University of Utah -AMDCC Steering Committee - March 2006

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THE UNIVERSITY OF UTAH

Diabetic Cardiomyopathy - Clinical Problem in Humans

- **In the absence of overt CAD, functional defects e.g. diastolic dysfunction or systolic dysfunction.**
- **Impaired myocardial reserve following exercise or inotropic stimuli**
- **Congestive heart failure without CAD(small clinical series)**
- **Mechanistic Studies - Decreased PCr/ATP ratios (T2DM), increased FAO and MVO_2 in obese females.**

Diabetic Cardiomyopathy - Increased Vulnerability to Injury

- **Increased risk of heart failure following coronary ischemia**
- **Increased likelihood of heart failure following LVH**
- **Many studies of tissue samples reveal interstitial fibrosis consistent with myocyte loss**
- **Increased local activation of the intra-cardiac Renin-Angiotensin System in late stage contributing to myocyte/endothelial cell apoptosis necrosis and replacement fibrosis.**

Diabetic Cardiomyopathy - Animal Models State of the Art 2000

- **Abnormal Substrate Metabolism Increased FAO, Decreased GLU-Ox, mainly STZ Diabetes Models**
- **Decreased Sarcoplasmic Reticulum Calcium Handling**
- **Increased pro-fibrotic signaling via PKCβ2**

Cardiomyopathy

Minimal Criteria for Mouse Models of Diabetic Cardiomyopathy

In the context of insulin resistance and hyperglycemia:

- Decreased ejection fraction and/or 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 and 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

University of Utah Strategy

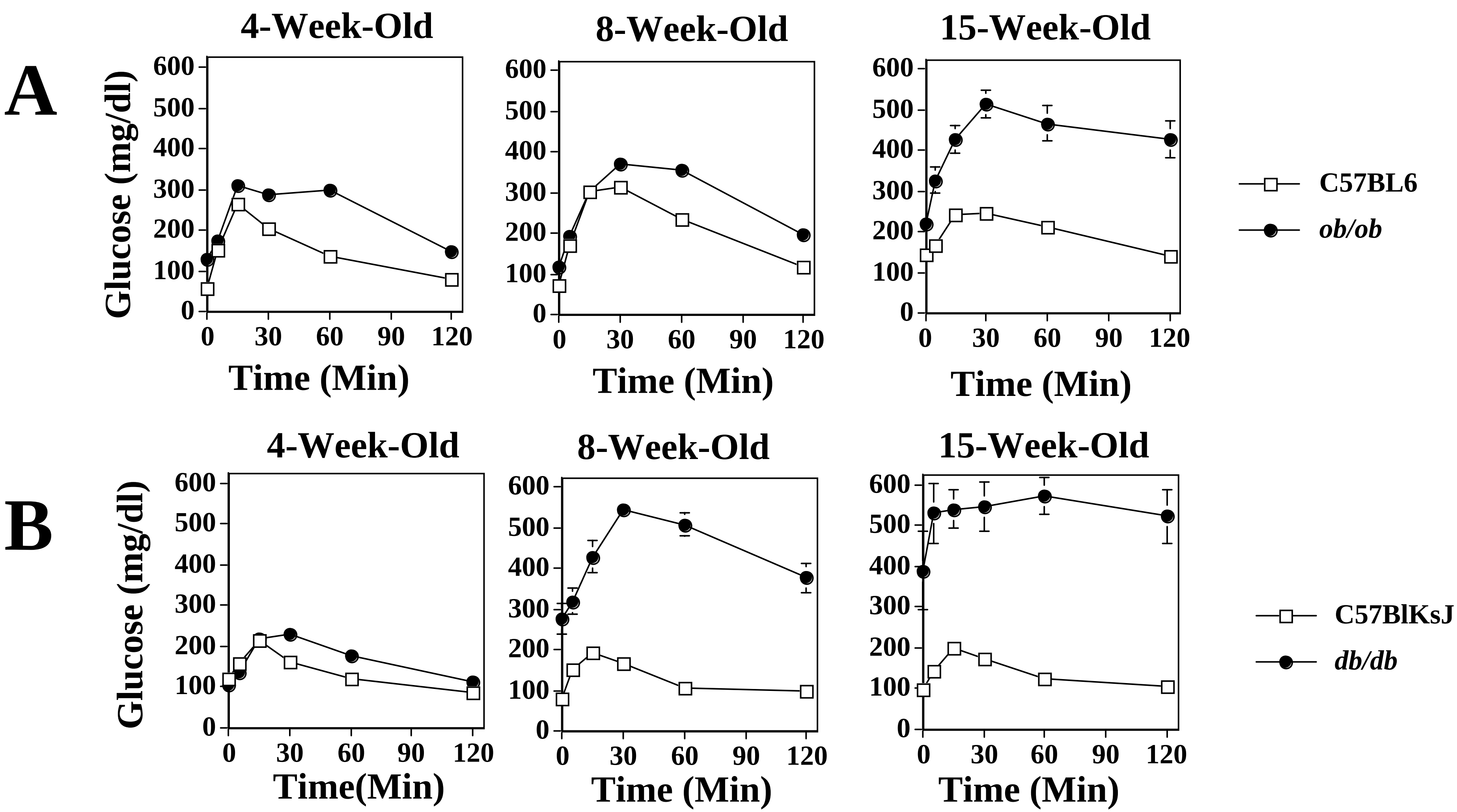
- **Model the consequences of impaired insulin action**
- **To discern in animal models the mechanisms responsible for abnormal cardiac metabolism by focusing primarily on early changes in hearts of mouse models of type 2 diabetes.**
- **To model the combined effect of diminished insulin action and increased FA delivery to the heart**

CIRKO Mice

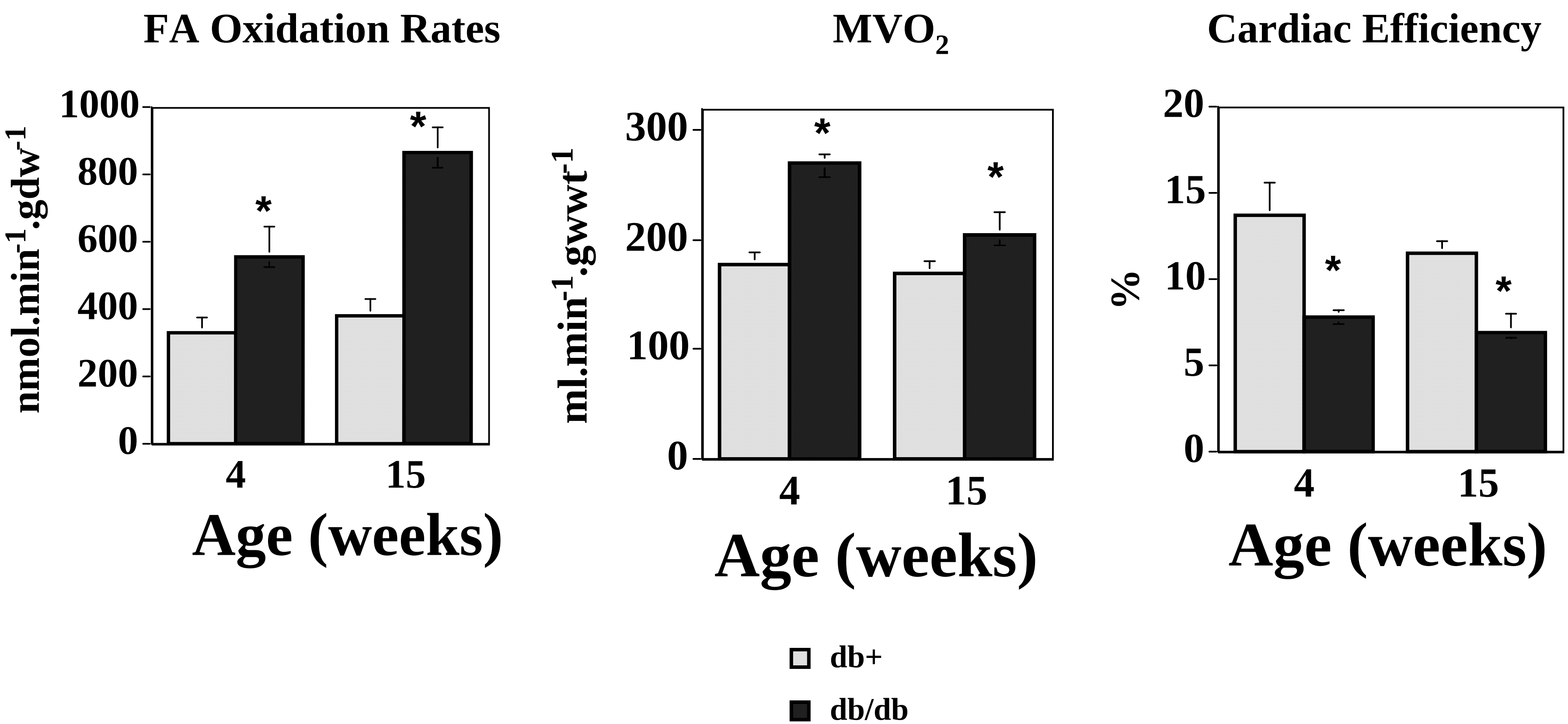
Criteria/Validation	Results
Ejection Fraction	Age related decline
Interstitial/replacement Fibrosis	There is a 2-fold ($p<0.05$) increase in interstitial fibrosis - picrosirius red. Magnified after LVH (banding or isoproterenol)
Cardiac Hypertrophy	Absent in young mice. Age related increase in LV mass relative to controls suggesting abnormal LV remodeling
Invasive Assessment of LV function	Modest increase in LVEDP (12±4 mmHg versus 6±3 mmHg)
Isolated Hearts	Age related decline in cardiac contractility. Impaired inotropic response
Altered Gene Expression	Myosin isoform switching
Cardiac Metabolism and Mitochondrial Dysfunction	Decreased rates of glucose oxidation at all ages, increased rates of fatty acid oxidation in young mice < 8 weeks. As they age, they develop progressive mitochondrial dysfunction, which leads to a reduction in fatty acid oxidative capacity. CIRKO hearts develop oxidative stress.
Altered response to stress	CIRKO mice develop severe LV dysfunction following pressure overload hypertrophy induced by transverse aortic constriction. This is associated with a more exuberant fibrotic response than similarly treated control hearts Similar observations have been observed in response to isoproterenol treatment. Following myocardial ischemia, CIRKO hearts develop abnormal LV remodeling and impaired LV function.

Early Mechanisms in Monogenic Models of Obesity, Insulin Resistance and Diabetes

Glucose Tolerance Tests ob/ob and db/db mice

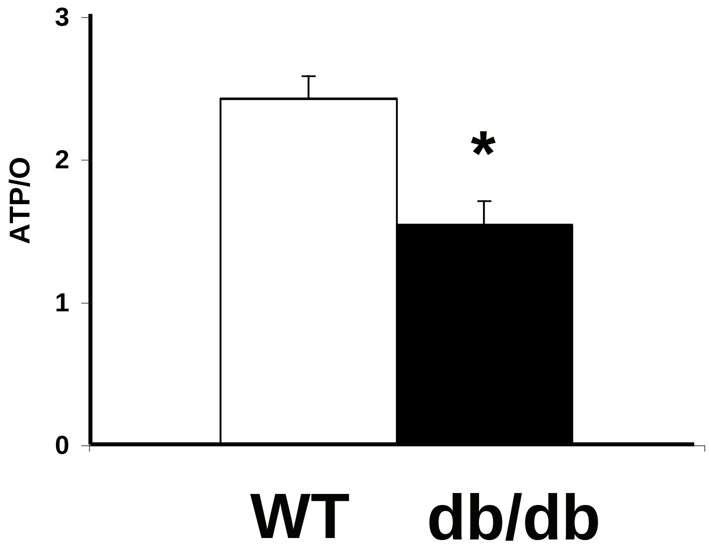


Abnormal Substrate Metabolism and Increased MVO_2 Precede the Onset of Hyperglycemia in db/db mice

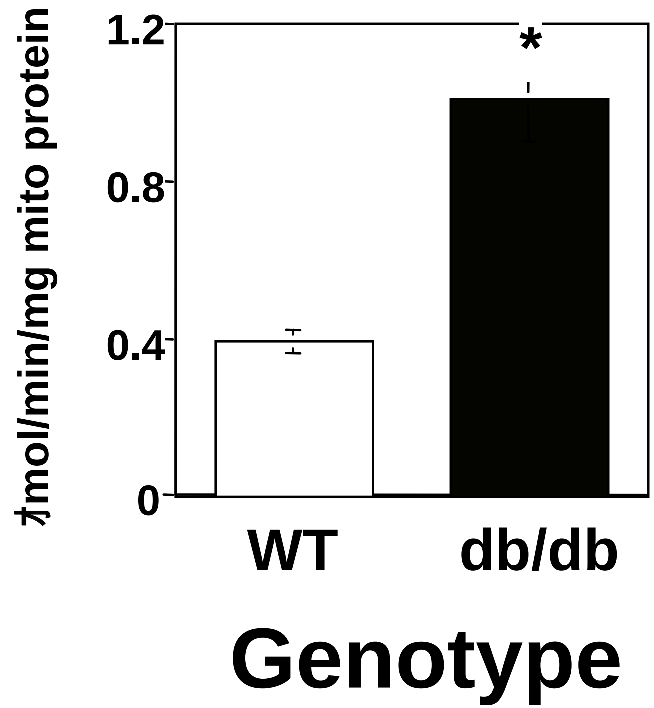


Mitochondria from obese and diabetic mice are uncoupled and generate excess oxidants

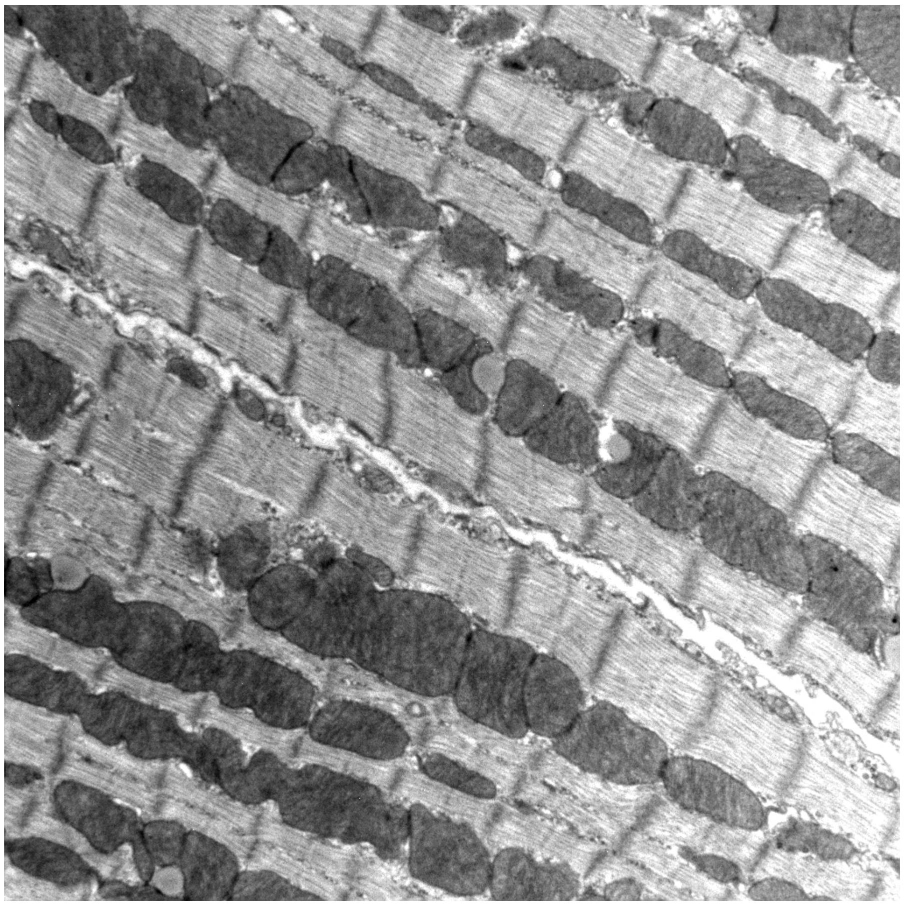
Mitochondrial ATP Generation



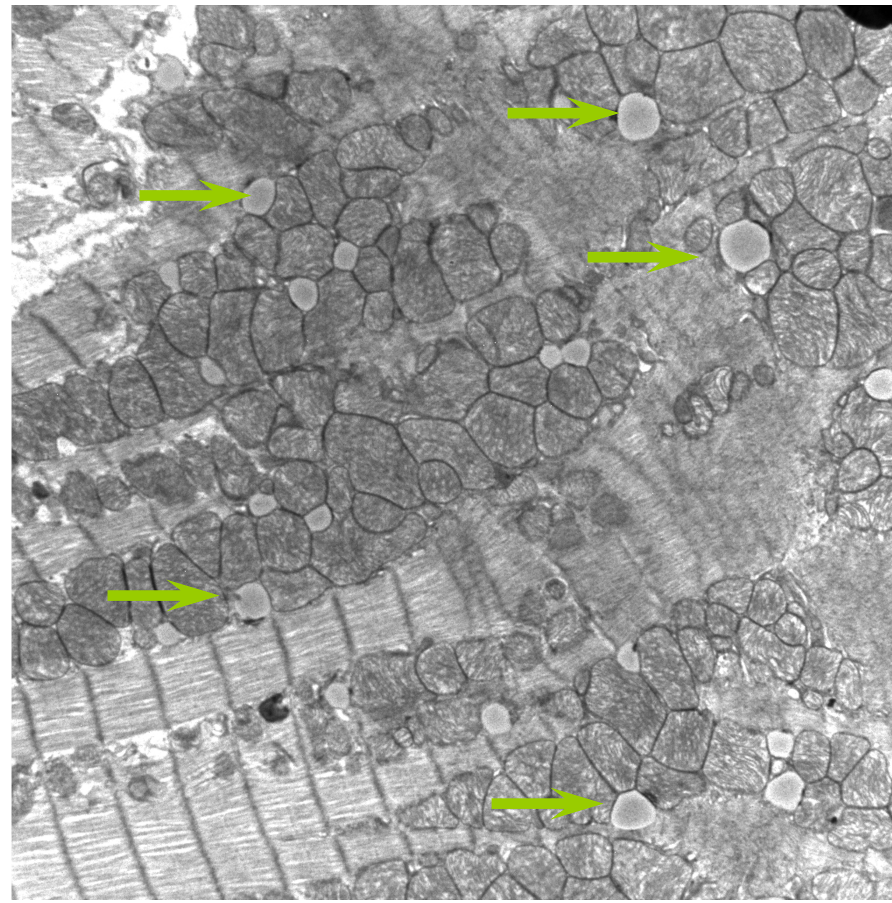
Mitochondrial H_2O_2 Generation



Abnormal Mitochondrial Number and Morphology Accompany Mitochondrial Dysfunction in db/db Mouse Hearts



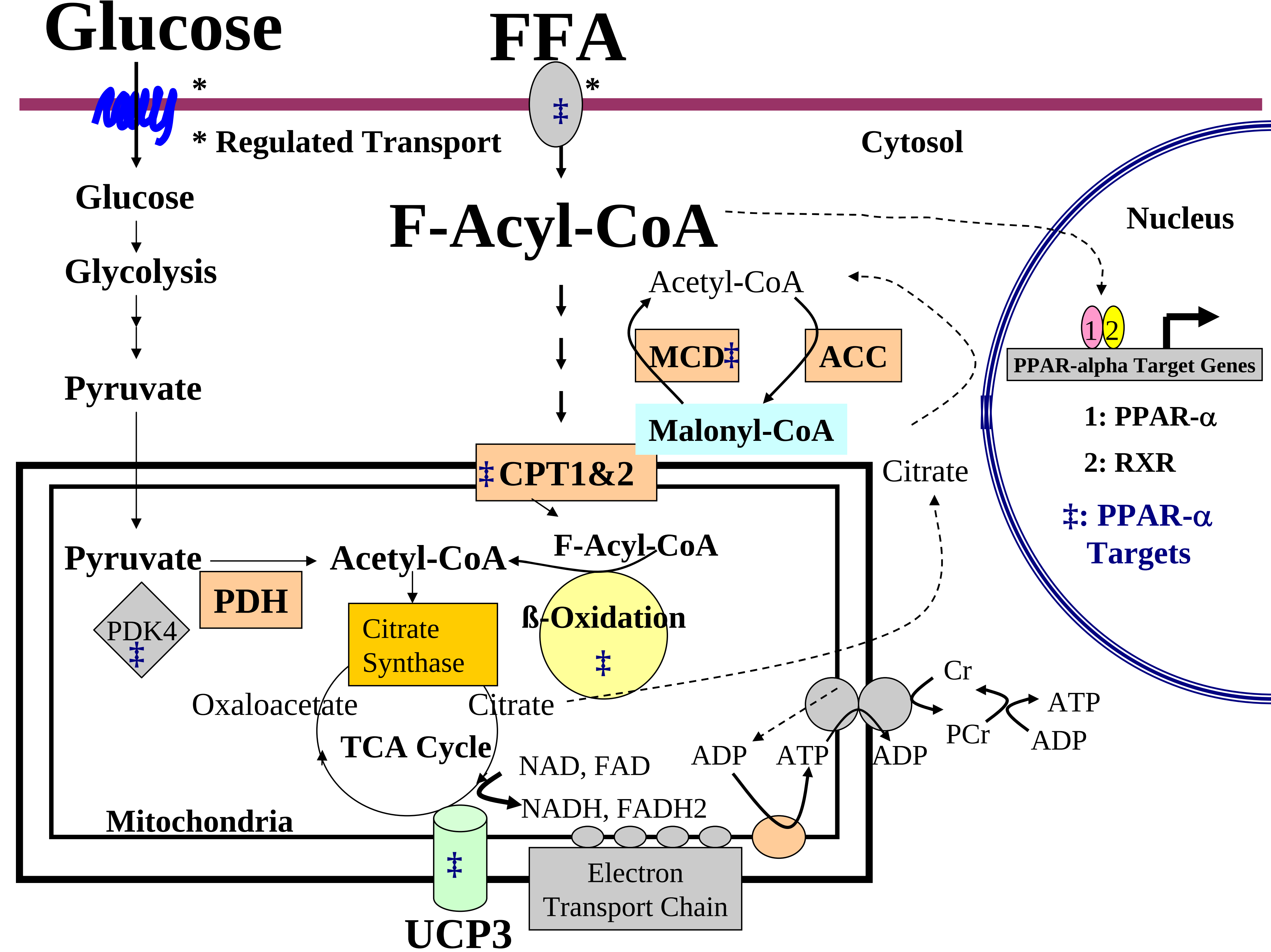
Wildtype



Db/db

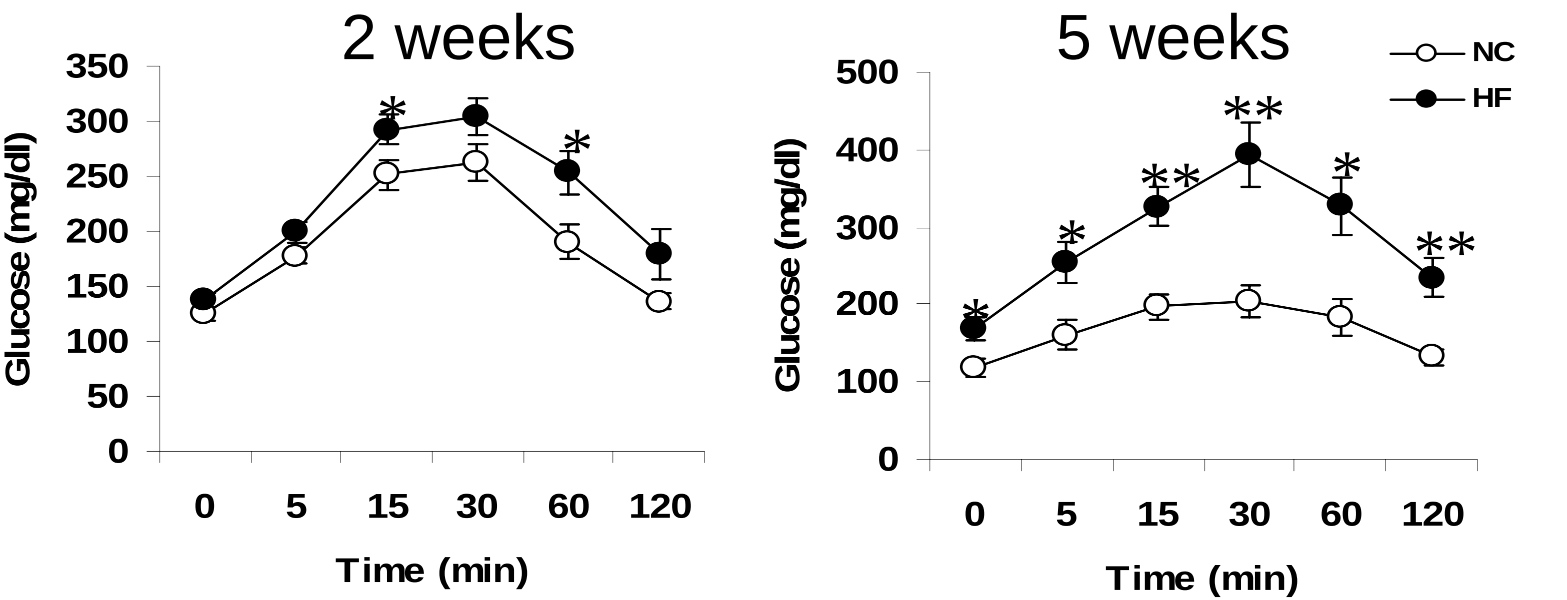
Transcriptional Mechanisms

- **Increase in FA metabolism and Mitochondrial Uncoupling Precede Transcriptional Changes such as increased expression of PPAR-α and PGC-1α co-activated genes.**
- **These pathways are activated later on and probably act to sustain the elevated FA utilization**

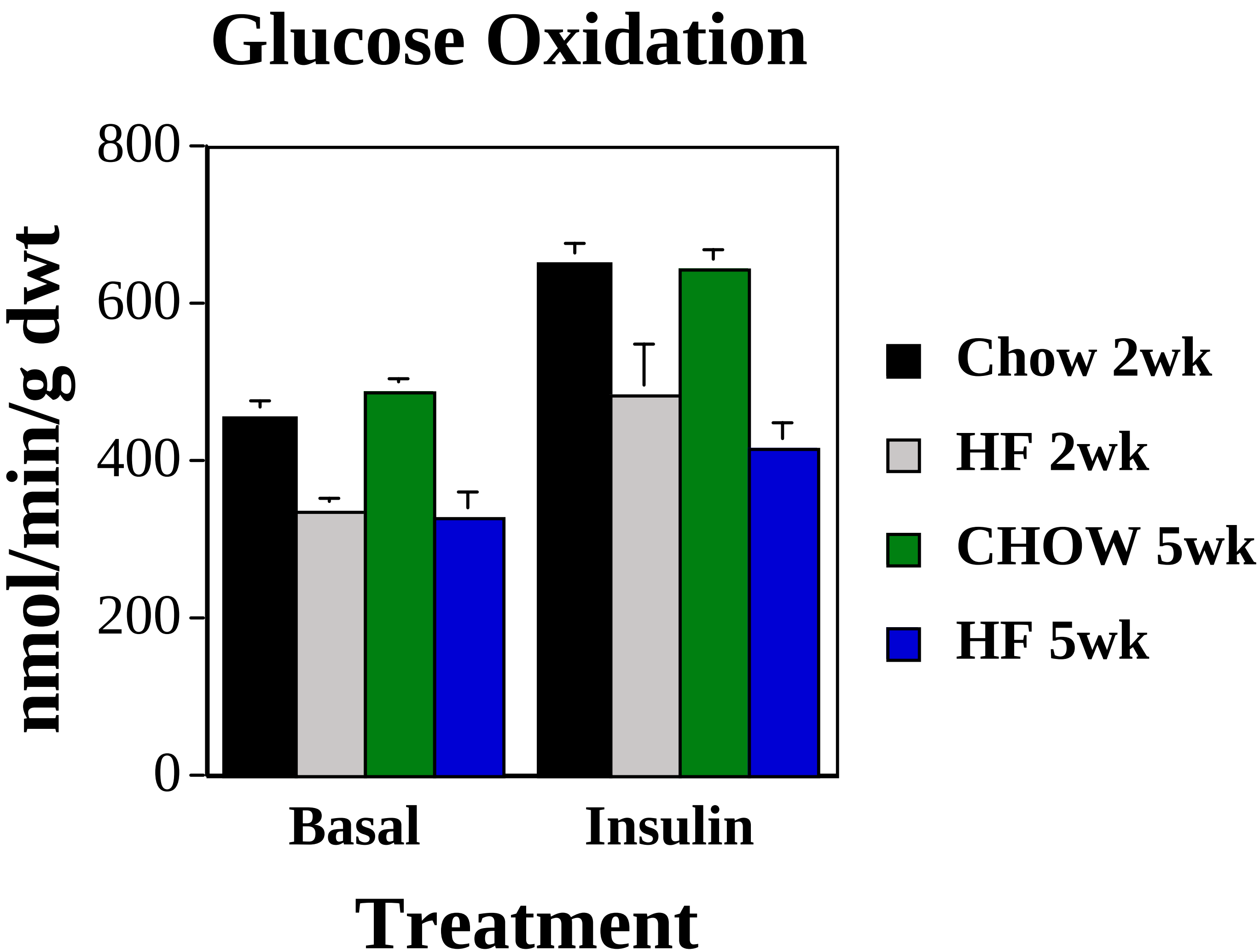

Glucose
FFA
*
*
* Regulated Transport
Cytosol
Glucose
Glycolysis
Pyruvate
F-Acyl-CoA
Acetyl-CoA
MCD‡
ACC
Malonyl-CoA
Nucleus
1
2
PPAR-alpha Target Genes
1: PPAR-α
2: RXR
‡: PPAR-α Targets
Citrate
‡CPT1&2
F-Acyl-CoA
Pyruvate
Acetyl-CoA
PDH
PDK4
Citrate Synthase
ß-Oxidation
Oxaloacetate
Citrate
TCA Cycle
NAD, FAD
NADH, FADH2
ADP
ATP
ADP
Cr
PCr
ATP
ADP
Mitochondria
UCP3
Electron Transport Chain

Early Mechanisms in Diet Induced Obesity and Insulin Resistance

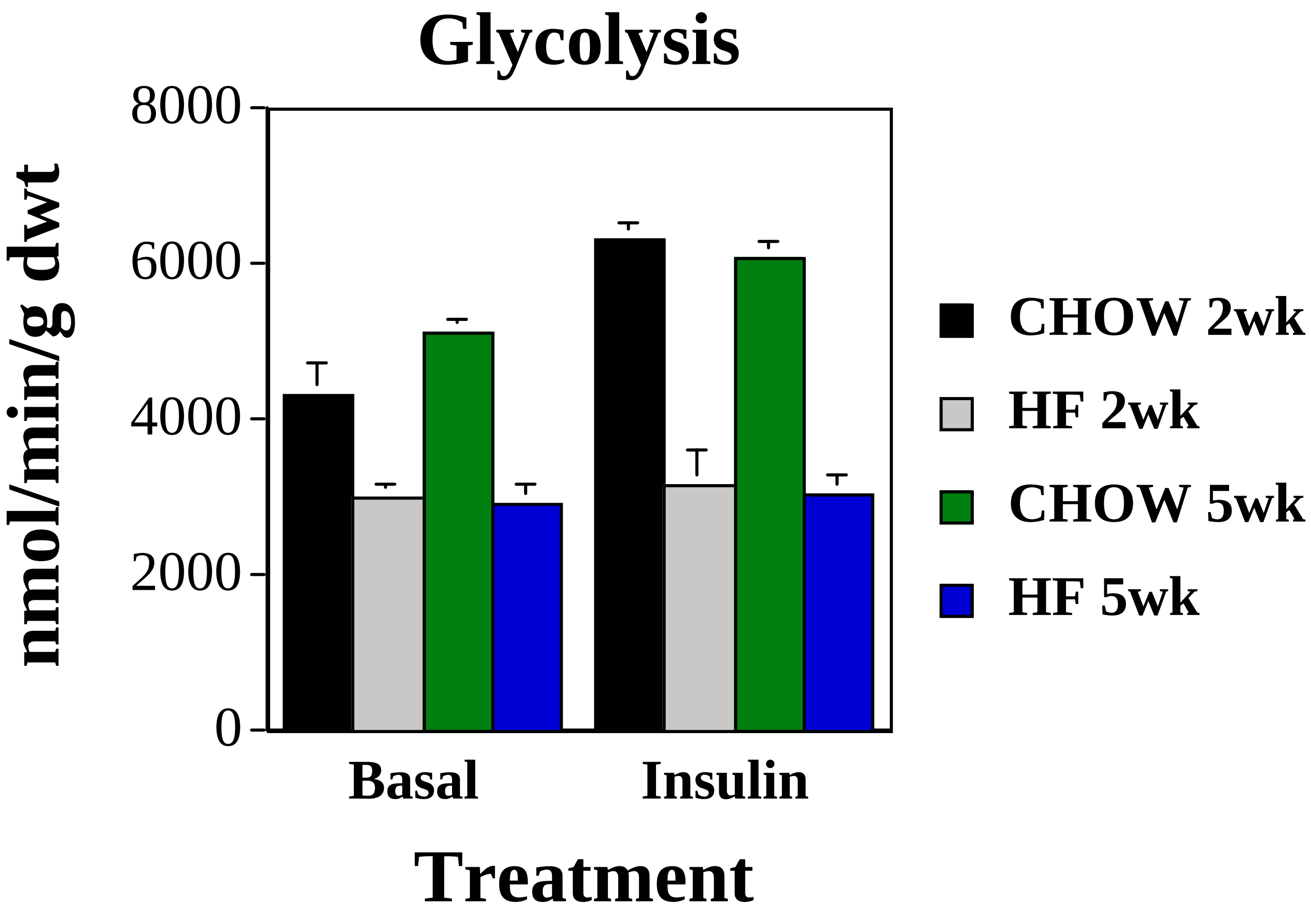
Glucose Tolerance Tests After High Fat Feeding



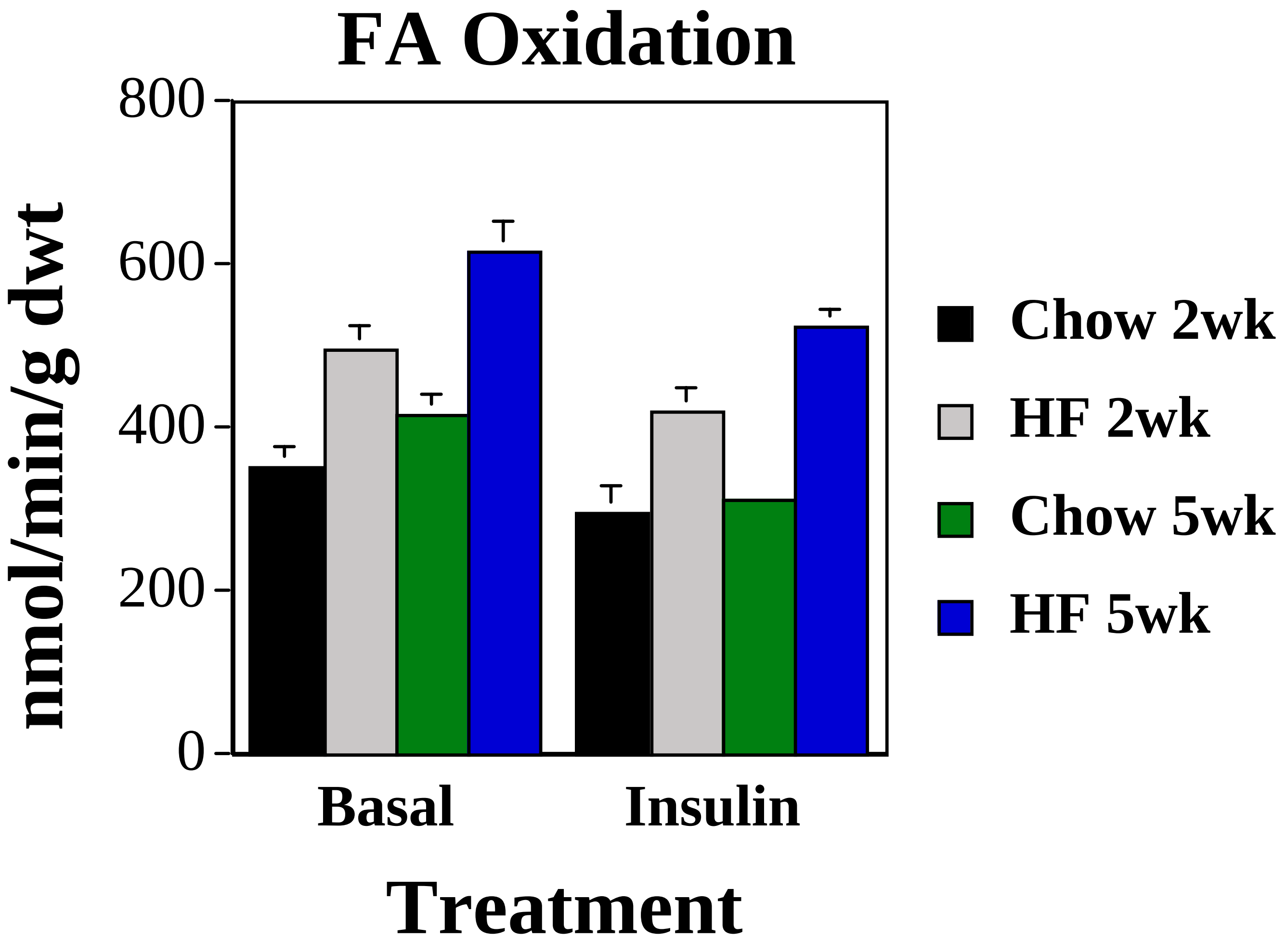
Substrate Metabolism Following High-Fat Feeding



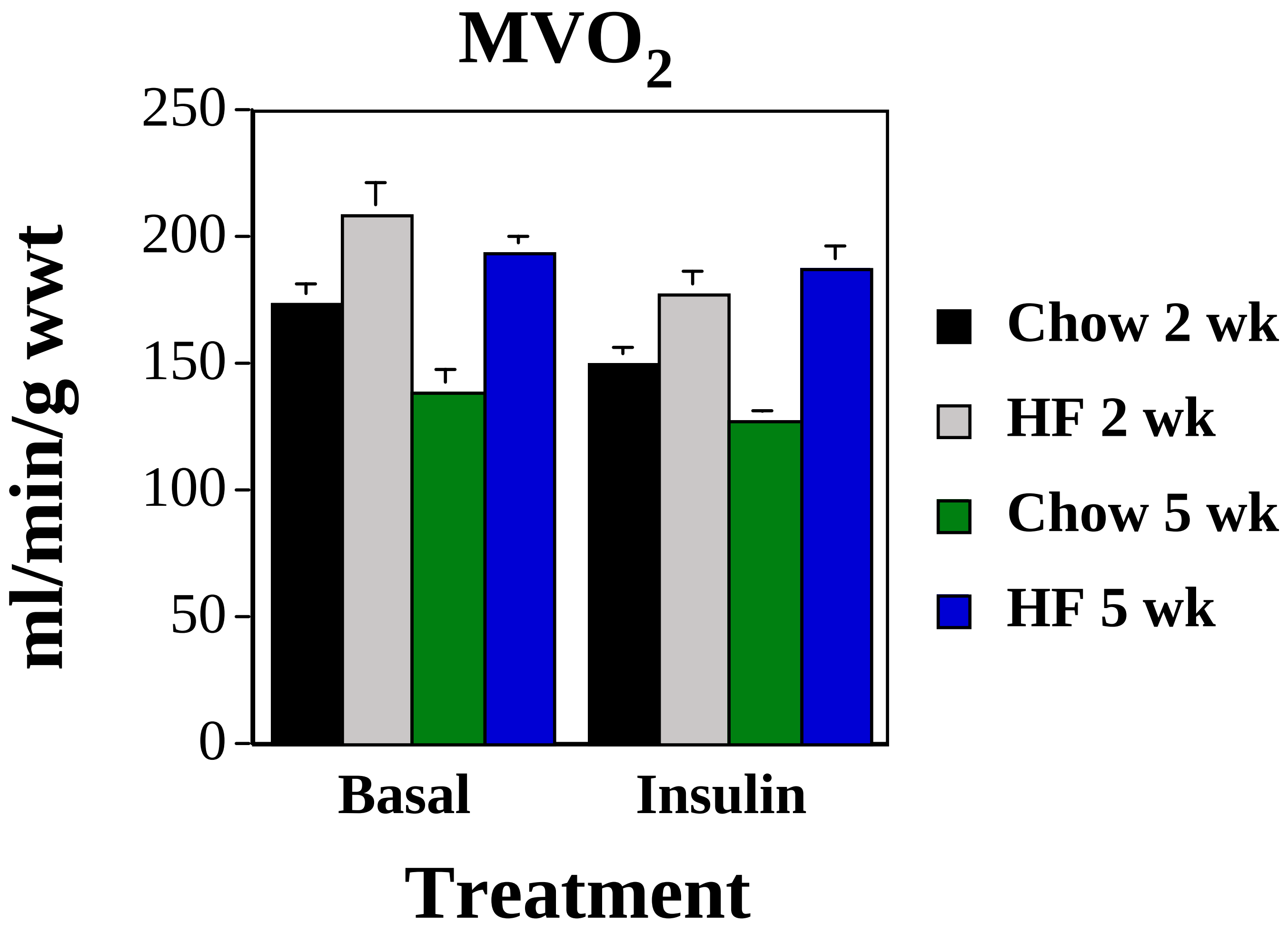
Substrate Metabolism Following High-Fat Feeding



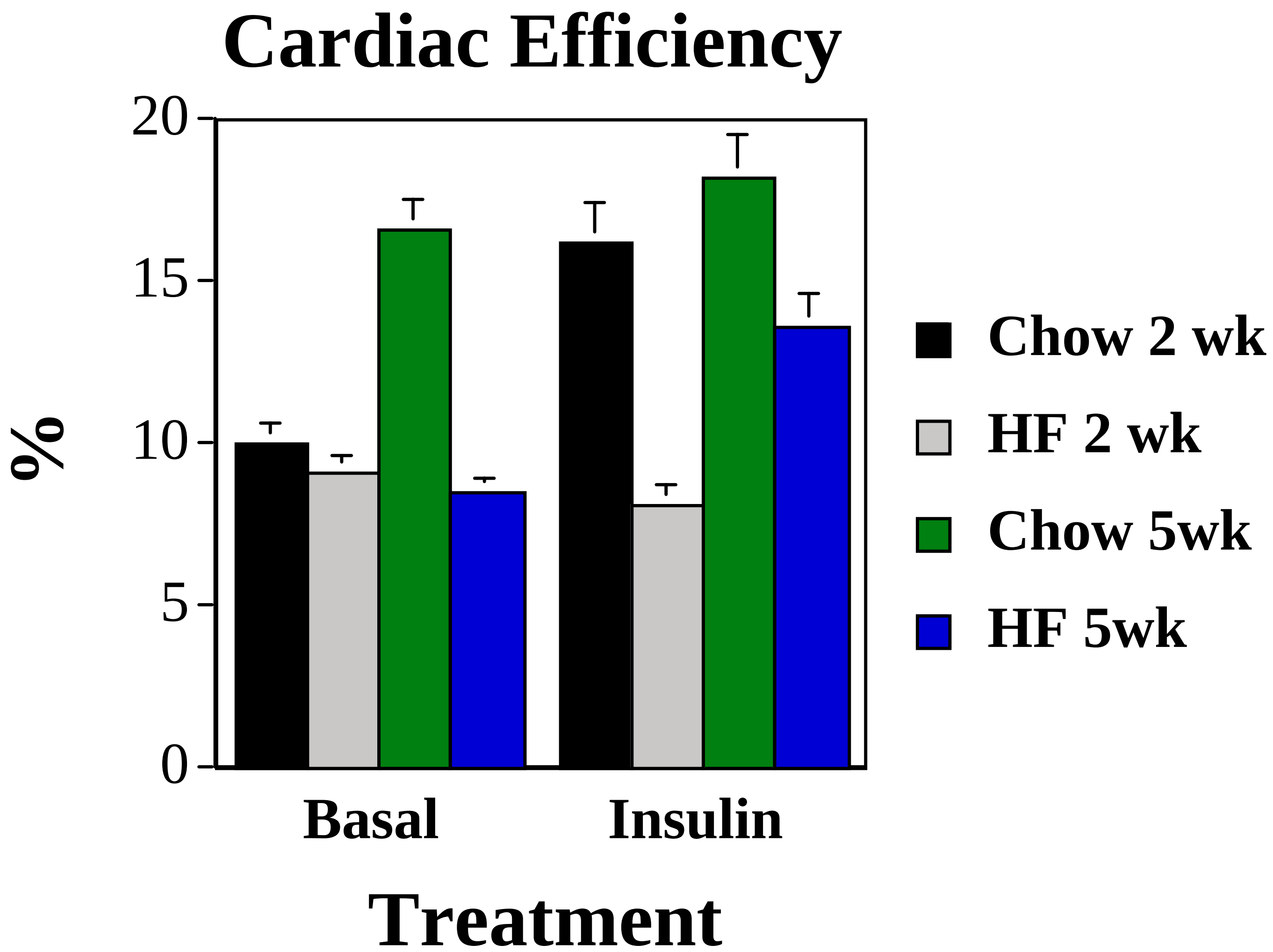
Substrate Metabolism Following High-Fat Feeding



Myocardial Oxygen Consumption Following High-Fat Feeding



Cardiac Efficiency Following High-Fat Feeding

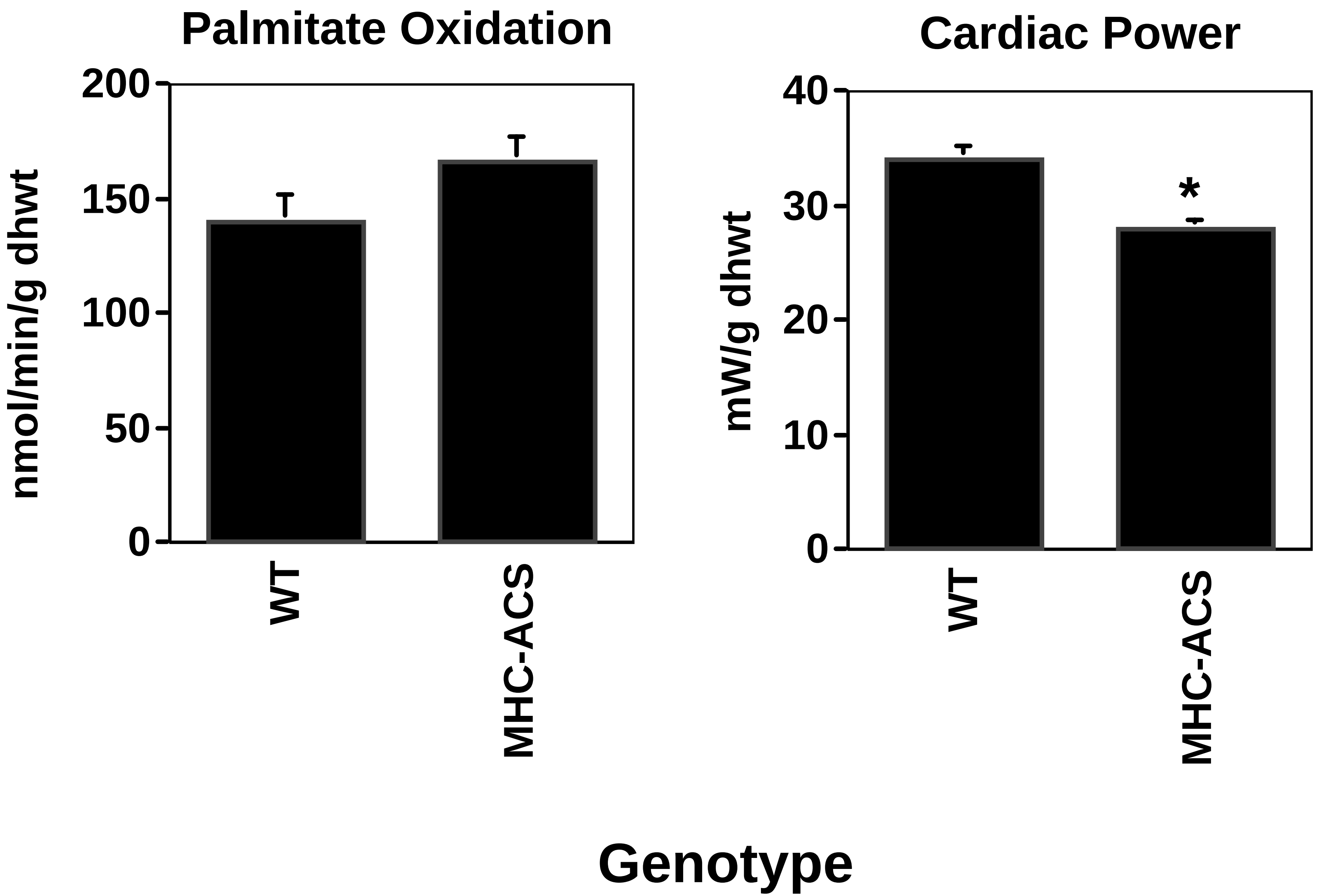


High-Fat Feeding Recapitulates Many of the Early Changes that Characterizes the Diabetic Heart

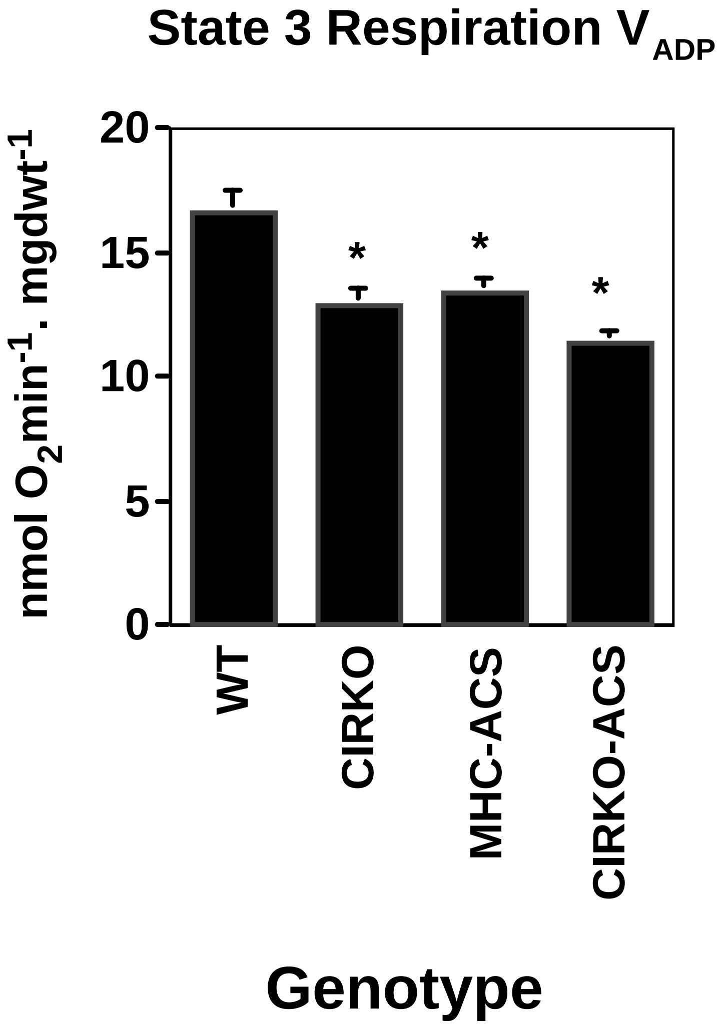
Modeling The Consequences of Increased Myocardial Lipid Delivery and Impaired Insulin Action

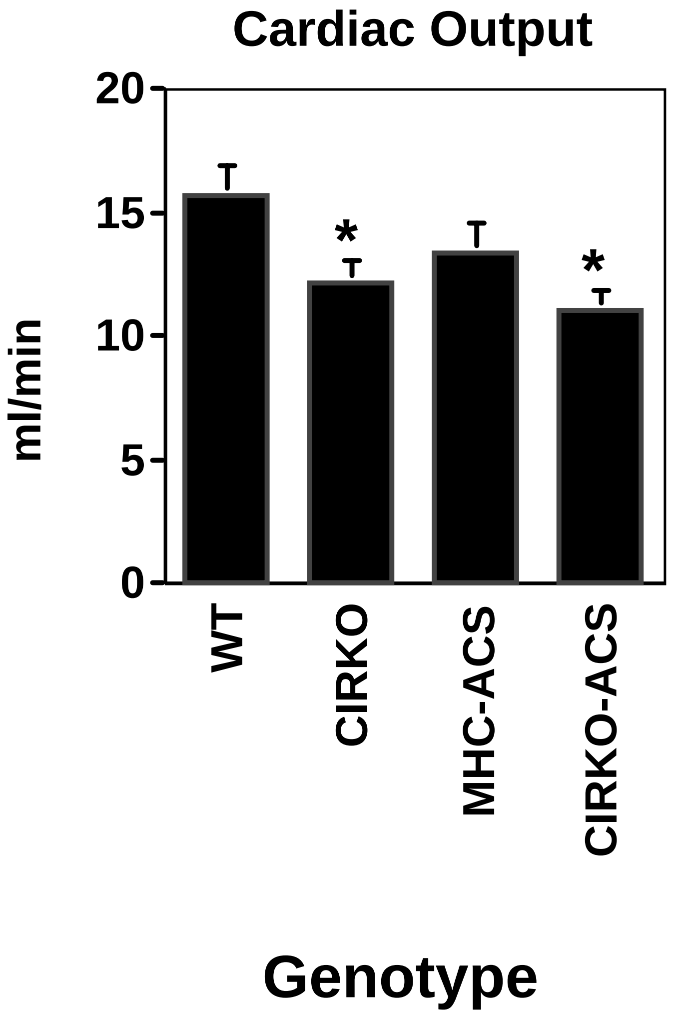
MHC -ACS X CIRKO
CIRKO-ACS

MHC -ACS, 24-week-old Mice

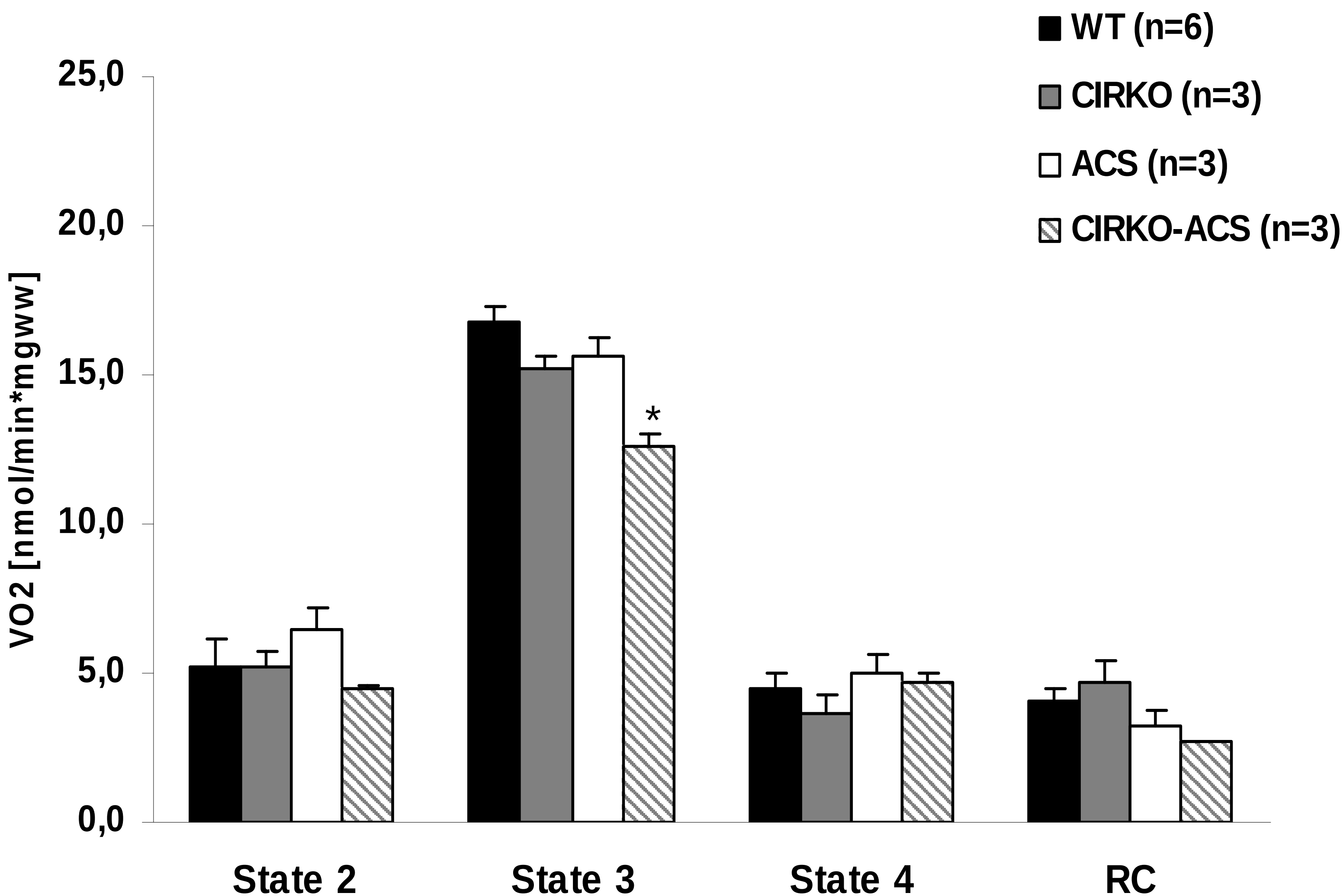


24-week-old Mouse Data

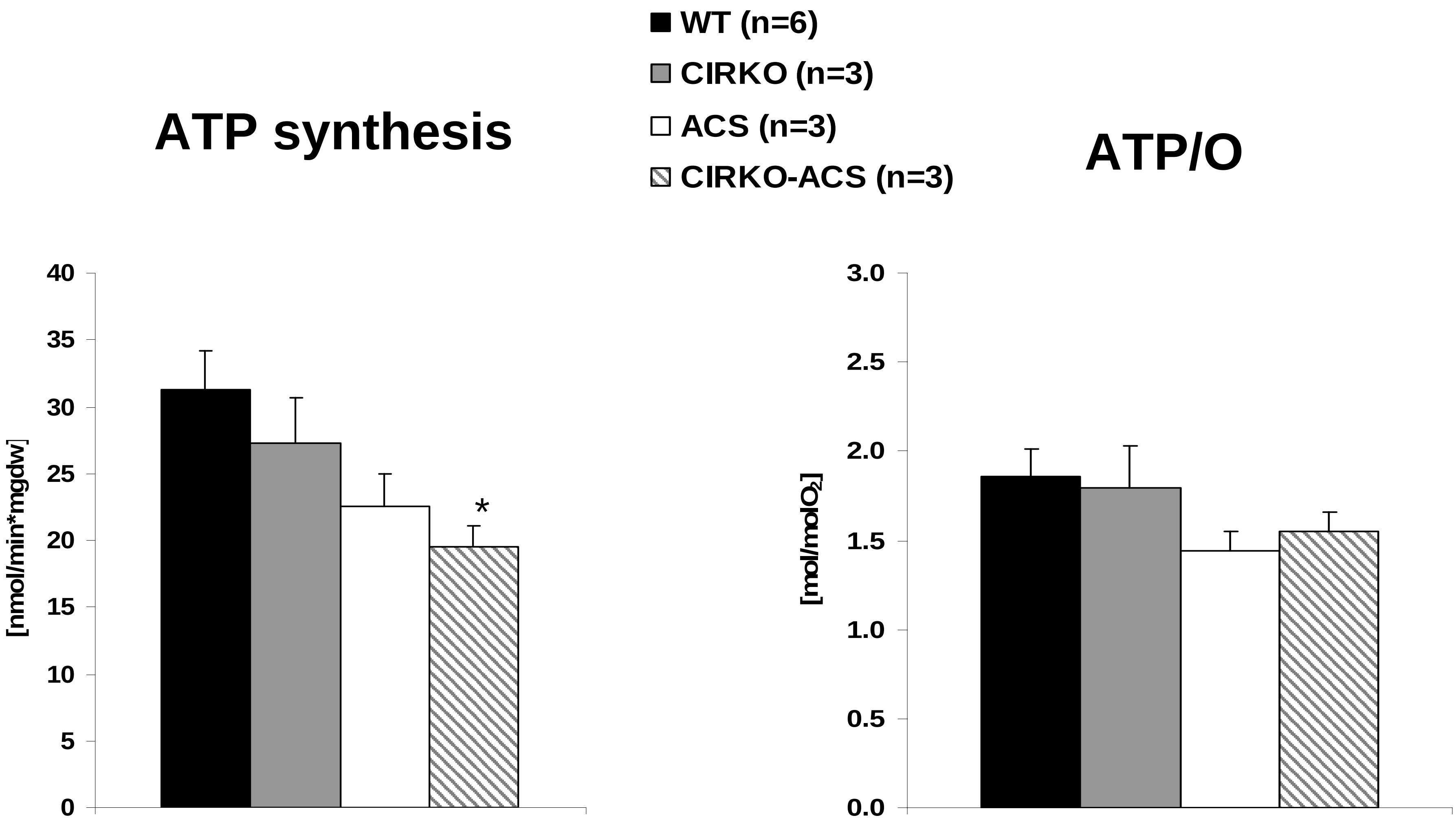




Reduced state 3 respiration in CIRKO-ACS, but not in CIRKO and ACS mice at 12 weeks



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

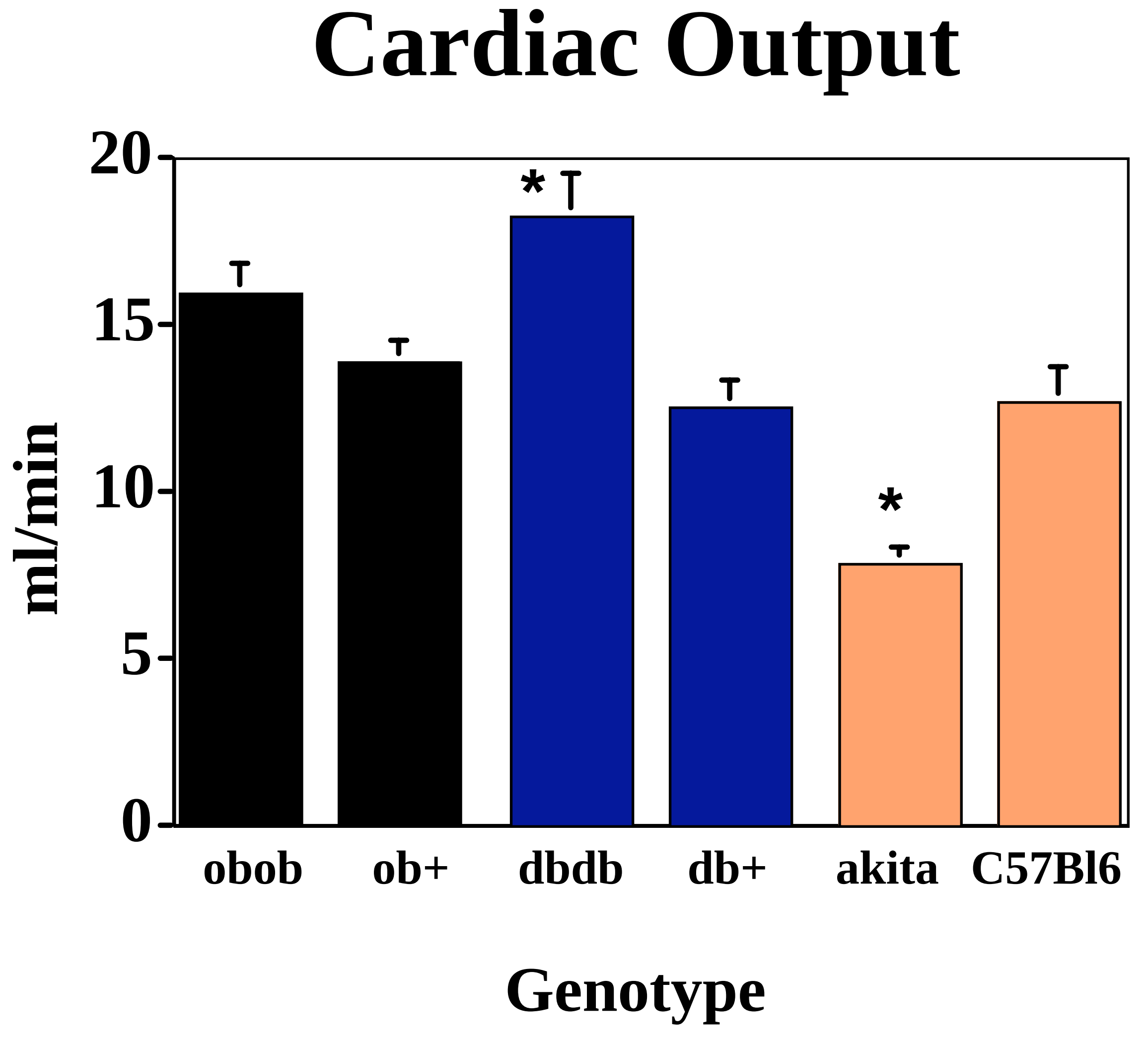


Increased Myocardial FA Delivery Precipitates Mitochondrial Dysfunction and this is Accelerated by the Presence of Insulin Resistance

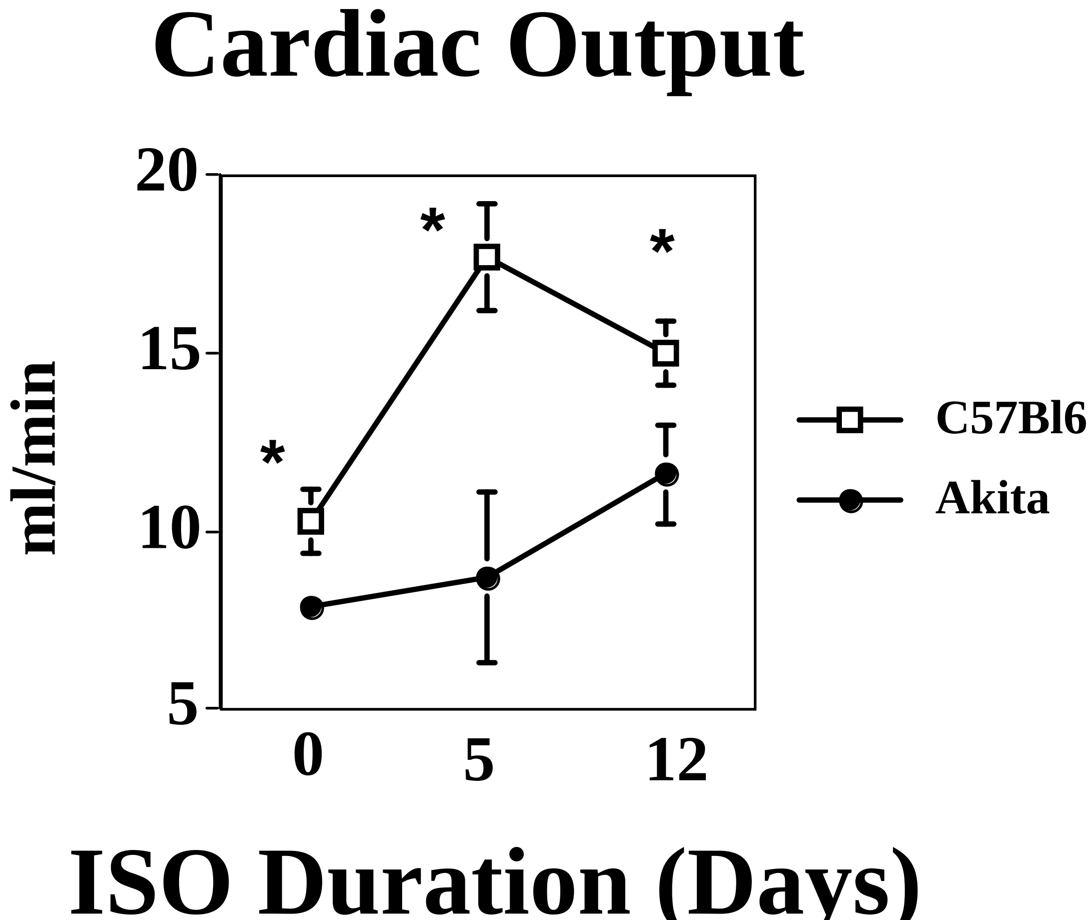
Cross Model Comparisons

Type 2 DM
versus
Type 1 DM

Cardiac Output Across 3-Models of Diabetes



Impaired Response of Akita Mice to Isoproterenol



Summary Feldman Table

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
Akita *	C57BL/6J	Ongoing Phenotyping	Completed	Pending	In Progress	Pending	Pending	In Progress	In Progress	Pending
ACS-CIRKO*	Mixed	Ongoing Phenotyping	Completed	Pending	Completed	Pending	Pending	In Progress	In Progress	Pending
High-Fat Feeding ¦	C57BL/6J	Ongoing Phenotyping	In Progress	Pending	In Progress	Pending	Pending	In Progress	Pending	In Progress
Dominant Negative PI3 Kinase *	FVB	Advanced Phenotyping	Completed	In Progress	Completed	In Progress	Pending	Completed	In Progress	In Progress
UCP-DTA¦	FVB	Ongoing Phenotyping	In Progress	Pending	In Progress	Pending	Pending	Completed	Pending	Pending
Ob/ob	C57BL/6J	Final Phenotyping	Completed	Completed	Completed	Completed	Completed	Completed	In Progress	Completed
Db/db	C57BLKS	Final Phenotyping	Completed	Completed	Completed	Completed	Completed	Completed	Pending	Completed
CIRKO	Mixed	Final Phenotyping	Completed	Completed	Completed	Completed	Completed	Completed	Completed	Completed

* Current top three Models; ¦ Next top two models; Original top three models.

Diabetic Cardiomyopathy - Animal Models State of the Art 2006

- Abnormal Substrate Metabolism, and Metabolic Inflexibility, Myocardial Insulin Resistance, Established in Various Models of T2DM
- Mitochondrial Dysfunction is a Clear Mechanism
- Oxidative Stress is an Important Consequence and Reversal of Oxidative Stress Ameliorates Cardiac Dysfunction
- High-Fat Feeding Precipitates Cardiac Dysfunction, Insulin Resistance, Abnormal Metabolism
- Mouse Models of Lipotoxicity - increased FFA availability or uptake or increased expression of transcription factors that upregulate FA metabolism
- Decreased Sarcoplasmic Reticulum Calcium Handling now shown in T2DM mouse hearts, and can be reversed by overexpressing SERCA2 or phospholamban
- Impaired Insulin Signaling Impairs Muscle and Vascular function in the heart and contributes to increased injury/fibrosis following ischemia
- Activated Protein Kinase C in diabetic myocardium may contribute to fibrosis and may be downstream of Renin-Angiotensin Activation

Acknowledgements

- **Fellow Co-Investigators from the other AMDCC Sites**
- **External Advisory Committee**
- **NIH Staff**