

# **Mouse Models of Diabetic Cardiomyopathy**

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# Model Justification

- **We have elected to model the diabetic cardiomyopathy in mice by analyzing existing models at the level of insulin signaling, substrate utilization and mitochondrial function - ob/ob and db/db mice**
- **By generating mouse models with altered insulin signaling and altered substrate flux - CIRKO, G4H-/- and Dn PI3- K transgenics**
- **By examining the response of these models to clinically relevant stresses namely cardiac hypertrophy and myocardial ischemia**

# Cardiomyopathy

## Minimal Criteria for Mouse Models of Diabetic Cardiomyopathy

In the context of insulin resistance and hyperglycemia:

- Decreased ejection fraction  $\pm$  evidence of diastolic dysfunction
- Interstitial or replacement fibrosis
- LV hypertrophy (models of type 2 diabetes)

## Validation Criteria for Mouse Models of Diabetic Cardiomyopathy

- Invasive assessment of LV function in vivo to confirm systolic  $\pm$  diastolic dysfunction
- Evidence of LV dysfunction in isolated perfused hearts
- Evidence of abnormal cardiac metabolism and mitochondrial dysfunction
- Altered gene expression e.g. increased expression of beta-MHC, decreased expression of alpha-MHC, decreased expression of glucose transporters (GLUT4 and GLUT1)
- Impaired response to stress such as pressure overload hypertrophy and myocardial ischemia

<b>Animal model</b>	<b>Background Strain</b>	<b>Current Status</b>
<b>Ob/ob</b>	<b>c57Bl/6J</b>	<b>Final/advanced phenotyping</b>
<b>Db/db</b>	<b>c57BlKS</b>	<b>Final/advanced phenotyping</b>
<b>CIRKO</b>	<b>Mixed</b>	<b>Final/advanced phenotyping</b>
<b>UCP-DTA</b>	<b>FVB</b>	<b>Ongoing phenotyping</b>
<b>Dominant Negative PI3Kinase</b>	<b>FVB</b>	<b>Phenotyping in Progress</b>
<b>Inducible CIRKO</b>	<b>Mixed</b>	<b>Early Phenotyping</b>
<b>ACS-CIRKO</b>	<b>Mixed</b>	<b>Breeding is in Progress</b>
<b>STZ</b>	<b>Various</b>	<b>CIRKO-STZ in progress</b>
<b>GLUT4 Heart Specific</b>	<b>Mixed</b>	<b>Ongoing phenotyping</b>

# Summary of Previous Presentation

- **In two models of type 2 diabetes (ob/ob and db/db mice) cardiac dysfunction is associated with**
  - **Myocardial Lipid Accumulation**
  - **Impaired Myocardial Insulin Signaling**
  - **Altered Substrate Utilization (increased Fatty Acid Oxidation, reduced Glucose Oxidation and increased Myocardial Oxygen Consumption)**
  - **Mitochondrial Dysfunction characterized by Reduced Respiratory Capacity, Mitochondrial Uncoupling, Increased ROS Production and Mitochondrial Proliferation.**
- **With the exception of Lipid Accumulation, all of these phenotypes are recapitulated in the CIRKO mouse (Cardiomyocyte Restricted KO of Insulin Receptors).**

# **The CIRKO Mouse is the Major Platform on which we will Model the Diabetic Cardiomyopathy**

- **Increasing Lipid Delivery via Transgenic Approaches and STZ Diabetes**
- **Evaluating the Early Consequences of Loss of Insulin Signaling in an Inducible CIRKO mouse**
- **Pursuing Mechanisms that are Responsible for Increased Myocardial Injury in this Model**
- **Evaluating the role of downstream signaling pathways such as PI-3 Kinase**

# EAC Recommendations and Responses

- **Functional (Echo) data of myocardial function is a good start. Should continue these studies with older animals, paying specific attention to insulin sensitivity and glucose measurements to correlate with function.**
- **Ob/ob and db/db mice have been studied (echo and cath) at 15 weeks, and a longer cohort is in progress.**
- **Ex vivo studies have been performed in CIRKO mouse hearts out to 1 year. Caths and Echos have been performed out to 15 weeks at baseline and following hypertrophic and ischemic stimuli. An aging cohort is in progress for 1 year in vivo studies.**

# EAC Recommendations and Responses

- Interesting data with the alteration in metabolism and mitochondrial function. The CIRKO and dNPI3K animals also look intriguing.
- **Our working hypothesis is that impaired myocardial insulin signaling will increase the susceptibility of the heart to fatty acid induced injury. To address this we are increasing FA delivery to CIRKO hearts in two ways.**
  - STZ diabetes - Studies have been completed. Echocardiographic data and mitochondrial function analyses are being completed.
  - Crossing CIRKO mice with transgenic mice that overexpress Acyl-CoA synthetase. - Breeding is in progress. ACS transgene has been successfully bred into the floxed IR background. Breeding is now in progress to generate double Cre/ACS transgenics.
- **Mitochondrial Phenotyping of dNPI3K mice has started.**



# EAC Recommendations and Responses

- While the observation that ob/ob and db/db mice have mitochondrial dysfunction and respiratory uncoupling is interesting, the relevance of these models to human diabetic cardiomyopathy is less straightforward. It appears that a great deal of descriptive data have been generated for models that may not be very relevant to humans with insulin resistance and intact leptin signaling pathways.

# EAC Recommendations and Responses

- Two recent human studies have provided evidence that similar changes may be taking place in humans.
- Scheuermann-Freestone M et. al. *Circulation* 107:3040-6 (2003)  
Myocardial high energy phosphate content was reduced in the hearts of type 2 diabetics and is inversely correlated with circulating FFA concentrations.
- Peterson LR et.al. *Circulation* 109: 2191-6 (2004)  
Young women with morbid obesity have LV hypertrophy, increased rates of FA oxidation, increased MVO<sub>2</sub> and decreased cardiac mechanical efficiency. Positive correlation between BMI and MVO<sub>2</sub>, glucose intolerance and fatty acid uptake.
- Myocardial insulin resistance occurs in leptin independent animal models such as the UCP-DTA mouse and the Goto-Kakizaki rat
- Desrois M et al. *JMCC* 37: 547-55 (2004) and *Cardiovasc. Res.* 61: 288-296 (2004)

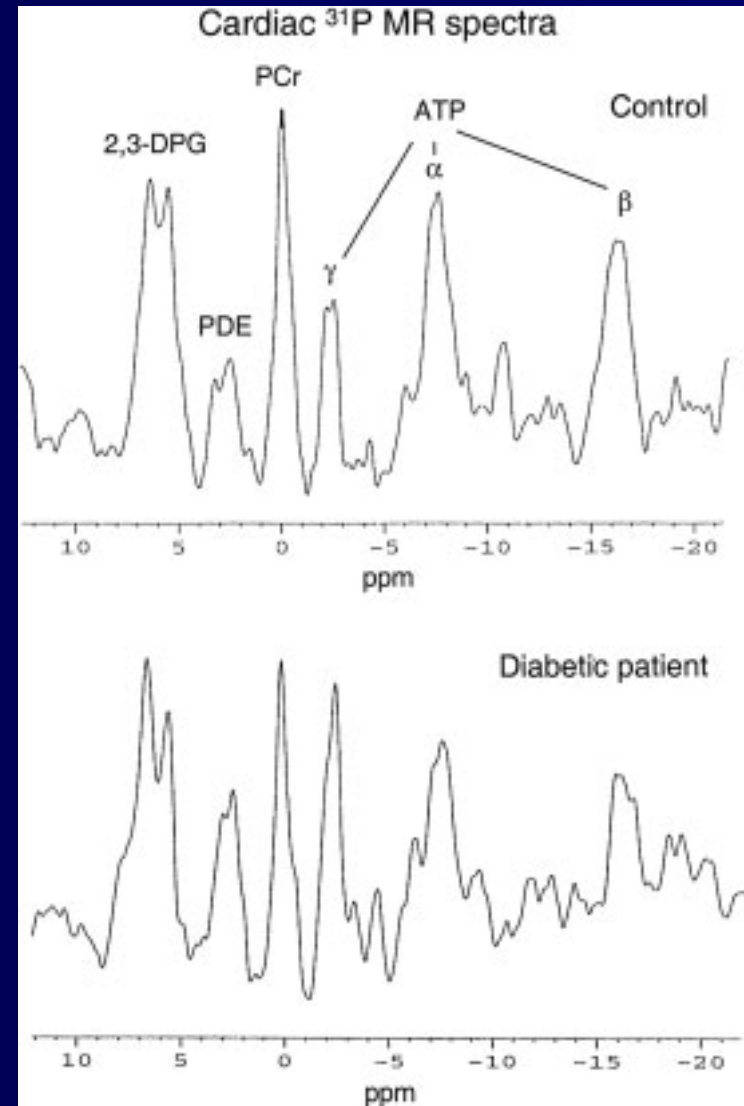
# **Mice versus Men**

**Evidence for Altered Myocardial  
Substrate Utilization, Insulin  
Resistance and Impaired  
Myocardial Energetics in Humans  
with Obesity and Diabetes**

# Mechanistic Observations Human Studies

--Using NMR spectroscopy individuals with type 2 diabetes were recently shown to have reduced myocardial high energy phosphate content that was inversely associated with circulating levels of FFA (Scheuermann-Freestone M et al. *Circulation* 107:3040, 2003), suggesting mitochondrial dysfunction

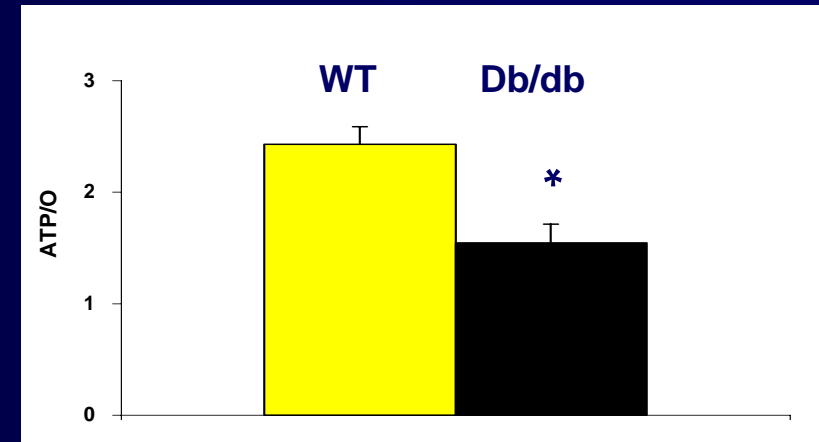
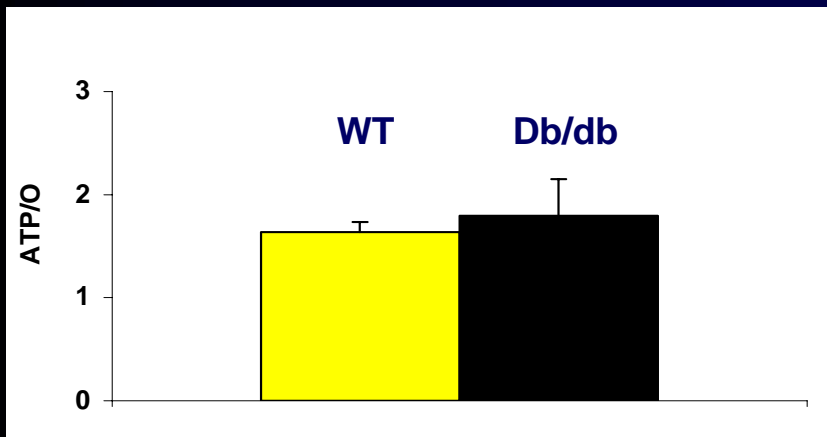
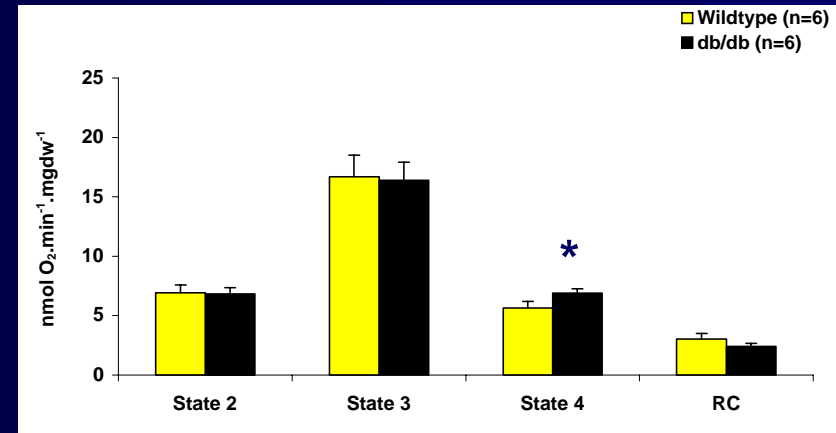
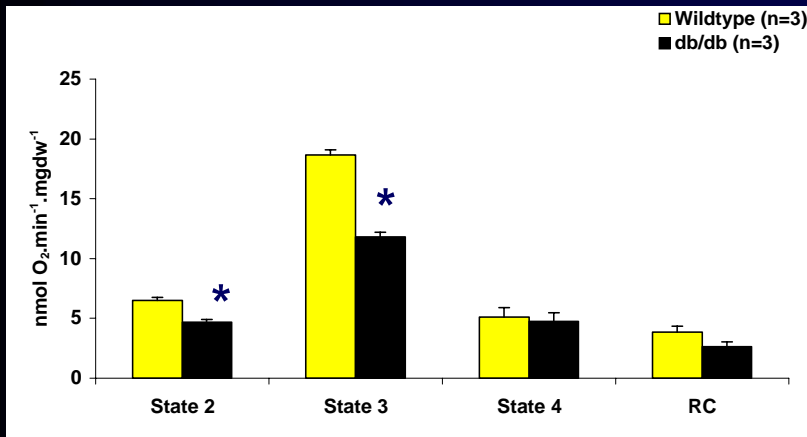
--Using MR spectroscopy a strong association between obesity and increased myocardial triglyceride accumulation was described. Indeed myocardial TG content was positively associated with LVH and inversely associated with LV function (Szczepaniak et al. *Magnetic Resonance in Medicine* 49:417, 2003).



# Mitochondrial Dysfunction and Increased Uncoupling in Cardiac Mitochondria from db/db Mice

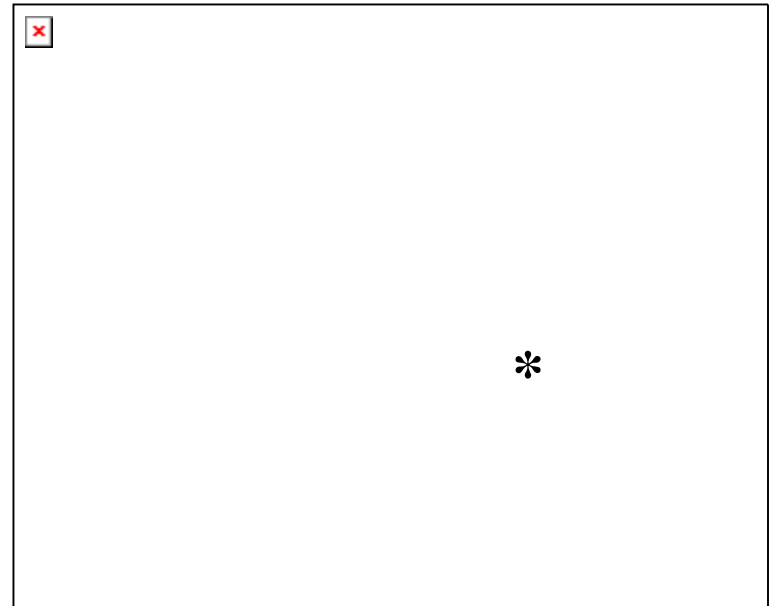
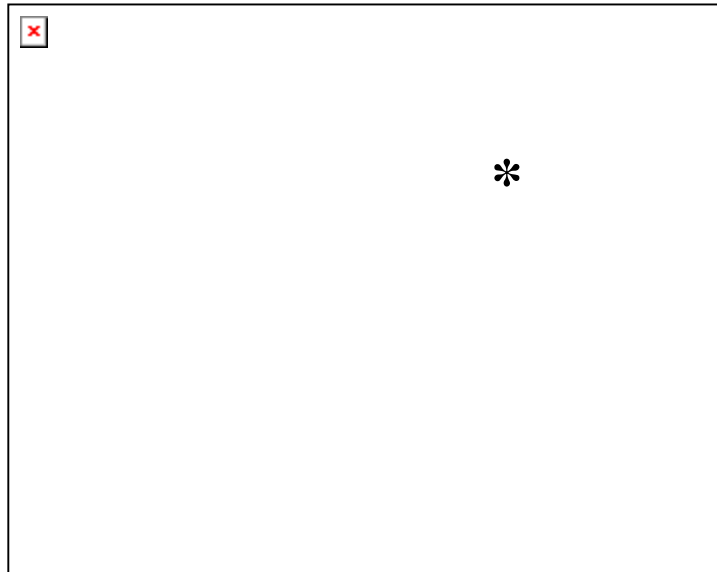
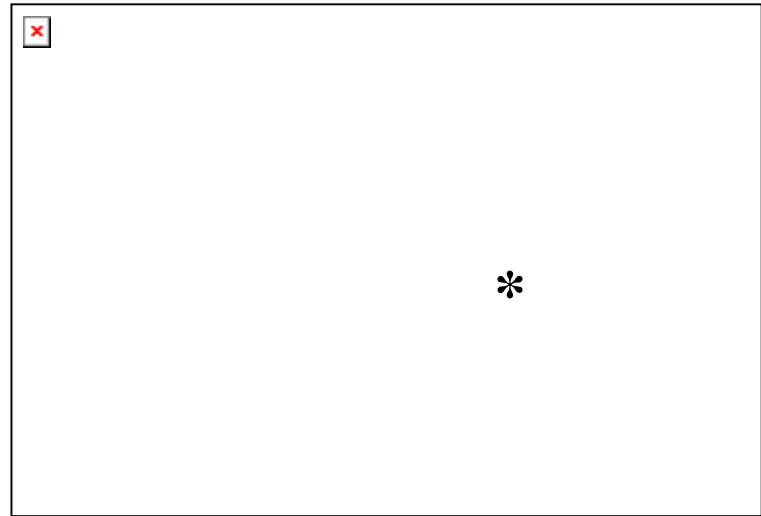
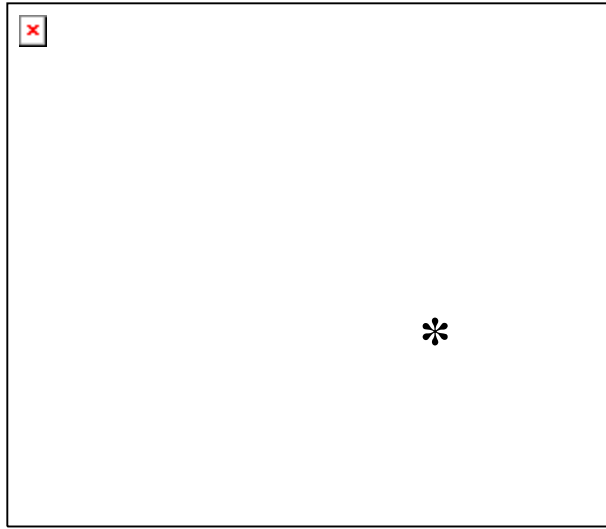
*Glucose Perfused*

*Glucose and Palmitate Perfused*



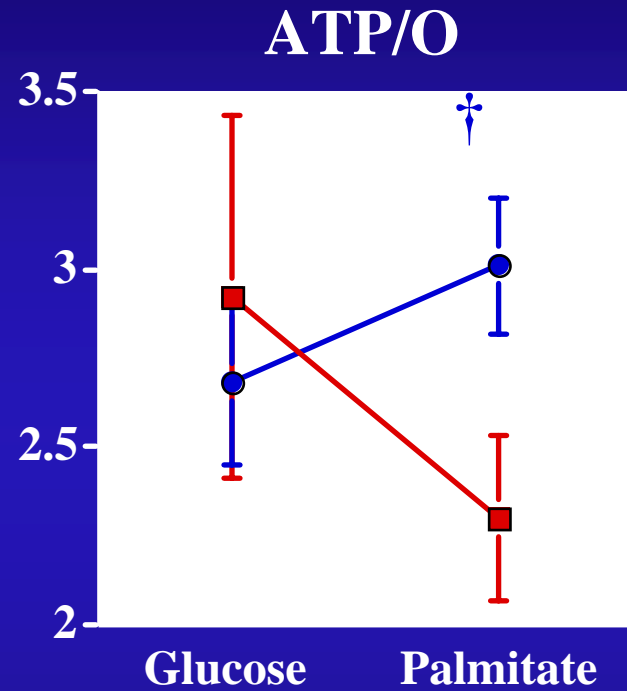
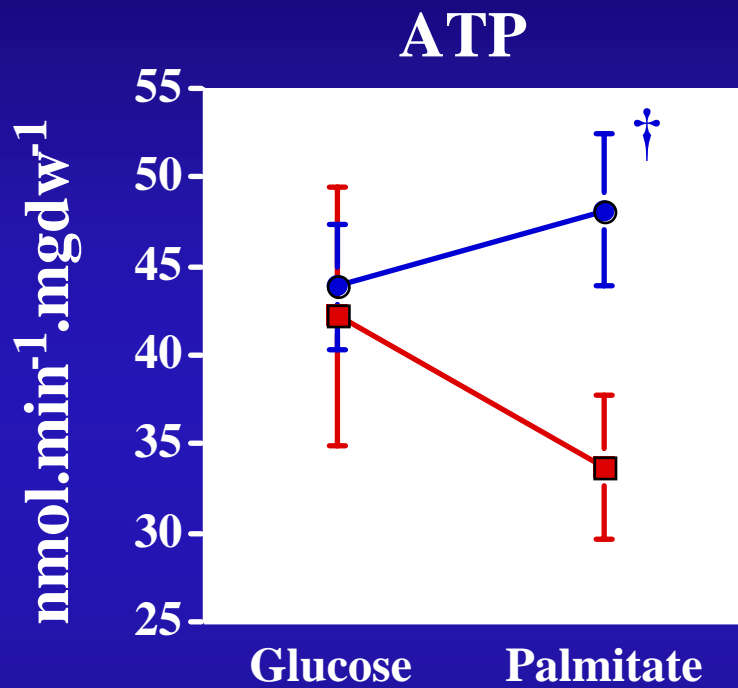
Mitochondrial respiration determined in the presence of 20  $\mu$ M palmitoyl-L-carnitine

# Targeted Proteomic Analysis of ob/ob Mitochondria



■ WT ■ ob/ob

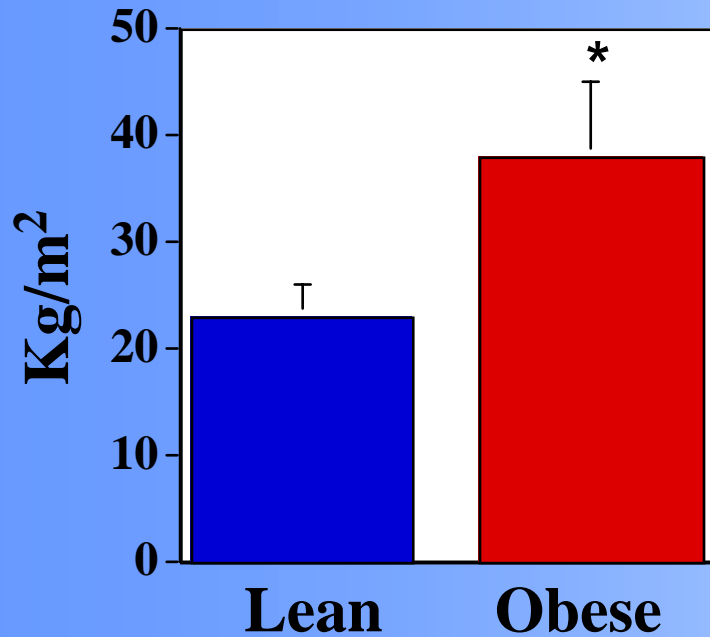
# Mitochondrial ATP Production in Glucose and Palmitate Perfused ob/ob Mouse Hearts



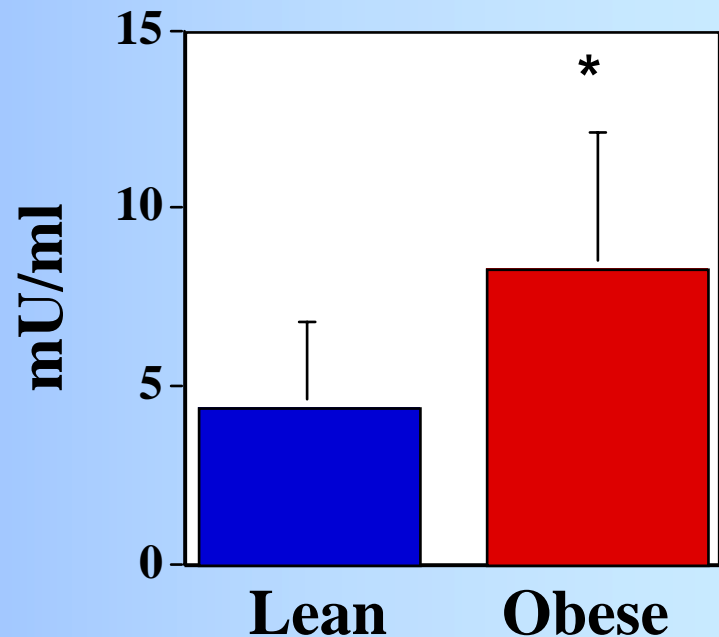
■ Ob/ob  
● WT

# Characteristics of Study Cohort

## Body Mass Index



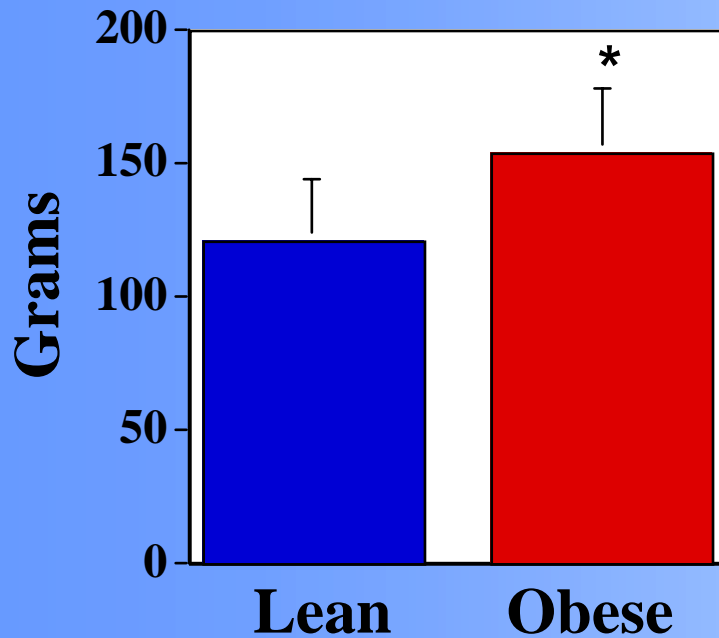
## Serum Insulin



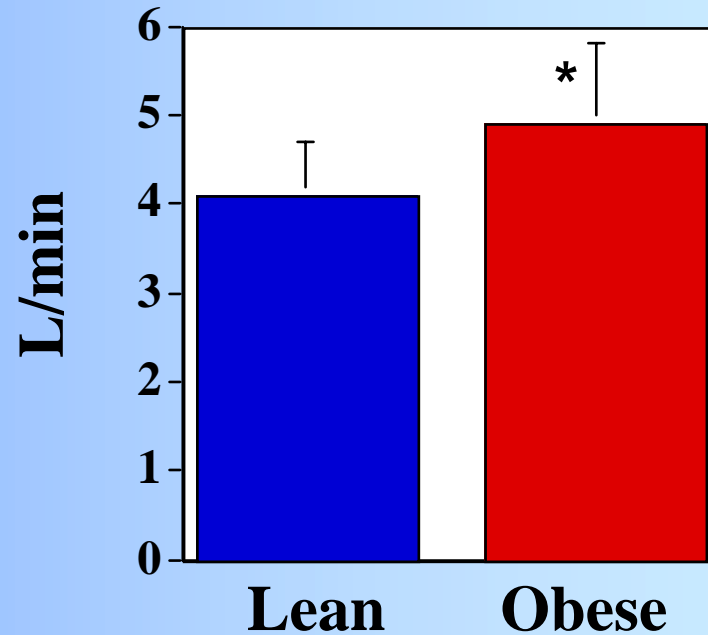


# Cardiac Structure/Function

## LV Mass

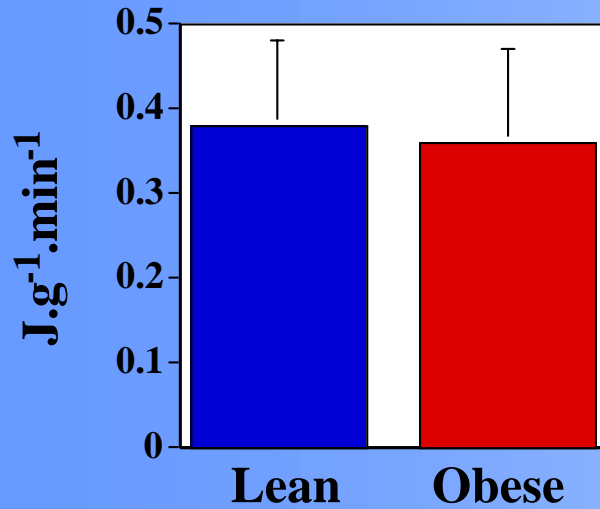


## Cardiac Output

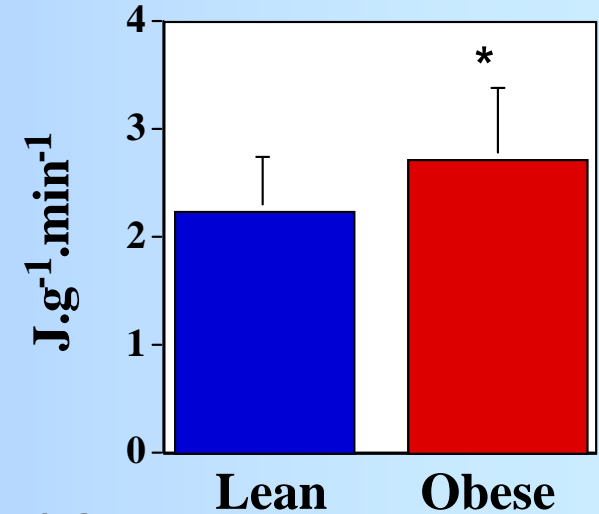


# Cardiac Bioenergetics

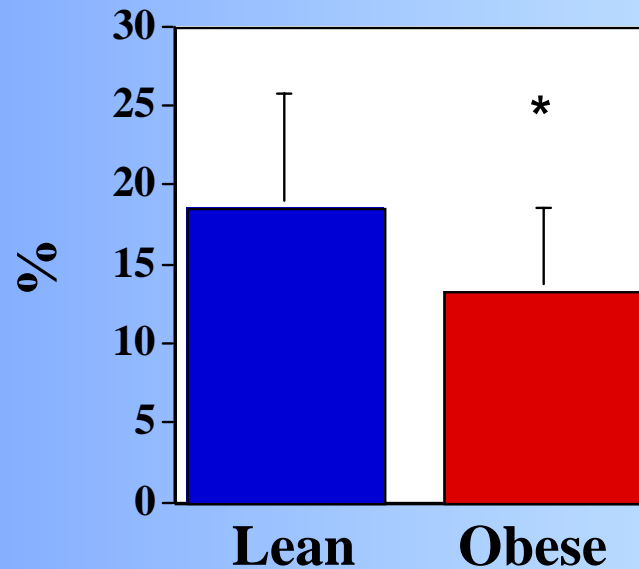
## Cardiac Work



## MVO<sub>2</sub>

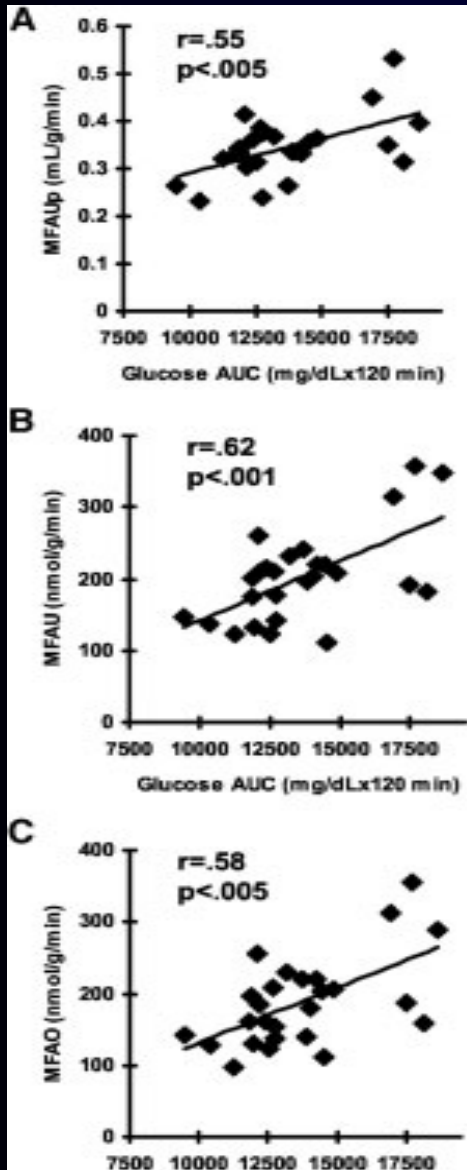


## Cardiac Efficiency

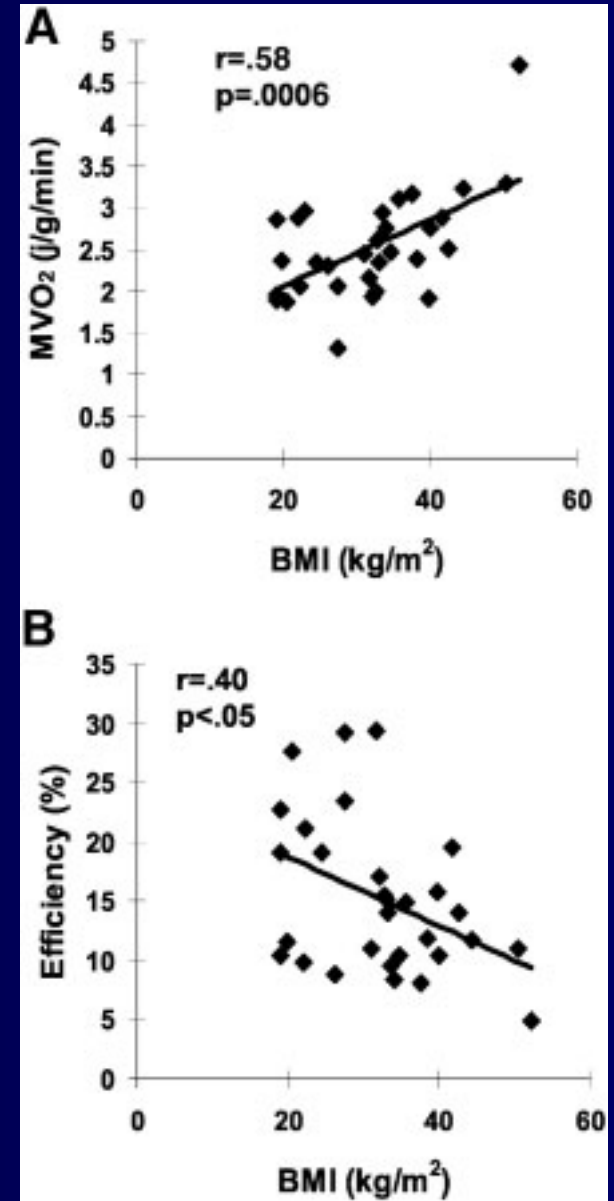


Peterson L et al -  
Circulation  
2004;109:2191-96

# Fatty Acid Utilization

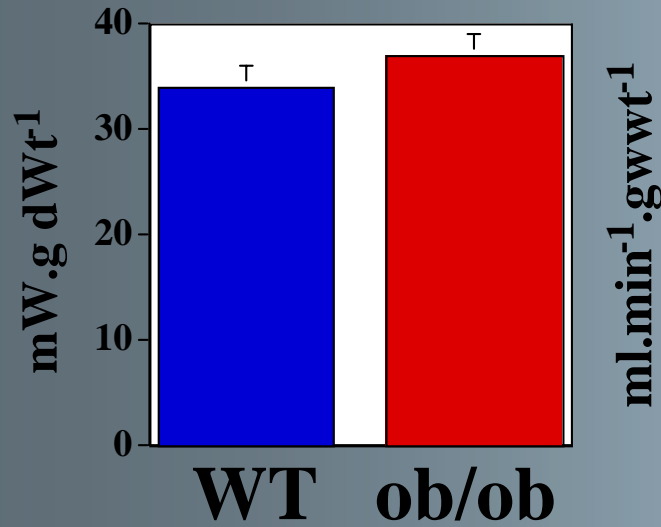


Peterson L et al -  
Circulation  
2004;109:2191-96

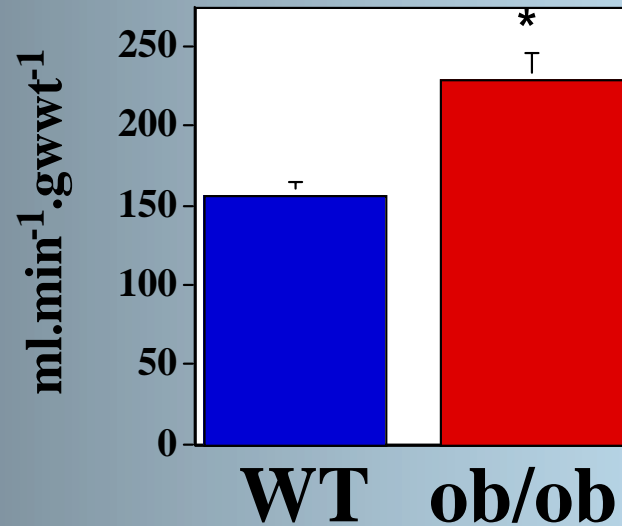


# Cardiac Performance and Myocardial Oxygen Consumption in the Hearts of 4-week-old ob/ob Mice

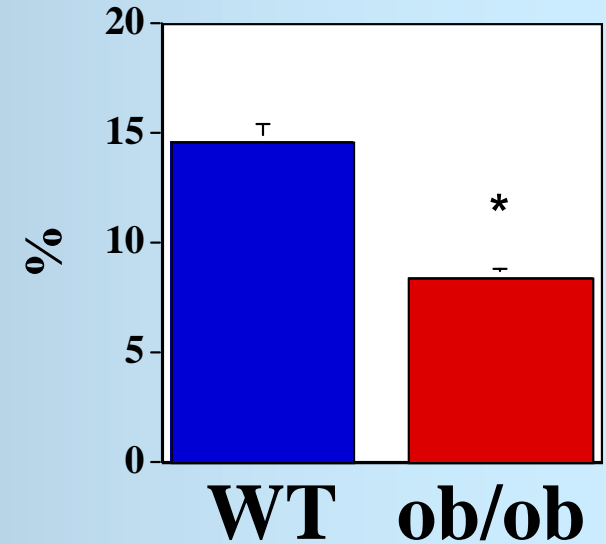
## Cardiac Power



## MVO<sub>2</sub>

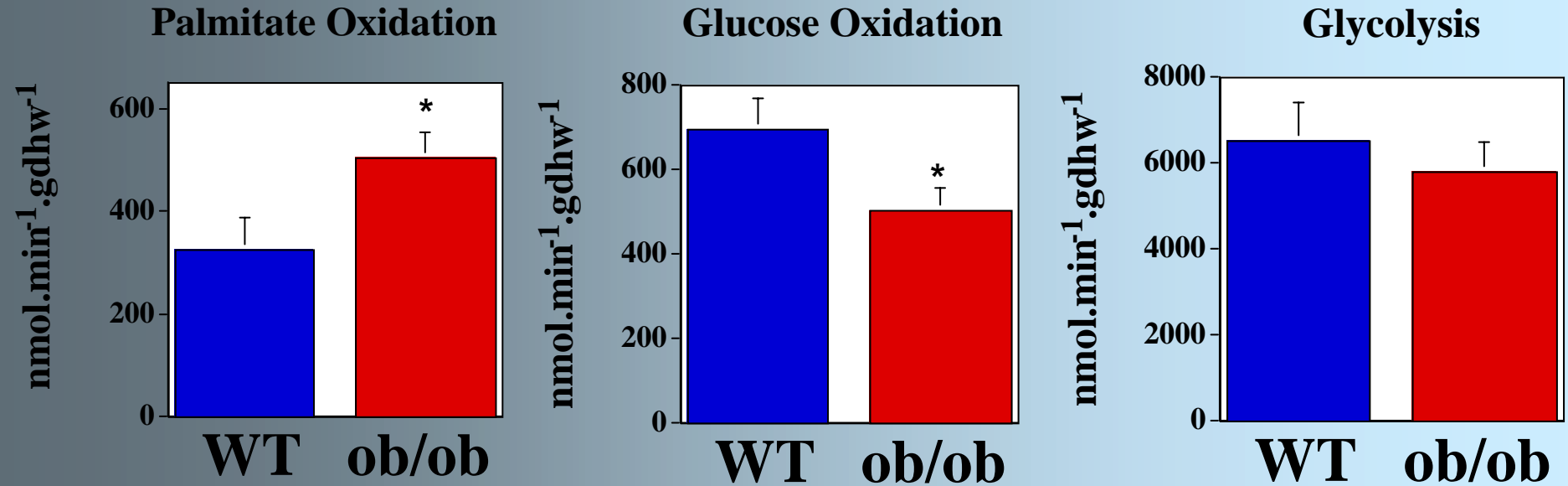


## Efficiency



Perfusion conditions: 11mM Glucose, 1mM Palmitate, 1nM Insulin

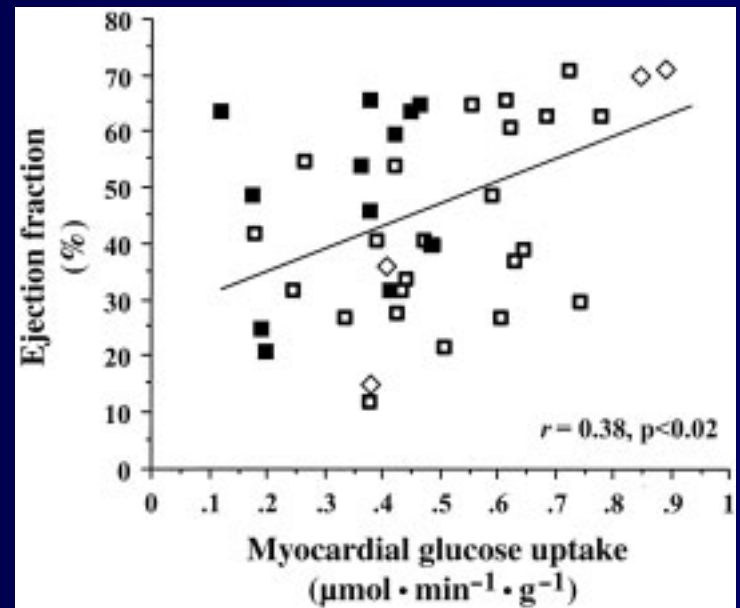
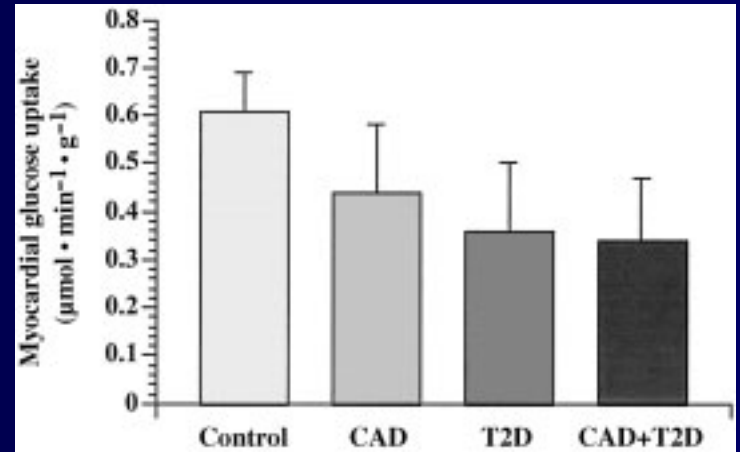
# Substrate Metabolism in Hearts of 4-week-old ob/ob Mice



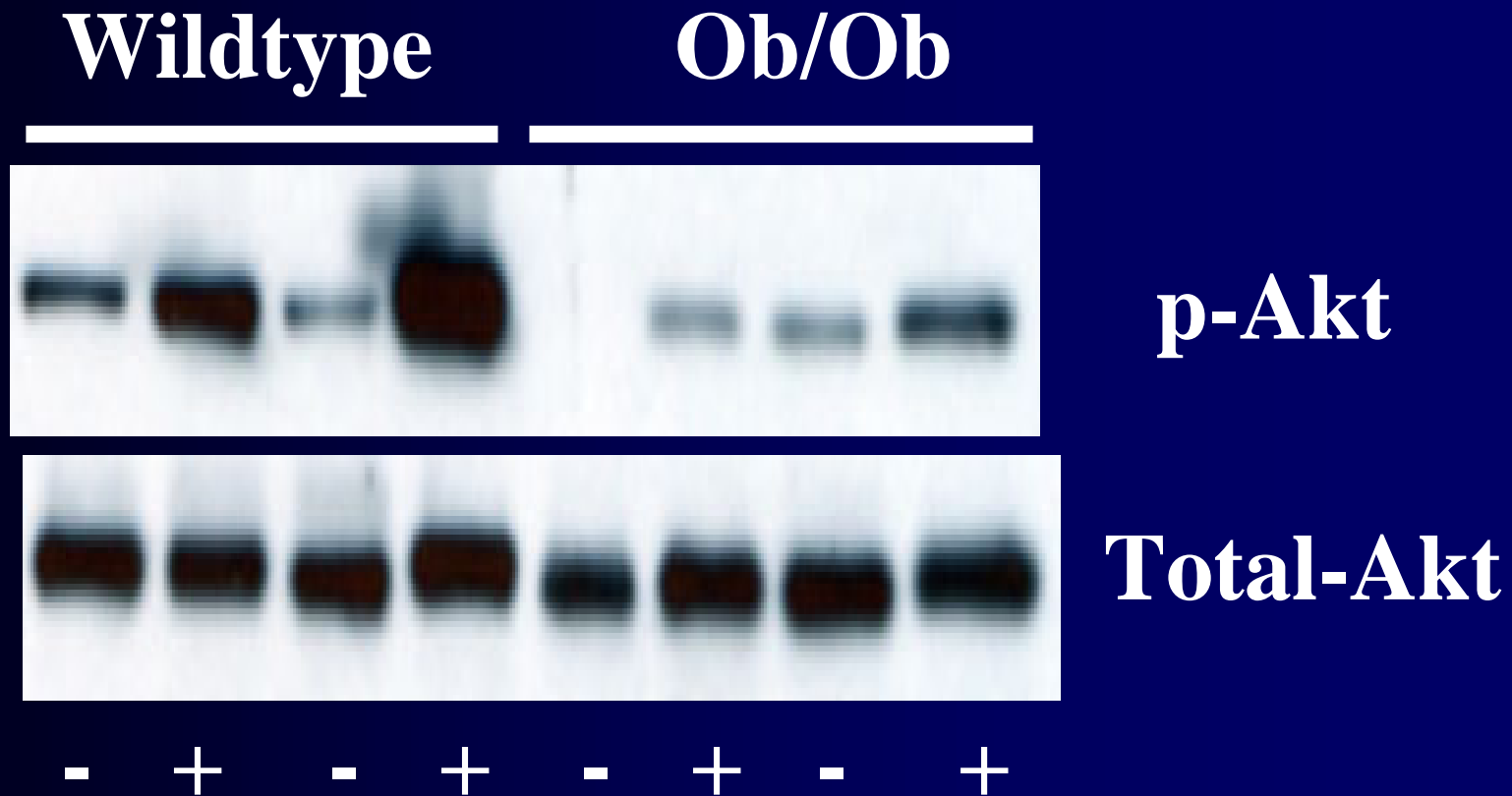
Perfusion conditions: 11mM Glucose, 1mM Palmitate, 1nM Insulin

# Does Insulin Resistance Occur in the Human Heart?

- Using euglycemic clamps and PET scanning (under physiological levels of insulin), the hearts of individuals with type 2 diabetes demonstrates reduced insulin stimulated glucose uptake that was equivalently decreased in subjects with and without CAD. And a positive correlation was observed between myocardial glucose uptake and LV ejection fraction (Iozzo P et al. Diabetes 51:3020, 2002).



# Impaired Insulin Signaling in Cardiomyocytes of Ob/Ob Mice



**GLUT4**



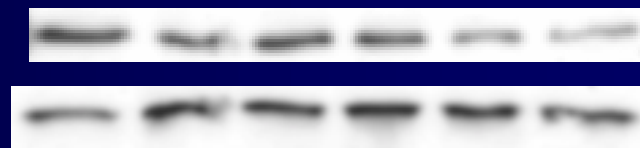
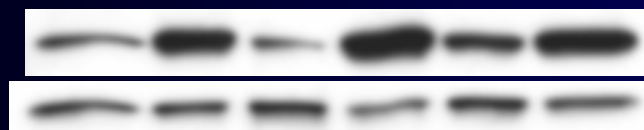
**Wildtype**

**UCP-DTA**

**A**

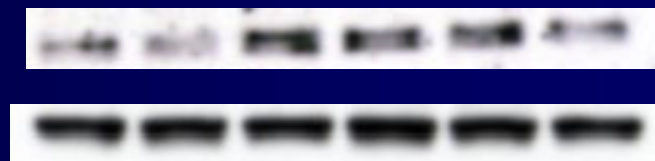
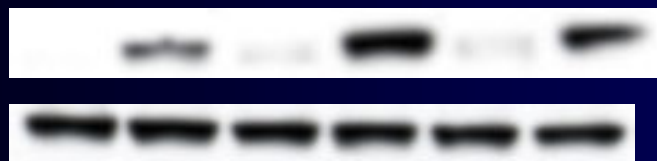
pAkt →

Akt →



pGSK3 →

GSK3 →



**Insulin:**

- + - + - +

- + - + - +

**Wildtype**

**UCP-DTA**

**B**



# EAC Recommendations and Responses

- **The idea of diastolic dysfunction as a criteria for diabetic cardiomyopathy is extremely questionable given current technologies.**
- We agree. It is for this reason, that the criteria that we have developed for diabetic cardiomyopathy includes evidence for systolic dysfunction (i.e. decreased ejection fraction), as well as evidence of myocardial injury such as interstitial fibrosis, and LV hypertrophy in models of type 2 diabetes.
- **Better models of increased lipid delivery to the heart should be pursued. Why not include transgenic PPARalpha mice in these studies? These animals appear to have a myocardial phenotype that resembles the diabetic state.**
- We agree that many models of increased myocardial lipid delivery now exist. We have initially chosen the ACS mice to evaluate the role of increased fatty acid entry per se.

# EAC Recommendations and Responses

- Are the CIRKO more likely to die prematurely, especially after aortic banding? Humans with diabetic cardiomyopathy have an increased rate of sudden death. Telemetry studies could address whether any of these models have cardiac arrhythmias.
- The aortic banding in CIRKO animals have so far been only extended to four weeks. Although we see significantly reduced cardiac function, we have not observed premature mortality. We would expect however, that CIRKO mice would die earlier than wildtype mice if we follow them for longer periods of time post-aortic banding.

# EAC Recommendations and Responses

- Are the CIRKO more likely to die prematurely, especially after aortic banding?  
Humans with diabetic cardiomyopathy have an increased rate of sudden death.  
Telemetry studies could address whether any of these models have cardiac arrhythmias.
- We did observe increased early/premature mortality in CIRKO mice following coronary artery ligation. Sudden death after myocardial infarction is more likely among diabetics. Moreover, we have recently published a paper showing that potassium currents are altered in the hearts of CIRKO mice, in a manner that is similar to that observed in db/db mice (Shimoni et al J. Physiol 2004, 555: 345-354). In a second study now underway using optical mapping we have seen that the propagation of the action potential is markedly attenuated in CIRKO mouse hearts following ischemia. These electrophysiological changes would definitely predispose CIRKO hearts to arrhythmias.
- We have just obtained EKG telemetry capability and will use this to address the question of arrhythmias in ischemic CIRKO hearts in vivo

# Phenotyping to Date



<b>Criteria/Validation</b>	<b>Db/db mice</b>
Ejection Fraction	Reduced
Replacement/Interstitial Fibrosis	Trichrome Stains in progress. Increased myocardial lipid.
Cardiac Hypertrophy	No
Invasive Assessment of LV Function	↑LVSP, ↑LVDP as early as 4-weeks of age, ↑dP/dt.
Isolated Hearts	Decreased LV Function much worse than ob/ob.
Cardiac Metabolism/Mitochondrial Function	↓Glucose Ox, ↑FA OX Mitochondrial Dysfunction is present.
Gene Expression	MHC isoforms switched.
Response to Stress	Pending
Electrophysiology	Prolonged action potential.

# Phenotyping to Date



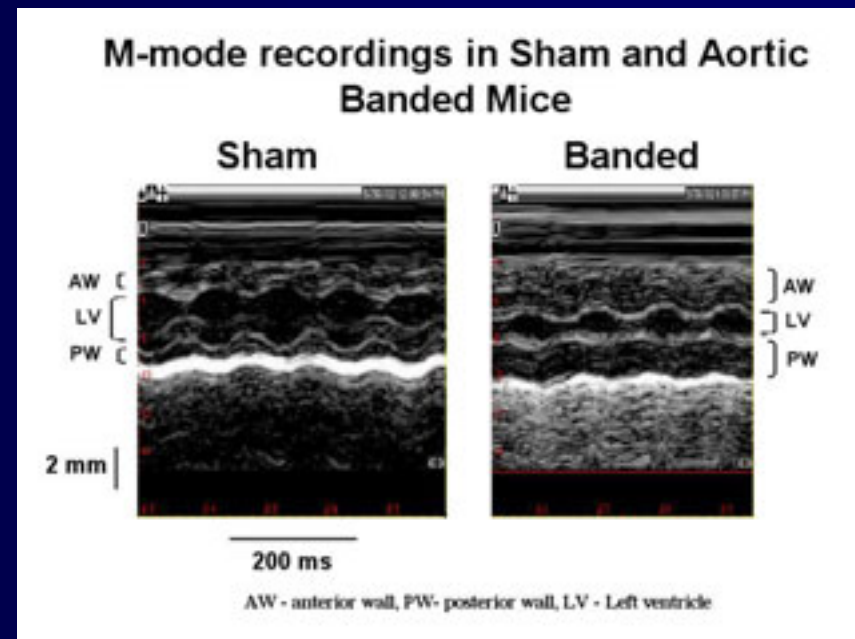
<b>Criteria/Validation</b>	<b>Ob/ob Mice</b>
Ejection Fraction	Reduced
Replacement/Interstitial Fibrosis	Trichrome pending, Increased myocardial lipid.
Cardiac Hypertrophy	Yes
Invasive Assessment of LV Function	↑LVSP, ↑LVEDP as mice age, dp/dt↑ at 4 weeks, ↓ at 8-weeks.
Isolated Hearts	Decreased LV Function.
Cardiac Metabolism/Mitochondrial Function	↓Glucose Ox, ↑FA- OX Mitochondrial dysfunction is present. Insulin signaling in cardiomyocytes is impaired.
Gene Expression	MHC isoforms switched.
Response to Stress	Impaired Inotropic Response
Electrophysiology	NA

# Phenotyping to Date

<b>Criteria/Validation</b>	<b>CIRKO Mice</b>
Ejection Fraction	Reduced
Replacement/Interstitial Fibrosis	Increased
Cardiac Hypertrophy	Age dependent
Invasive Assessment of LV Function	LV Function reduced, $\pm$ Increased Diastolic Pressures.
Isolated Hearts	Decreased LV Function.
Cardiac Metabolism/Mitochondrial Function	$\downarrow$ Glucose Ox, $\uparrow$ Fa OX (young) $\downarrow$ Fa OX (Old). Mitochondrial dysfunction is present.
Gene Expression	MHC isoforms switched.
Response to Stress	Impaired response and increased injury following ischemia, pressure overload and chronic catecholamine stimulation.
Electrophysiology	Prolonged action potential

# **Mechanisms for Increased Cardiac Injury in CIRKO Mice**

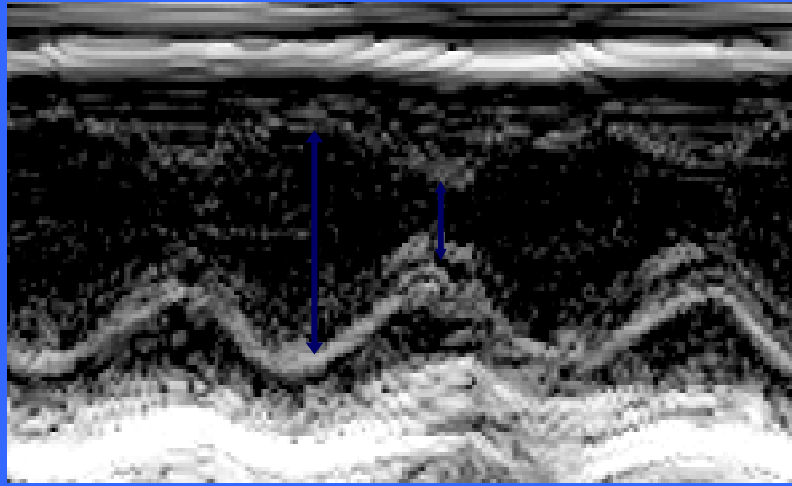
# Development of a Minimally Invasive Method for Inducing Pressure Overload Hypertrophy in Mice



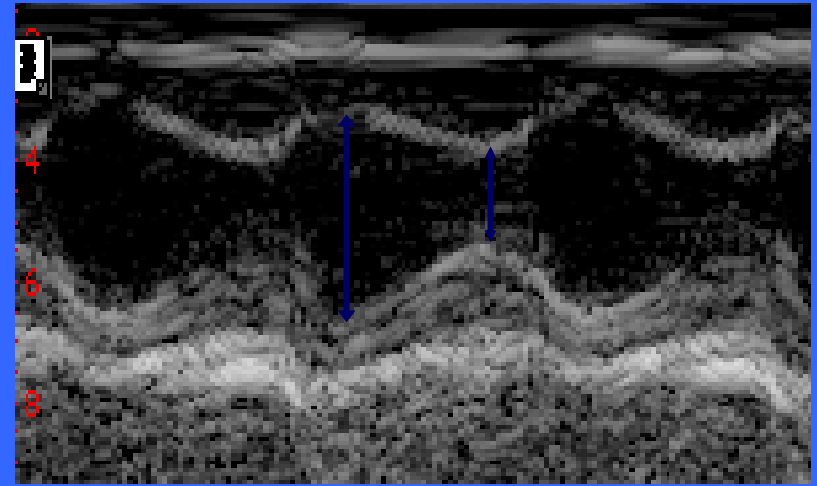


# Aortic Banding in CIRKO Mice

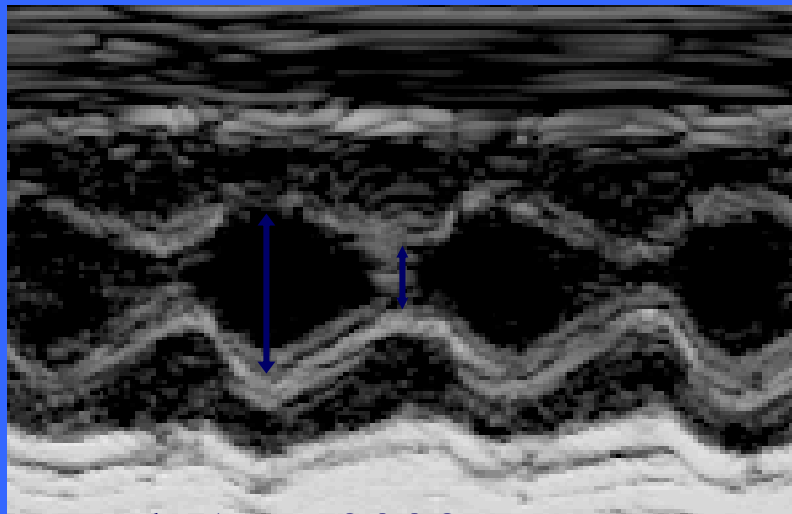
WT Sham



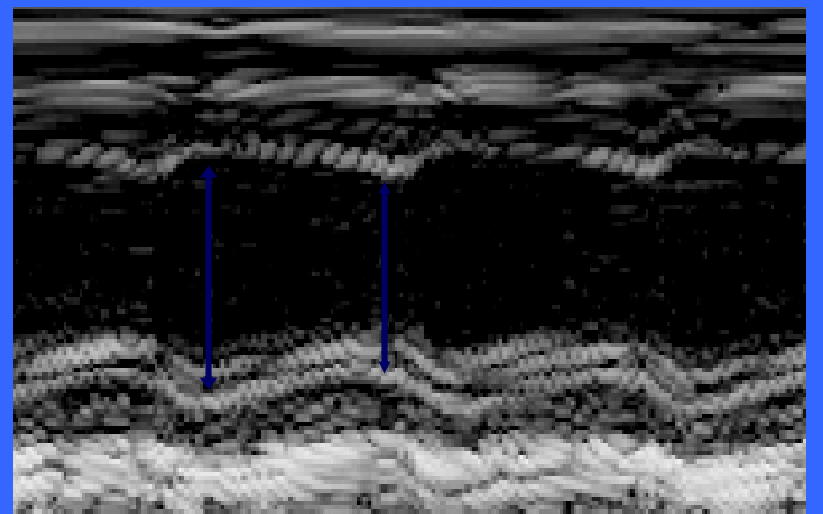
CIRKO Sham



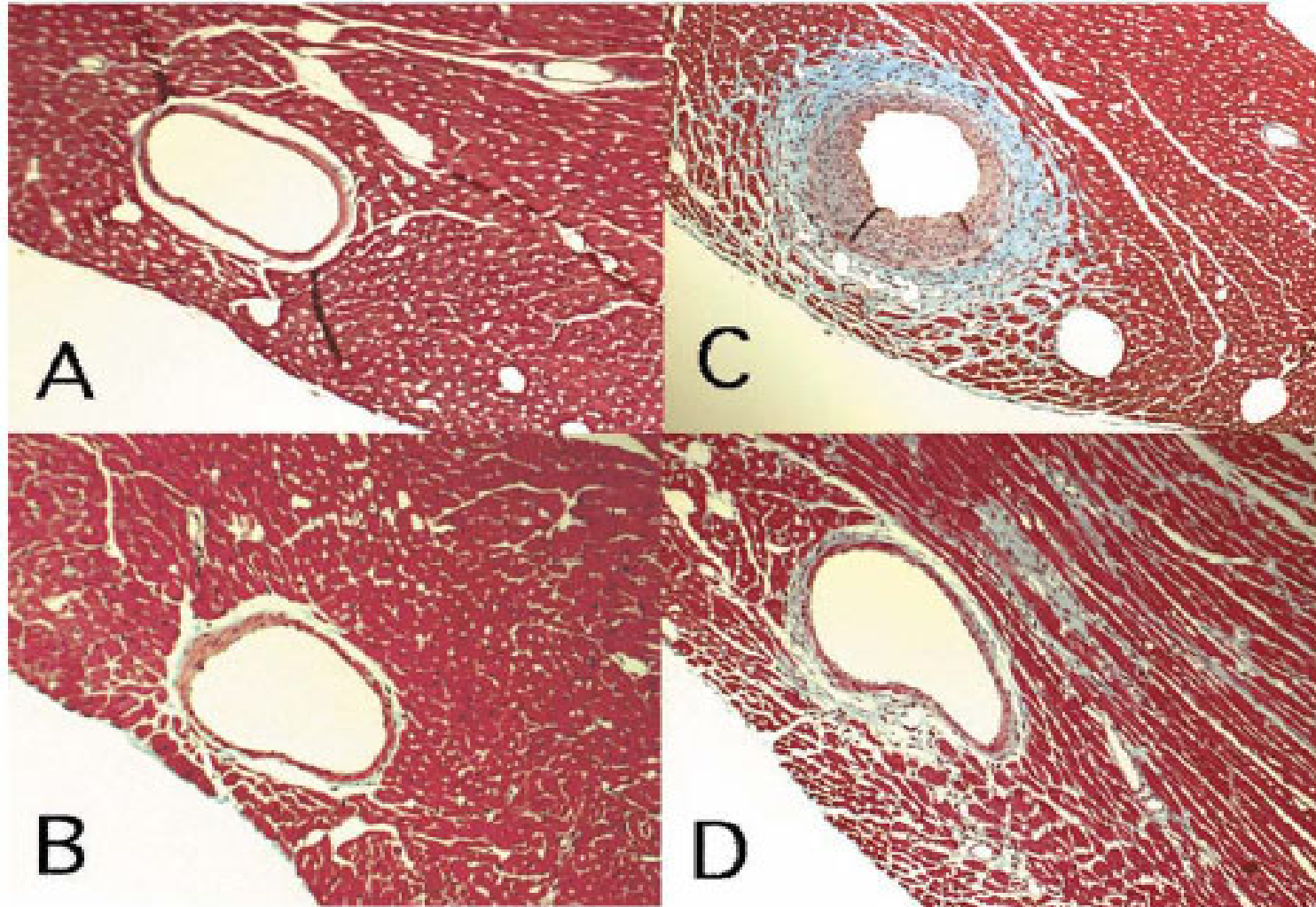
WT MTAB



CIRKO MTAB



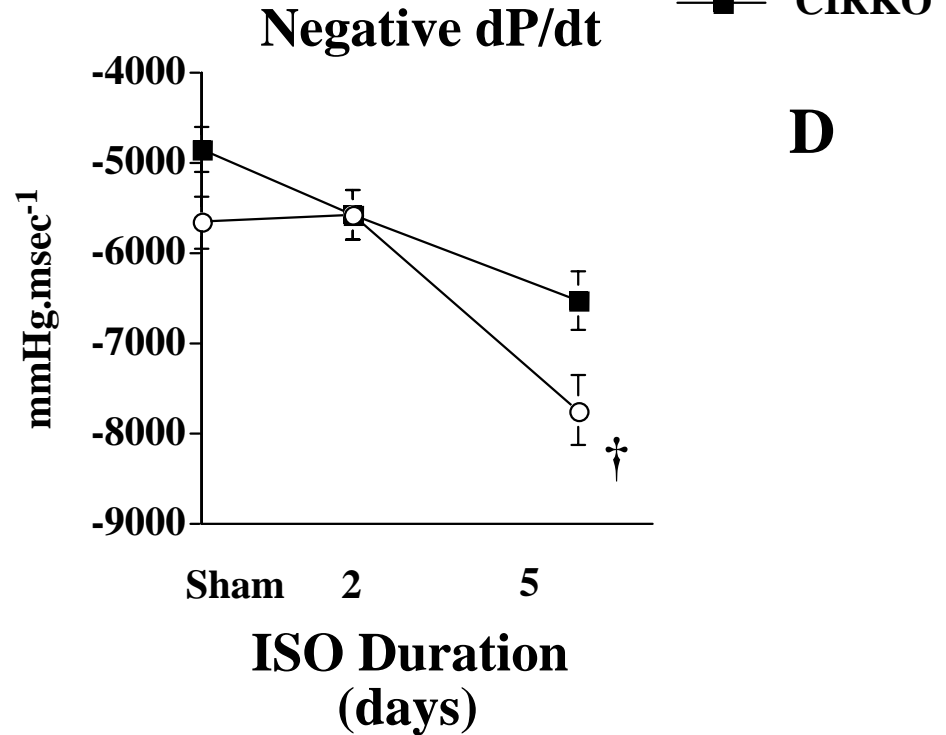
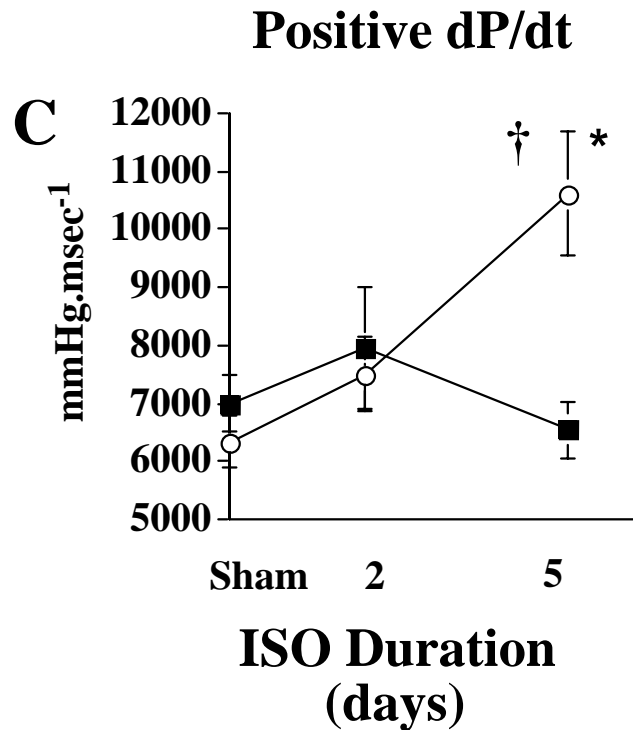
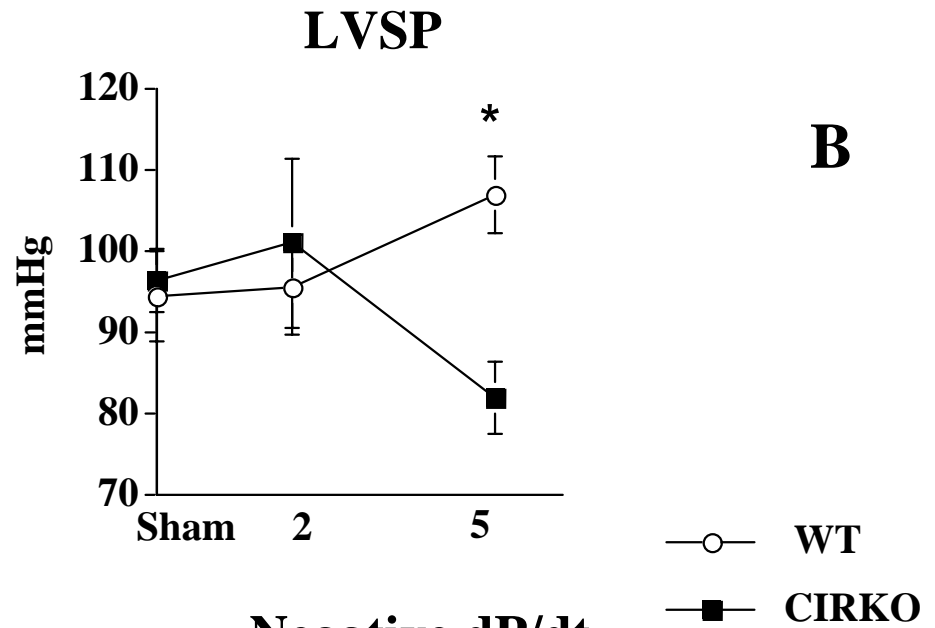
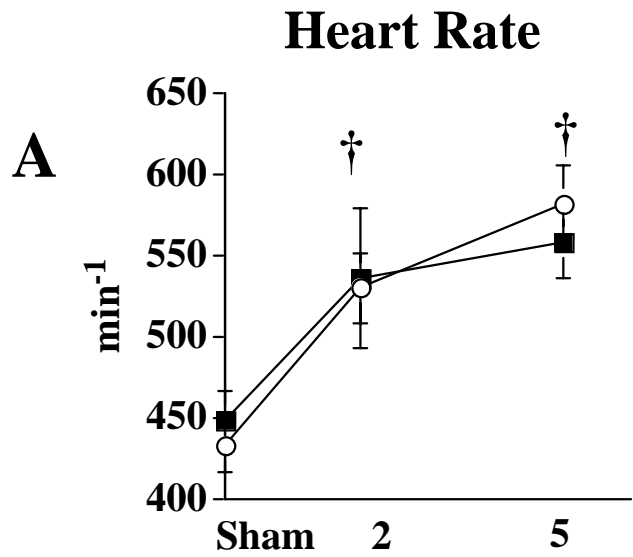
# Banded CIRKO Hearts Develop Increased Fibrosis



**WT**

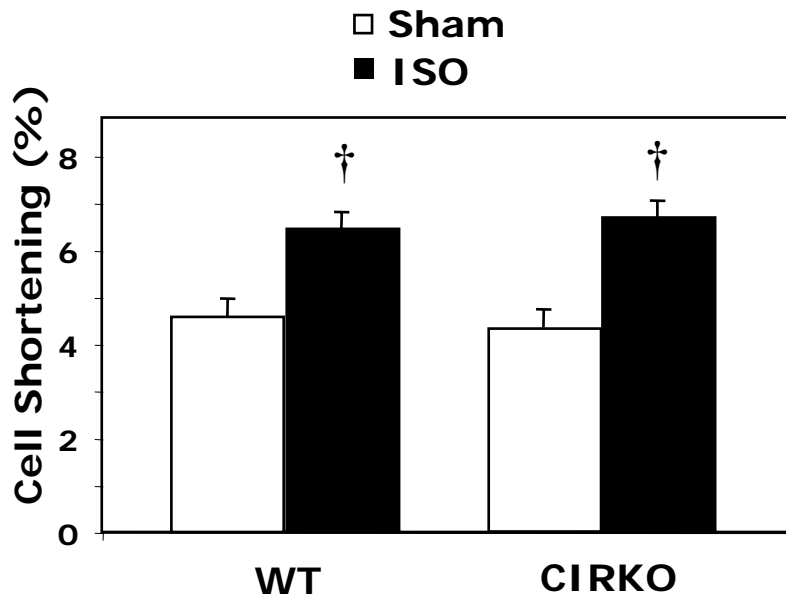
**CIRKO**

**Response of CIRKO  
Hearts to Isoproterenol  
Infusion**

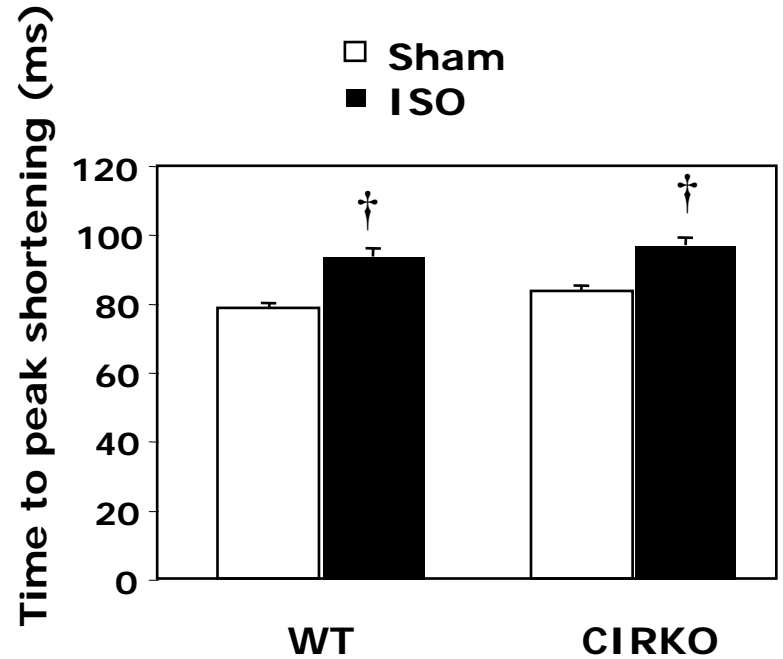


# Intrinsic Contractility of Myocytes Isolated from ISO Treated CIRKO Is Normal

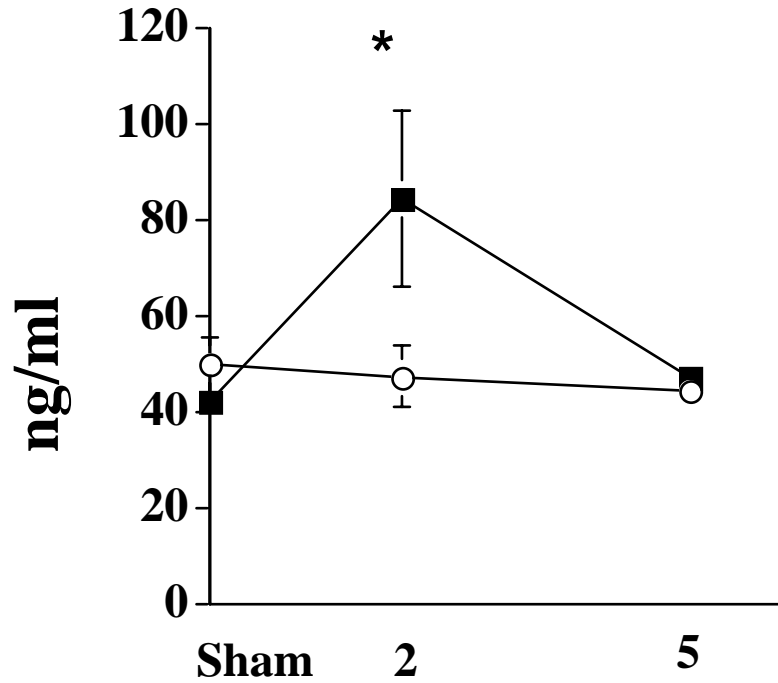
## A



## B



# Serum Troponin



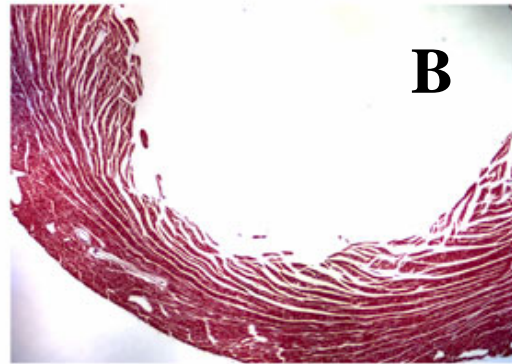
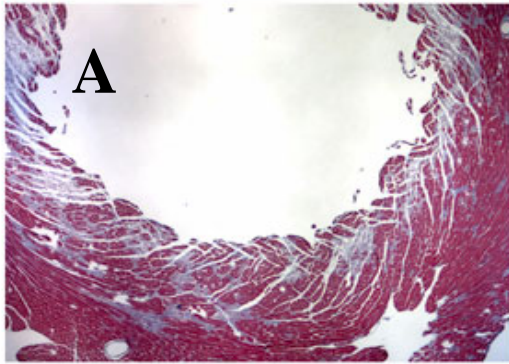
**Duration of Isoproterenol  
(Days)**

—○— WT  
—■— CIRKO

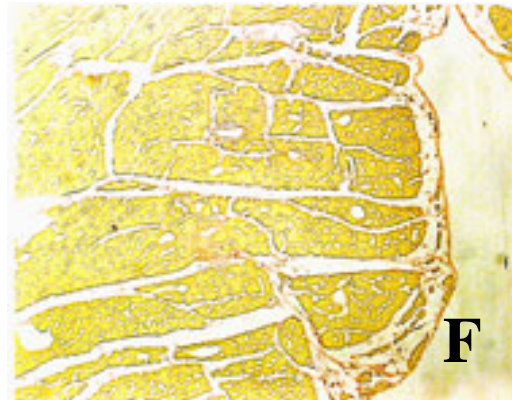
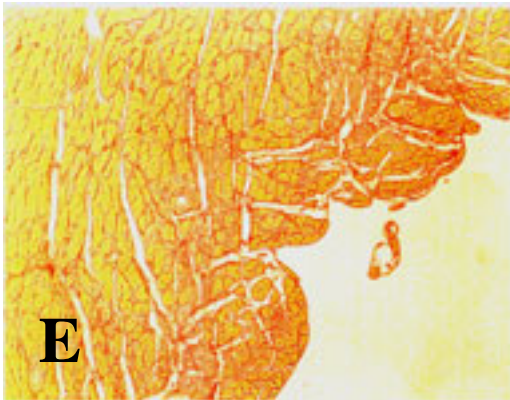
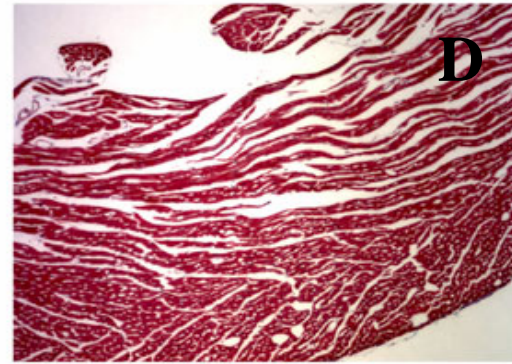
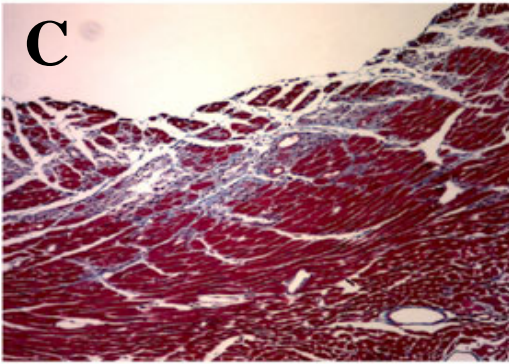
CIRKO ISO

WT ISO

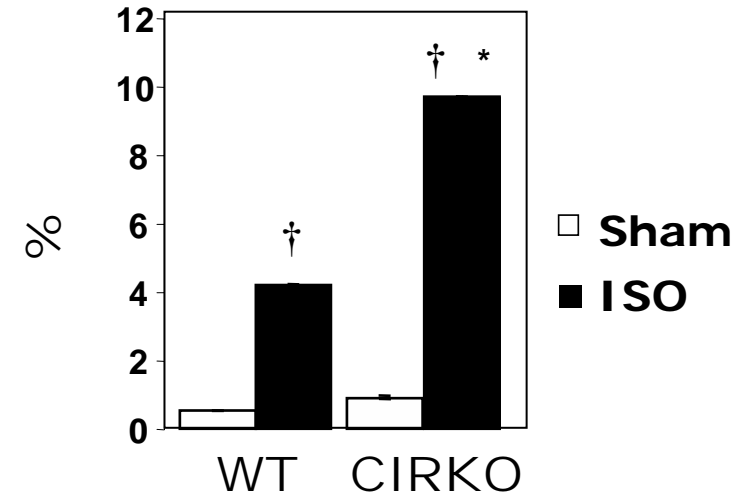
4X



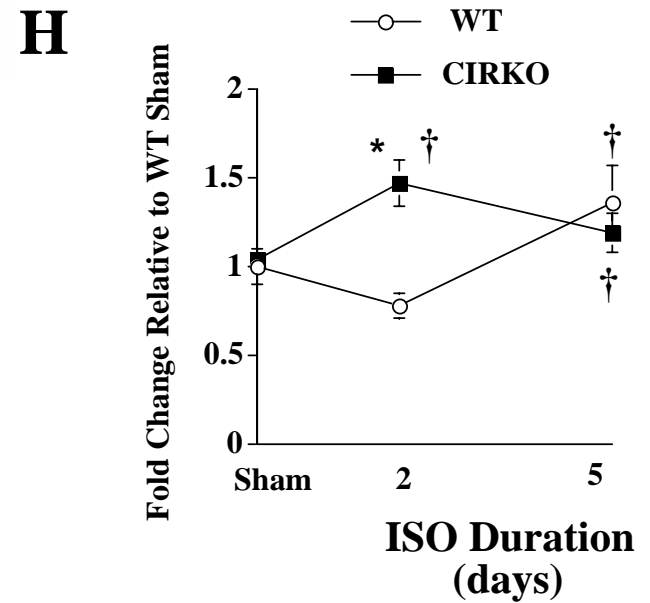
20X



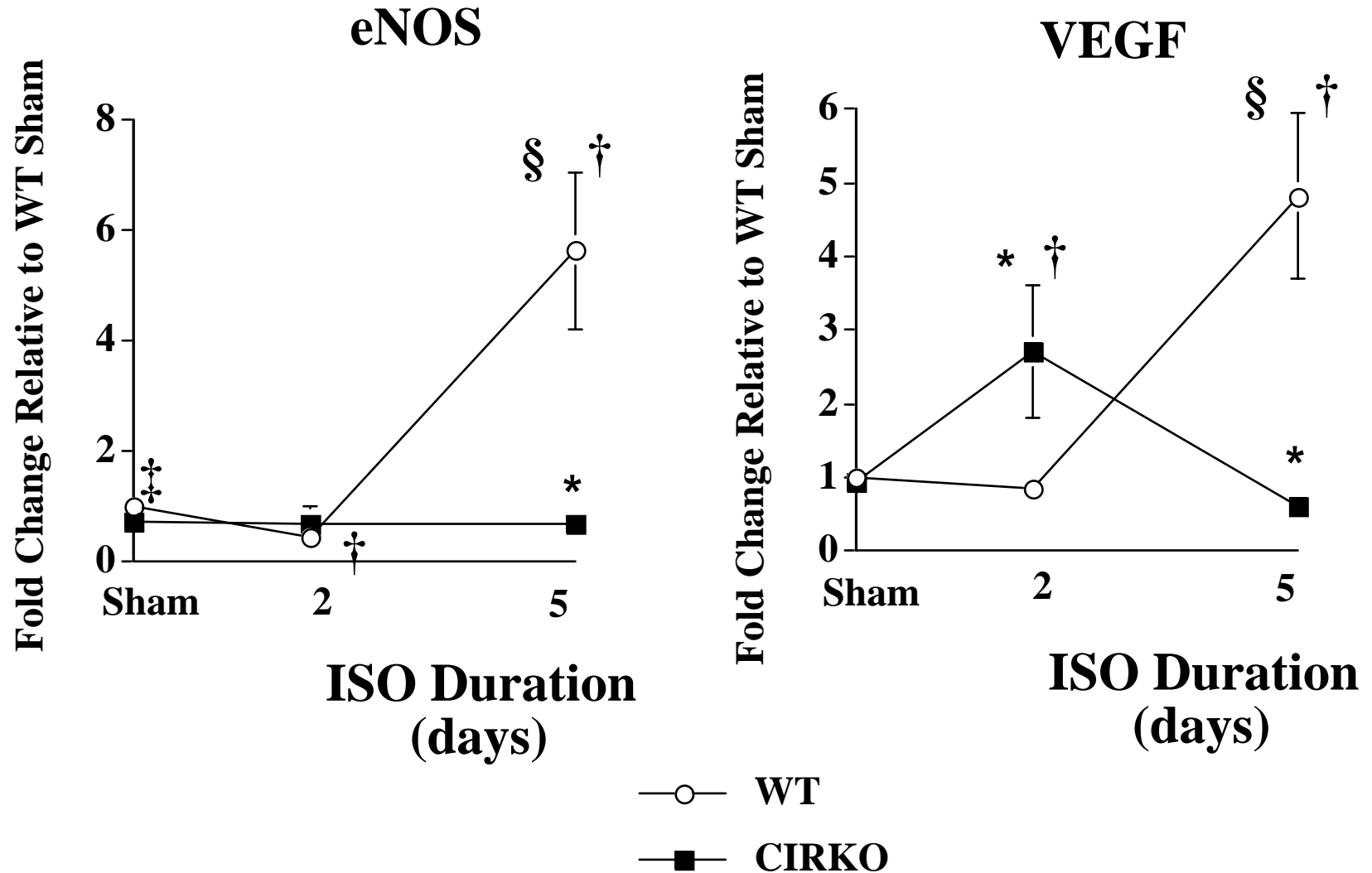
## G Collagen Volume Fraction



## H CTGF mRNA Abundance

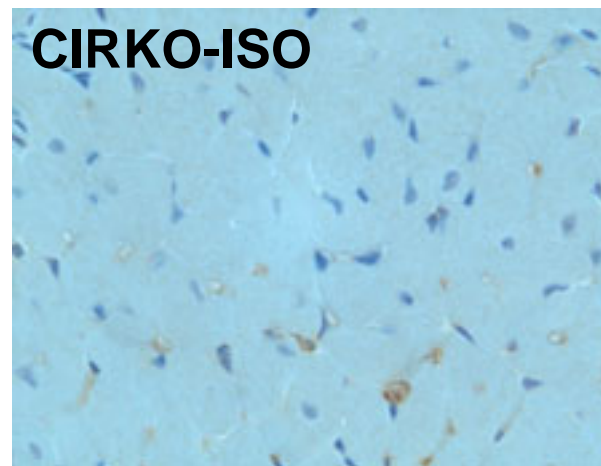
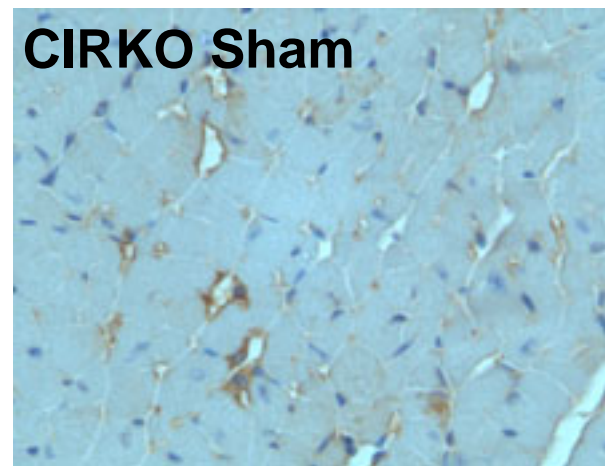
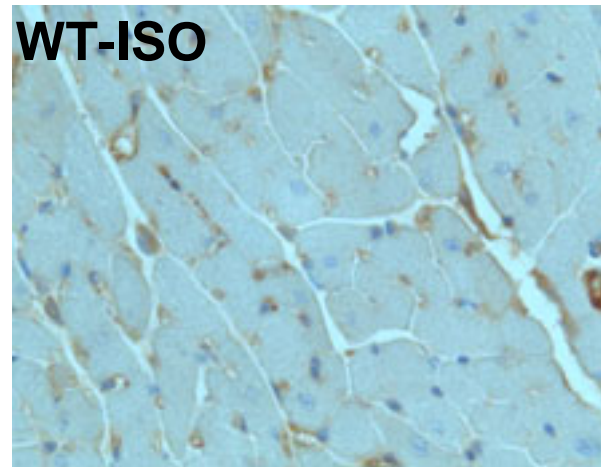
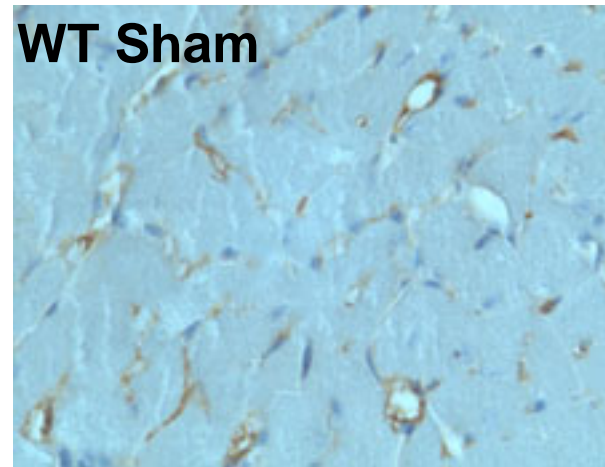


# Impaired Angiogenic Gene Response in ISO Treated CIRKO Hearts

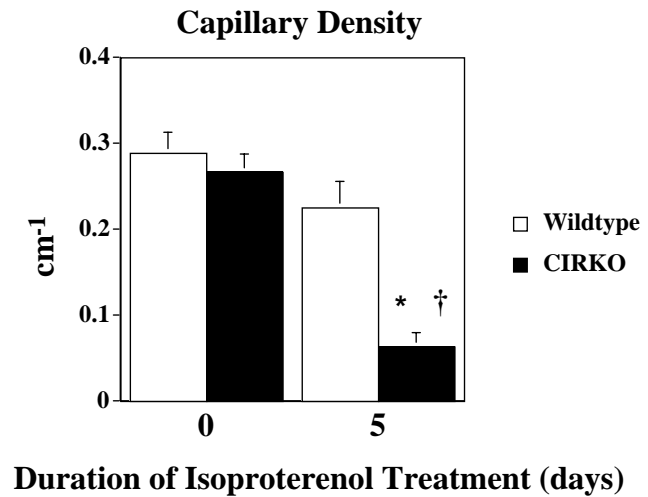




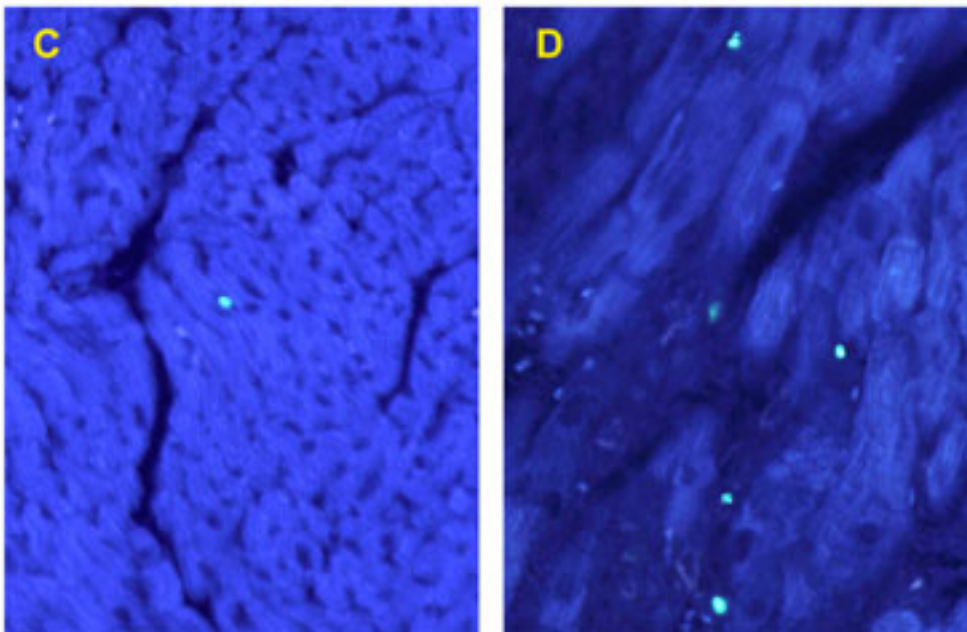
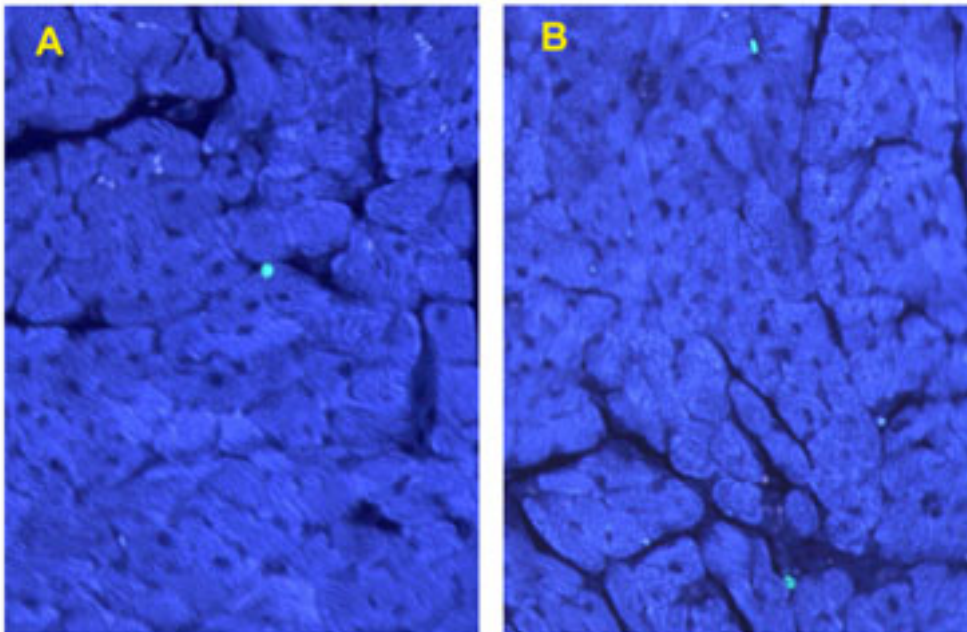
# Reduced Capillary Density in ISO Treated CIRKO Hearts



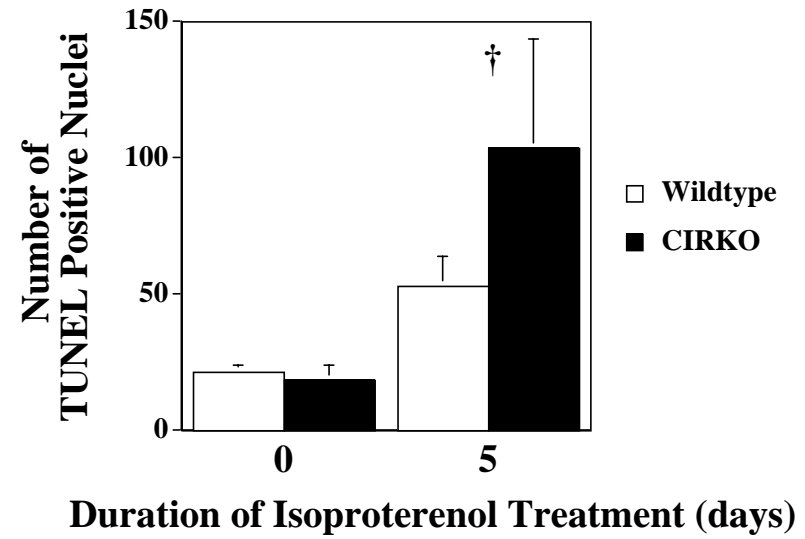
**E**



# TUNEL STAINING OF CIRKO HEARTS

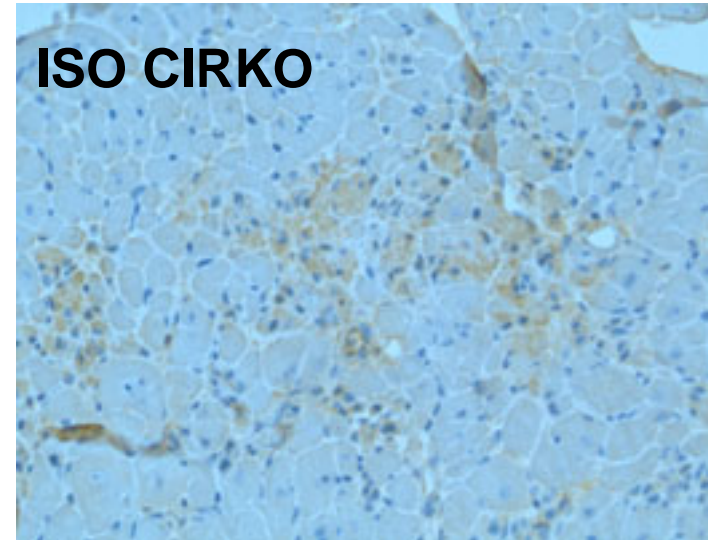
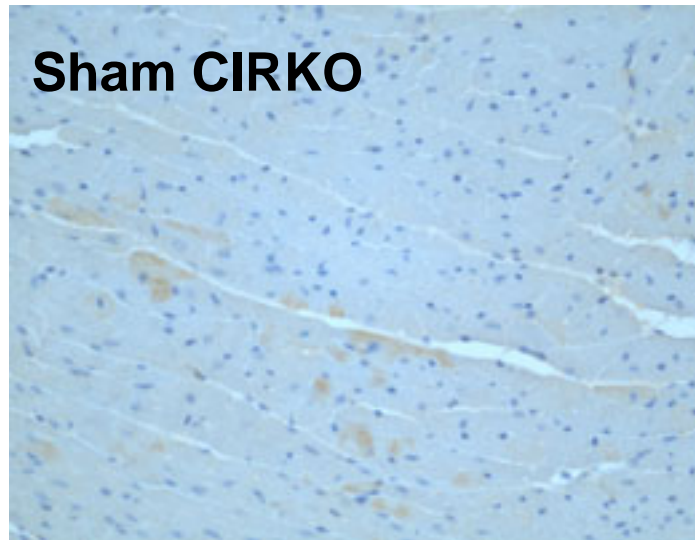
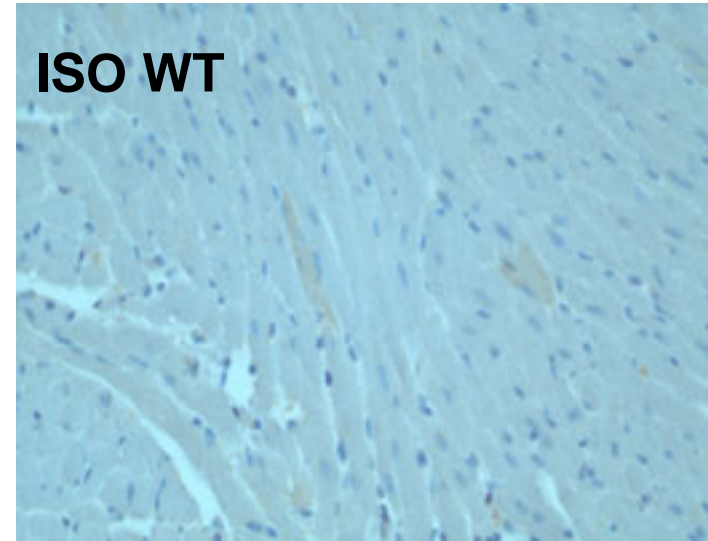
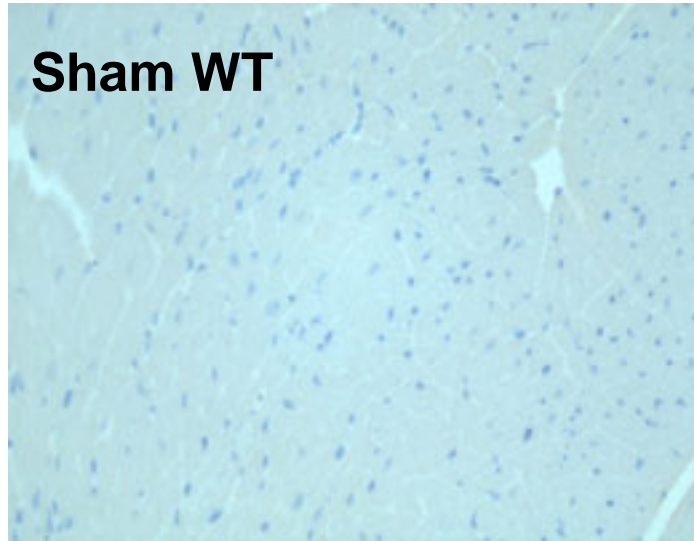


**E**



A - WT Sham  
B- WT ISO  
C- CIRKO Sham  
D- CIRKO ISO

# Immunohistochemistry for Cleaved Caspase 3





# Summary

- **In addition to changes in mitochondrial function - Altered FA utilization and increased mitochondrial ROS production.**
- **CIRKO Hearts are more susceptible to injury in the face of hemodynamic stress because of**
- **Impaired Adaptation of the Coronary Vasculature to Hypertrophic Growth of Cardiomyocytes Leading to Reduced Capillary Density**
- **Increased Ischemic Injury**
- **Increased Susceptibility to Apoptosis**

# Phenotyping to Date



<b>Criteria/Validation</b>	<b>CIRKO+ STZ</b>
Ejection Fraction	In Progress
Replacement/Interstitial Fibrosis	In Progress
Cardiac Hypertrophy	Pending
Invasive Assessment of LV Function	Pending
Isolated Hearts	Decreased LV Function.
Cardiac Metabolism/Mitochondrial Function	Pending
Gene Expression	In Progress
Response to Stress	Pending
Electrophysiology	Pending

# Phenotyping to Date

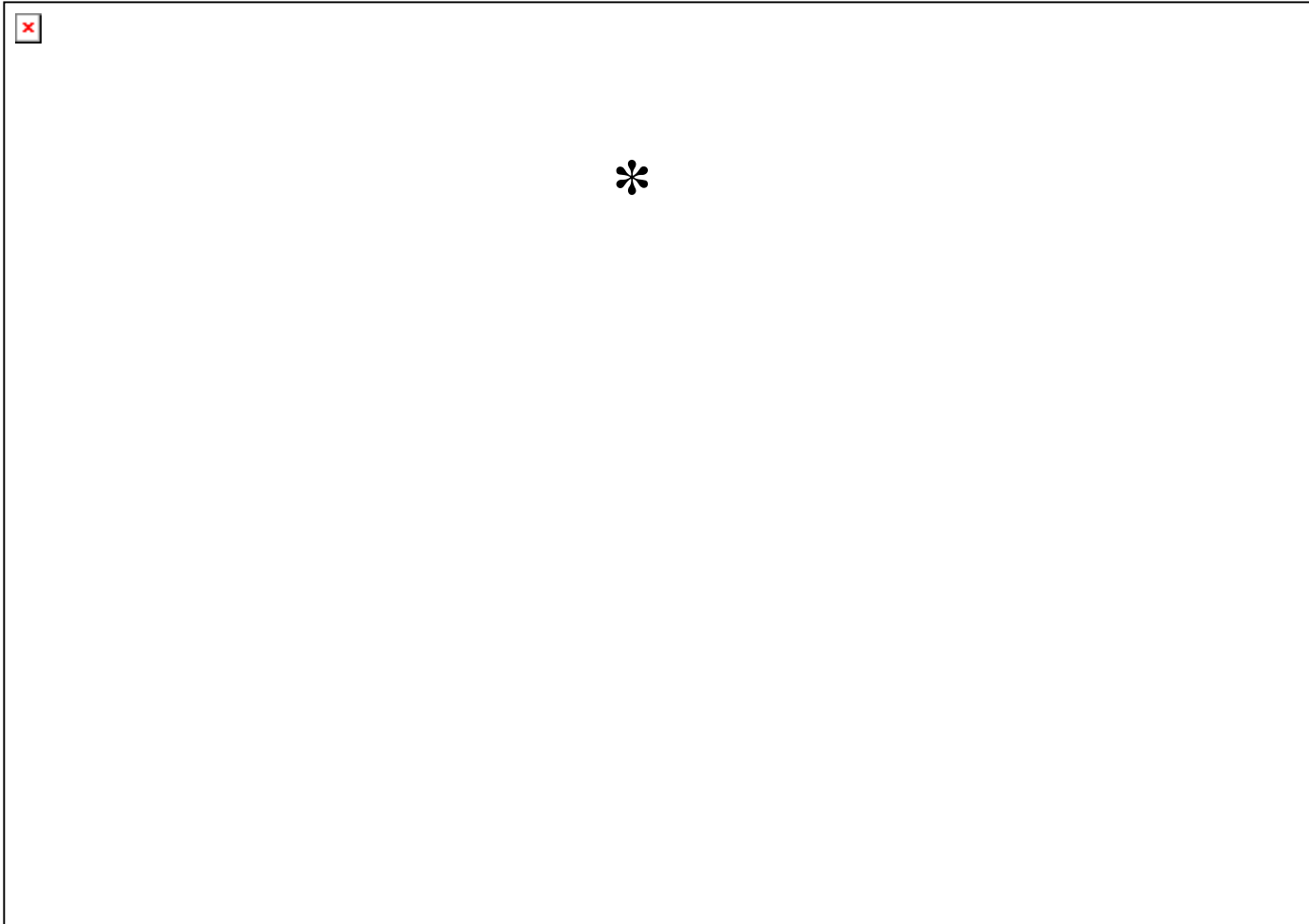
<b>Criteria/Validation</b>	<b>CIRKO+ ACS Mice</b>
Ejection Fraction	Pending
Replacement/Interstitial Fibrosis	Pending
Cardiac Hypertrophy	Pending
Invasive Assessment of LV Function	Pending
Isolated Hearts	Pending
Cardiac Metabolism/Mitochondrial Function	Pending
Gene Expression	Pending
Response to Stress	Pending
Electrophysiology	Pending

# Phenotyping to Date



<b>Criteria/Validation</b>	<b>dNPI3K</b>
Ejection Fraction	Normal, but reduced after Aortic banding
Replacement/Interstitial Fibrosis	Increased after aortic banding
Cardiac Hypertrophy	Not at baseline but increased after aortic banding
Invasive Assessment of LV Function	Pending
Isolated Hearts	Decreased LV Function.
Cardiac Metabolism/Mitochondrial Function	Similar to older CIRKO mice Mitochondrial dysfunction is present.
Gene Expression	MHC isoforms not switched. ANF is increased
Response to Stress	Impaired response to aortic banding
Electrophysiology	NA

# Carnitine-Palmitate Respirations are Reduced in Mice with Reduced PI-3Kinase Activity





# **Summary of Phenotyping Capabilities that are Available to Other Consortium Members**

- **Whole Animal Metabolic Phenotyping: Glucose Clamps, Serum Analysis- Insulin, FFA, Leptin, Adiponectin**
- **Intact Animal Cardiovascular Phenotyping: Invasive LV Catheterization, Mouse Echocardiography, Aortic Banding and Coronary Artery Ligation Surgery, EKG telemetry.**
- **Isolated Hearts: Isovolumic and Working Heart Preparations for Determination of Cardiac Function, Substrate Metabolism and Oxygen Consumption**
- **Mitochondrial Phenotyping: Bioenergetics, Respiration, ATP, Mitochondrial Enzyme Assays**
- **Histological Assessment: Light Microscopy, Immunofluorescence, Electron Microscopy**

# **Current Collaborations with Other Consortium Members**

- **Generation of podocyte restricted GLUT4 KO mice (Utah +Michigan)**
- **Metabolic Phenotyping of mouse model of lipotoxic cardiomyopathy -Heart-restricted overexpression or KO of LPL. (Utah + Columbia)**
- **Metabolic Phenotyping and pressure overload hypertrophy studies of mice with heart-restricted KO of PPAR-gamma. (Utah + UCLA)**
- **Histological and Ultrastructural analysis of Diabetic Pig Hearts (Utah + UNC)**