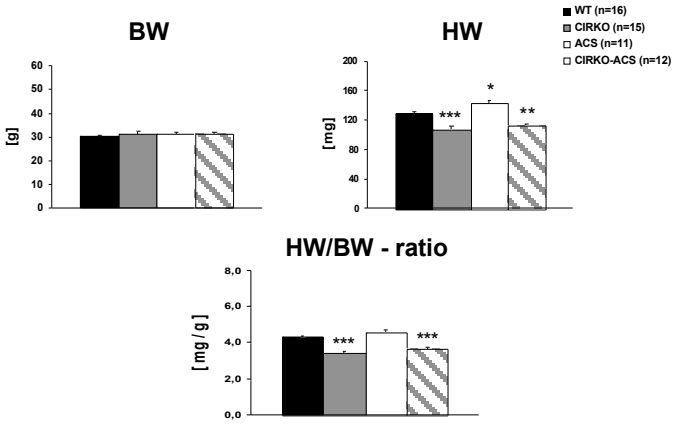
4. Boudina S**,** Sena S, O'Neill BT, **Abel ED**. Lack of Insulin Signaling in Cardiomyocytes Leads to Progressive ROS-mediated Defects in Mitochondrial Function.

(Presented Endocrinology Canada-International Symposium on the Science of Diabetes Complications: Implication for Novel Therapy. Toronto, Canada 2004.)

Figures and Tables for Project 3

Figure 7:

Heart Weights (HW), Body Weights (BW) and HW:BW Ratios in 24 week-old CIRKO and CIRKO-ACS Mice



*** p<0.05 vs. WT, * p<0.05 vs. CIRKO

Table 6:

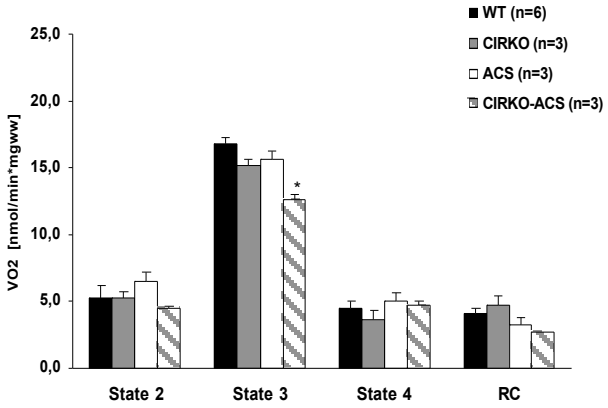
Echocardiography in 24-week-old CIRKO, ACS, or CIRKO-ACS mice

	WT (n=5)	CIRKO (n=6)	ACS (n=4)	CIRKO-ACS (n=6)
LVDd [cm]	0.40 ± 0.06	0.38 ± 0.03	0.40 ± 0.04	0.40 ± 0.02
LVDs [cm]	0.26 ± 0.07	0.24 ± 0.03	0.25 ± 0.04	0.26 ± 0.02
LVPWd [cm]	0.10 ± 0.03	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
FS [%]	36.8 ± 9.4	35.7 ± 4.2	38.4 ± 6.7	35.3 ± 4.5
EF	0.74 ± 0.11	0.73 ± 0.05	0.76 ± 0.08	0.73 ± 0.06
LVOTvti [cm]	4.50 ± 0.80	4.18 ± 1.03	3.42 ± 0.43	3.51 ± 0.77
HR [bpm]	448 ± 59	378 ± 32	499 ± 46	402 ± 27
CO [ml/min]	15.7 ± 2.7	12.2 ± 2.1*	13.4 ± 2.3	11.0 ± 2.2**

* p<0.05 vs. WT, ** p<0.05 vs. WT and ACS

Figure 8:

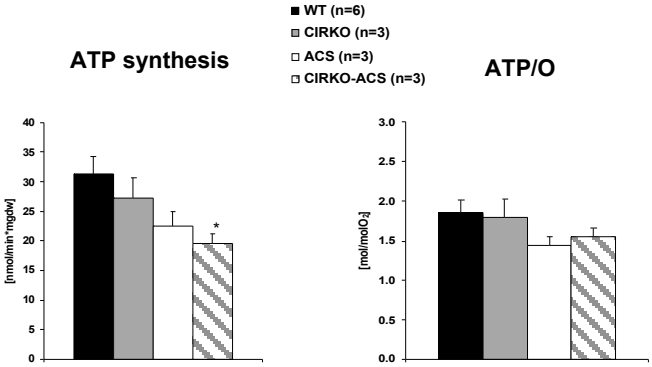
Reduced state 3 respiration in CIRKO-ACS, but not in CIRKO and ACS mice at 12 weeks



* p<0.05 vs. all other genotypes

Figure 9:

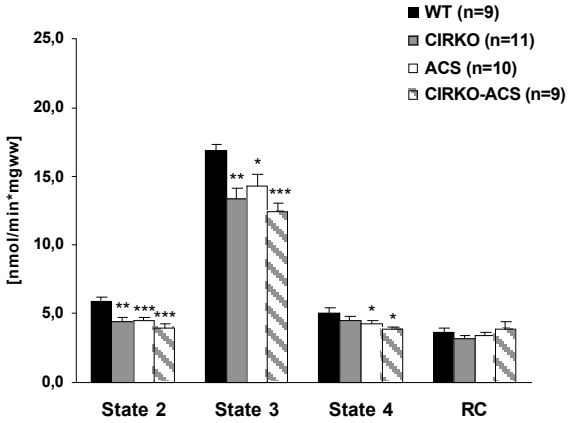
ATP synthesis and ATP/O ratio in CIRKO, ACS, and CIRKO-ACS mice (12 weeks)



* p<0.05 vs. all other genotypes

Figure 10:

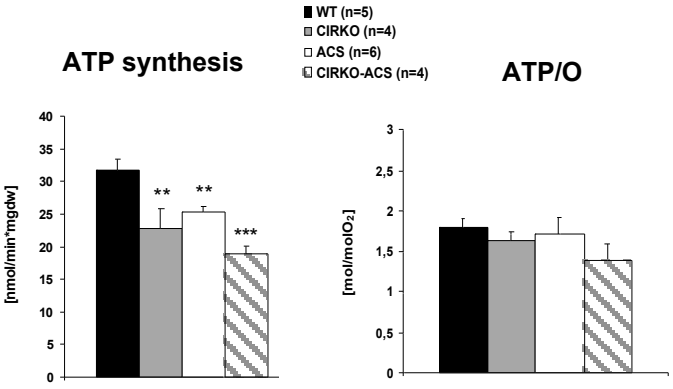
Equivalent Impairment in palmitoyl-carnitine supported state 3 respiration in 24-week-old CIRKO, ACS, and CIRKO-ACS mice



*, **, ***, p<0.05, 0.01 and 0.005 vs. WT

Figure 11:

Reduced ATP synthesis, but unchanged ATP/O ratio In 24-week-old CIRKO, ACS, and CIRKO-ACS mice



**, ***, p<0.01 and 0.005 vs. WT

Table 7:

ACS mice show contractile dysfunction *in vitro* *(Isolated Working Hearts)*

	WT (n=4)	ACS (n=4)
Palmitate oxidation	141 ± 12	166 ± 11
MVO_2	152 ± 11	114 ± 5 **
cardiac efficiency	16.5 ± 1.9	15.1 ± 1.5
LVDevP	36.0 ± 0.8	31.2 ± 1.3 **
Cardiac power	33.9 ± 0.8	27.6 ± 1.3 ***
Cardiac output	15.6 ± 0.5	13.0 ± 0.3 ***

**, *** p<0.05 and 0.01 vs. WT respectively

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Animal Model	Background Strain	Current Status	Echocardiography (Ejection Fraction)	Histology (Interstitial or Replacement Fibrosis)	Cardiac Morphology (L.V. Hypertrophy?)	Invasive Assessment of L.V. Function	Isolated Heart Analysis	Cardiac Metabolism/ Mitochondrial Function	Response to Stress	Gene Expression	Electrophysiology
Akita *	C57BL/6J	Ongoing Phenotyping	Completed	Pending	In Progress	Pending	Pending	In Progress	Pending	Pending	Pending
Dominant Negative PI3 Kinase *	FVB	Advanced Phenotyping	Completed	In Progress	Completed	In Progress	Pending	Completed	In Progress	In Progress	Pending
ACS-CIRKO*	Mixed	Ongoing Phenotyping	Completed	Pending	Completed	Pending	Pending	In Progress	In Progress	Pending	Pending
High-Fat Feeding ¶	C57BL/6J	Ongoing Phenotyping	In Progress	Pending	In Progress	Pending	Pending	In Progress	Pending	In Progress	Pending
UCP-DTA¶	FVB	Ongoing Phenotyping	In Progress	Pending	In Progress	Pending	Pending	Completed	Pending	Pending	Pending
Ob/ob†	C57BL/6J	Final Phenotyping	Completed	Completed	Completed	Completed	Completed	Completed	In Progress	Completed	Pending
Db/db†	C57BLKS	Final Phenotyping	Completed	Completed	Completed	Completed	Completed	Completed	Pending	Completed	Completed
CIRKO†	Mixed	Final Phenotyping	Completed	Completed	Completed	Completed	Completed	Completed	Completed	Completed	In Progress

* Current top three Models; ¶ Next top two models; † Original top three models.