AMDCC Annual Report (2011)

PI: ABEL, E. DALE
Project Title: Modeling Diabetic Cardiomyopathy and Microangiopathy
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Abstract: This proposal is submitted to become a pathobiology site in the renewal of the Animal Models of Diabetic Complications Consortium (AMDCC). The University of Utah site proposes to generate two mouse models. The first will address mechanisms that are responsible for diabetic cardiomyopathy, and the second will model the role of impaired angiogenesis and arteriogenesis in the pathogenesis of ischemic complications of diabetes. Our studies in the first phase of the consortium revealed that diabetic cardiomyopathy (particularly in type 2 diabetes) was characterized by impaired myocardial insulin action, mitochondria! dysfunction, oxidative stress, increased FA utilization, decreased glucose utilization and lipotoxicity. Mice with cardiomyocyte-restricted deletion of insulin receptors (CIRKO), developed many of the features of diabetic cardiomyopathy but did not have a persistent increase in FA oxidation, did not develop lipotoxicity and had modest defects in cardiac function. We therefore propose to introduce into CIRKO mice an Acyl-CoA synthetase transgene that will modestly increased myocardial FA delivery in a sensitized background of mitochondrial superoxide (Sod2) haploinsufficency. We will then determine if these mice meet established AMDCC criteria for diabetic cardiomyopathy, determine if they exhibit characteristic defects in mitochondrial function and substrate utilization and test the hypothesis that they will be more susceptible to dysfunction in the face of LV hypertrophy. This model represents a powerful platform with which to test the direct effect of various therapeutic strategies on diabetic cardiomyopathy, independently of effects on systemic metabolism. We will also generate a mouse in the type -1 diabetes (Akita) background with temporal and cell-type restricted expression of a novel angiogenic factor netrin 1. We will use this mouse to determine if the maintenance of arteriogenesis and angiogenesis, by activating this transgene in the heart or the hind-limb will be sufficient to ameliorate the accelerated LV remodeling that characterizes diabetic hearts following coronary artery ligation, or reverse impaired recovery of hind-limb perfusion that occurs in diabetic animals following femoral artery occlusion. This model will also be a powerful platform with which to evaluate the role of impaired angiogenesis (in the pathogenesis), or therapeutic angiogenesis (in the treatment) of other ischemic complications of diabetes such as impaired wound healing, neuropathy or nephropathy.

1. **Program Accomplishments:**

The University of Utah's participation in the Animal Models of Diabetes Complications Consortium proposed the generation of two mouse models. Model –1: *Modeling the role of insulin resistance, lipotoxicity and oxidative stress in the pathogenesis of diabetic cardiomyopathy* - ***CIRKO-ACS-sod2$^{+/-}$***
Model –2: *Modeling the role of impaired angiogenesis/arteriogenesis in the pathogenesis of microvascular complications of diabetes and to model the potential utility of increasing angiogenic potential as a strategy for preventing or reversing microvascular complications of diabetes.* – ***Inducible Netrin-Akita***

In addition we proposed hypothesis driven aims for both of these models.

MODEL 1: CIRKO-ACS-sod2$^{+/-}$.
The overall hypothesis that will be evaluated by this model is: *Diabetic cardiomyopathy is characterized by impaired myocardial insulin signaling, lipotoxicity and oxidative stress.* The proposed studies will test the following specific hypotheses:

1. The CIRKO-ACS-sod2$^{+/-}$ will meet the validation criteria for diabetic cardiomyopathy in terms of decreased contractile function, increased intramyocellular lipid and increased myocyte loss and fibrosis.
2. CIRKO-ACS-sod2$^{+/-}$ will exhibit increased rates of FA oxidation, decreased rates of glucose oxidation, increased MVO_2 and decreased cardiac efficiency.
3. The mechanism responsible for impaired myocardial function and substrate utilization in CIRKO-ACS-sod2$^{+/-}$ mice will be mitochondrial uncoupling on the basis of increased FA-mediated superoxide generation, leading to impaired mitochondrial energetics.
4. CIRKO-ACS-sod2$^{+/-}$ will develop rapid functional deterioration following hemodynamic stress such as pressure overload hypertrophy.

MODEL 2: Inducible-Netrin-Akita (*Tam-b-actinCRE.ROSA26* $^{netrin1/lacZ}$*.ins2*$^{+/C96Y}$).

The overall hypothesis that will be tested in this model is: *Impaired adaptive angiogenesis and arteriogenesis contributes to impaired myocardial remodeling following coronary ischemia, and to increased limb loss following femoral artery occlusion in diabetes.* These studies will utilize the inducible-netrin-akita mouse and take advantage of our ability to upregulate netrin expression in a temporal fashion by inducible activation of cre-recombinase following treatment of mice with tamoxifen. If inducible cardiomyocyte-restricted Cre-Netrin Akita mice are also developed, we can additionally determine if this approach will hold true in an organ-restricted manner as well. The studies proposed in this aim will initially determine the fidelity of the temporal (tamoxifen-inducible) gene expression system in inducible-netrin-akita mice. Based on preliminary data that we have obtained with the tamoxifen-regulated MHC Cre mouse (MCM-MHC) we are confident that we will be able to increase netrin expression in cardiomyocytes of netrin-Akita mice, and deem it likely that more widespread netrin activation will be obtained with the inducible beta-actin driven tamoxifen cre transgenic (*Tam-b-actinCRE.ROSA26* $^{netrin1/lacZ}$*.ins2*$^{+/C96Y}$).

The following hypotheses will be tested:

1. Tamoxifen treatment of *Tam-b-actinCRE.ROSA26* $^{netrin1/lacZ}$*.ins2*$^{+/C96Y}$ mice will increase netrin1 expression ubiquitously, including cardiomyocytes and skeletal muscle. Tamoxifen treatment of *MCM-MHC.ROSA26* $^{netrin1/lacZ}$*.ins2*$^{+/C96Y}$ (if generated) will increase netrin expression in cardiomyocytes only.
2. Diabetic animals will exhibit accelerated myocardial remodeling following coronary artery occlusion and relative to control animals and the promotion of angiogenesis and arteriogenesis by netrin1 will reverse this phenotype
3. Diabetic animals will exhibit reduced recovery of hind-limb perfusion following femoral artery ligation relative to non-diabetic animals and the promotion of angiogenesis and arteriogenesis by netrin1 will reverse this phenotype

Recent Progress and Major Accomplishments

The **Inducible-Netrin-Akita (*Tam-b-actinCRE.ROSA26* $^{netrin1/lacZ}$*.ins2*$^{+/C96Y}$)** was the model that the consortium chose to develop and initially characterize at the Jackson Laboratories.

Generation of this model by JAX is now completed, and mice have been transferred to the University of Utah. The first part of this report will summarize new findings made in models related to the **CIRKO-ACS-sod2$^{+/-}$** project (Model 1) and the second part will summarize progress with the Netrin-Akita model (Model 2).

Model 1:

At the time that proposal was written our view of diabetic cardiomyopathy was one of impaired myocardial insulin action, coupled with lipotoxicity that was driven in part by increased FA delivery to the heart and mitochondrial oxidative stress. This view provided the rationale for generating a mouse model in which all of these components were present in the heart. Recent studies in our laboratory in the past 1-2 years have led us to revise our views somewhat. (1) We have obtained and published evidence that myocardial insulin sensitivity remains intact in the heart in models of type 1 diabetes and in the diet-induced mouse model of type 2 diabetes [1, 2]. This is in contrast to what occurs in more severe genetic models of type 2 diabetes such as the ob/ob mouse [3]. However, we have recently observed ad published that disturbances in myocardial metabolism, mitochondrial function and insulin action in these models are driven in part by hypothalamic leptin signaling [4]. (2) We also recently published that the increase in myocardial FA utilization that occurs fairly early in the course of diet-induced obesity occurs in part as a result of a primary impairment in glucose utilization, which is driven in part by a reduction in GLUT4 trafficking [2]. (3) In a recently published collaborative study with the Goldberg lab we observed that myocardial dysfunction in a mouse model of lipotoxic cardiomyopathy could be reversed by a genetic manipulation that actually increased rates of FA oxidation and the size of intramyocardial lipid droplets [5]. This study suggests that lipid accumulation and increased rates of myocardial FA oxidation can by dissociated from LV dysfunction. (4) We also recently published a collaborative paper in which we observed that cardiomyocyte stretch as occurs in pressure overload hypertrophy auto-activated insulin signaling, which exacerbated the transition to heart failure, and that this could be reversed by partially limiting myocardial insulin action [6]. Taken together, these observations have led us to shift our focus going forward towards understanding the mechanisms by which hyperinsulinemia might be contributing to some of the cardiac pathologies of type 2 diabetes, and potentially to that of insulin-treated type 1 diabetic subjects.

We are completing proposed studies in the CIRKO-ACS-sod2$^{+/-}$ project with the caveat that these models will reveal fundamental roles of these respective pathways in cardiovascular biology, but might not completely inform the pathophysiology of the diabetic cardiomyopathy.

The **CIRKO-ACS-sod2$^{+/-}$** model has three component mice that will be used to generate a compound transgenic/gene targeted model. The respective components are (1) Cardiomyocyte Insulin receptor KO mice (CIRKO), (2) Cardiomyocyte-restricted low-level overexpression of Acyl CoA Synthase (MHC-ACS1), and (3) Germline heterozygous mice for the mitochondrial superoxide dismutase (sod2$^{+/-}$). Additional updates related to these models are summarized below.

CIRKO: Studies described in last year's report regarding the regulation or myocardial autophagy by insulin signaling that were precipitated by observations made in CIRKO mice, as well as in mutant mice that lack IRS1/2 or PDK4 continue. The regulation of autophagy by insulin signaling is complex and may occur independently of insulin's ability to regulate mTOR or FOXO1. These observations now form the basis of a new NIH proposal that was recently funded.

MHC-ACS: We submitted the manuscript describing the impact of ACS overexpression on mitochondrial dynamics (fusion/fission) in the heart and in cultured cells to Cell Metabolism and the manuscript is now in revision.

Oxidative Stress and Diabetic Cardiomyopathy: Studies of the impact of diet-induced obesity in sod2 heterozygotes in the heart have not yet been completed.

Model 2:

This model represents the approved AMDCC model that is being generated at the Jackson Laboratories. Heterozyous *ROSA26* $^{netrin1/lacZ}$ mice were transferred to the Jackson Laboratories. Backcross to the C57BL6 background and crossing to the Akita strain to generate *ROSA26* $^{netrin1/lacZ}$*.ins2*$^{+/C96Y}$ is now completed. These mice have now been shipped to the University of Utah and we are expanding the colony here.

Plans for the Upcoming Year

1. Complete analysis of the impact of sod2 haploinsufficiency on cardiac function and metabolism and mitochondrial function in response to high-fat feeding.
2. Continue to characterize the role of autophagy in diabetic cardiomyopathy.
3. Perform initial characterization of the *Tam-b-actinCRE*.*ROSA26* $^{netrin1/lacZ}$*.ins2*$^{+/C96Y}$ mice, once a sufficient number of animals have been generated. This will entail documenting tamoxifen-inducible netrin expression in the heart and hind limb, followed by studies to determine if netrin-1 overexpression will enhance recovery of hind-limb circulation following femoral artery ligation, and cardiac function following coronary artery ligation. The model will also be useful in determining if netrin-1 overexpression will retard the progression of diabetic neuropathy.

2. Collaborations:

Within AMDCC

1. The collaborative project with Ira Goldberg examining the interaction of PPARα and PPARγ in lipotoxic cardiomyopathy was published in the JCI in October of 2010 [5].

With Jax

ROSA26 $^{netrin1/lacZ}$ mice were transferred to JAX and were backcrossed to the C57BL6 and Akita strains as described above. We have now received these mice from the Jackson

Laboratories and are increasing the size of the colony to generate animals for the studies as proposed.

With the MMPCs
None.

With other non-AMDCC PIs

1. Collaborative studies with Gary Sweeney at York University to determine the impact of adiponectin on myocardial fatty acid utilization and oxygen consumption was published in the AJP in August of 2010 [7].
2. Our ongoing collaboration with David Symons yielded another publication in which we examined the impact of loss of myocardial insulin signaling in CIRKO mice on coronary artery vascular reactivity in response to pressure overload [8]. We have also now elucidated the mechanism by which ceramide accumulation in the vessels of high-fat-fed mice impairs nitric oxide bioavailability by increased eNOS-mediated dephosphorylation by PP2A.
3. We are now collaborating with Mukesh Jain at Case Western where we are examining the mechanism by which members of the KLF transcription factors regulate cardiac metabolism.

3. Address previous EAC comments:

- The CIRKO-ACS-sod2+/- will be the result of three separate mice and work is summarized with each of these components. The CIRKO mouse is currently available and has been studied by Dr. Abel for a number of years. Work in the past year has centered on the effect of impaired insulin action on myocardial autophagy. While no data was reported, this will inevitably be novel and informative given the role of autophagy in diabetic cardiomyopathy is unknown. For the MHC-ACS animal, they have previously reported alterations in fatty acid uptake in myocardial mitochondria. A manuscript is in preparation now indicating that this effect is due to palmitate-induced ceramide generation. As for the third component, attempts to produce the MHC-SOD2 knockout animal resulted in early lethality, but the het mice are viable. Plans are to test whether high fat feeding will result in increased mitochondrial ROS generation and eventually accelerate cardiomyopathy. In the meantime, they have demonstrated that pharmacological antioxidants have beneficial effects upon myocardial function. Other work in the lab also supports the idea that proximal insulin signaling remains intact in the myocardium and thus provide a mechanisms whereby hyperinsulinemia may induce negative consequences. This hypothesis will have to be pursued in models other than the proposed CIRKO-ACS-sod2+/- as insulin signaling is abolished.

We agree with the advisors assessment and we are actively studying the consequences of increased myocardial signaling on cardiac autophagy using high-fat fed mice (Wildtype) and mice with inducible overexpression of GLUT4 to elucidate the contribution of impaired myocardial glucose utilization to the increase in myocardial autophagy that takes develops

in response to high-fat feeding.

- The second model proposed by the Abel lab is the inducible-netrin-Akita. Heterozyous ROSA26 netrin1/lacZ mice have been transferred to JAX. These animals have been backcrossed to the C57Bl6 background and crossed with the Akita. The final cross to introduce the tamoxifen-inducible cre has now begun with the hope of having mice available in the next couple months. These mice will be of value to several different complications and cross phenotyping should be pursued.

The mice have now been transferred to the University of Utah and we are expanding the colony for future studies.

- Dr. Abel's group continues to collaborate with other Consortium members. They have performed myocardial metabolism and mitochondrial function assays with models from the Goldberg and Smithies' laboratories.

- As stated above, the SC is strongly encouraged to routinely review the "protocols" section of the website to ensure that they are up-to-date and that new protocols are added as needed. In particular, there have been several requests from the community for additional protocols related to the electrophysiology of CV disease. We encourage Dr. Abel and the CV working group to lead an effort to identify any such gaps and work with Dr. McIndoe to add them to the website.

We will endeavor to complete this review prior to the end of the funding cycle.

- The goal of this project is to generate and characterize two mouse models: one is to study cardiomyopathy (CIRKO-ACS-sod2) and the other is to study angiogenesis (netrin-akita). For the CIRKO-ACS-sod2 mouse, the PI found that cardiac-restricted deletion of sod2 was lethal. They plan to study the het mice in the upcoming year. The second model is being generated at JAX; they anticipate having mice in the next year for study.

These mice have now arrived and we are expanding the colony.

- Overall, the project is on track to complete the goals; at this point, the PI is waiting on the netrin mice for study and is collaborating with other AMDCC investigators in the interim. No additional clarifications or suggestions are needed.

- The group is pursuing two lines of investigation. First, they are studying the role of insulin signaling, lipotoxicity and oxidative stress in cardiomyopathy. Data are described in only the most general terms, without tables or figures, and while these sound promising, it is not possible to evaluate progress. A manuscript has been submitted on the CIRKO mouse, but is not provided. Second, the will study vascular remodeling using an inducible netrin Akita mouse – this is under construction at JAX and thus no

experiments have been possible. Without data to review, it is not possible to offer substantive recommendations.

- Below is a list of your AMDCC publications from the website. Should any publications be added or subtracted? Has all of the relevant data from these publications been uploaded to the website? Please work with Dr. Rick McIndoe to ensure that the website and database are up-to-date and complete.

The listed references are a correct and the following new publications will be added. (See references [4, 8-10] in the bibliography.

1. Excessive cardiac insulin signaling exacerbates systolic dysfunction induced by pressure overload in rodents.
   Shimizu I, Minamino T, Toko H, Okada S, Ikeda H, Yasuda N, Tateno K, Moriya J, Yokoyama M, Nojima A, Koh GY, Akazawa H, Shiojima I, Kahn CR, Abel ED, Komuro I
   The Journal of clinical investigation, 2010 (120), 1506 – 1514
2. Diabetic cardiomyopathy, causes and effects.
   Boudina S, Abel ED
   Reviews in endocrine & metabolic disorders, 2010 (11), 31 – 39
3. Lipotoxicity in the heart.
   Wende AR, Abel ED
   Biochimica et biophysica acta, 2010 (1801), 311 – 319
4. Rodent models of diabetic cardiomyopathy.
   Bugger H, Abel ED
   Disease models & mechanisms, 2009 (2), 454 – 466
5. Tissue-specific remodeling of the mitochondrial proteome in type 1 diabetic akita mice.
   Bugger H, Chen D, Riehle C, Soto J, Theobald HA, Hu XX, Ganesan B, Weimer BC, Abel ED
   Diabetes, 2009 (58(9)), 1986 – 1997
6. Tissue-specific remodeling of the mitochondrial proteome in type 1 diabetic akita mice.
   Bugger H, Chen D, Riehle C, Soto J, Theobald HA, Hu XX, Ganesan B, Weimer BC, Abel ED
   Diabetes, 2009 (58), 1986 – 1997
7. Contribution of insulin and Akt1 signaling to endothelial nitric oxide synthase in the regulation of endothelial function and blood pressure.
   Symons JD, McMillin SL, Riehle C, Tanner J, Palionyte M, Hillas E, Jones D, Cooksey RC, Birnbaum MJ, McClain DA, Zhang QJ, Gale D, Wilson LJ, Abel ED
   Circulation research, 2009 (104), 1085 – 1094
8. Impaired insulin signaling accelerates cardiac mitochondrial dysfunction after myocardial infarction.
   Sena S, Hu P, Zhang D, Wang X, Wayment B, Olsen C, Avelar E, Abel ED, Litwin SE.
   Journal of molecular and cellular cardiology, 2009 (46(6)), 910 – 918
9. Contribution of impaired myocardial insulin signaling to mitochondrial dysfunction and oxidative stress in the heart.
   Boudina S, Bugger H, Sena S, O'Neill BT, Zaha VG, Ilkun O, Wright JJ, Mazumder PK, Palfreyman E, Tidwell TJ, Theobald H, Khalimonchuk O, Wayment B, Sheng X, Rodnick KJ,

Centini R, Chen D, Litwin SE, Weimer BE, Abel ED
Circulation, 2009 (119(9)), 1272 – 1283

10. Mechanisms for increased myocardial fatty acid utilization following short-term high-fat feeding
    Wright JJ, Kim J, Buchanan J, Boudina S, Sena S, Bakirtzi K, Ilkun O, Theobald HA, Cooksey RC, Kandror KV, Abel ED
    Cardiovascular research, 2009
11. Type 1 diabetic akita mouse hearts are insulin sensitive but manifest structurally abnormal mitochondria that remain coupled despite increased uncoupling protein 3
    Bugger H, Boudina S, Hu XX, Tuinei J, Zaha VG, Theobald HA, Yun UJ, McQueen AP, Wayment B, Litwin SE, Abel ED
    Diabetes, 2008 (57), 2924 – 2932
12. Captopril normalizes insulin signaling and insulin-regulated substrate metabolism in obese (ob/ob) mouse hearts
    Tabbi-Anneni I, Buchanan J, Cooksey RC, Abel ED.
    Endocrinology, 2008 (149), 4043 – 4050
13. Cardiac Remodeling in Obesity
    Abel ED, Litwin SE, Sweeney G
    Physiological reviews, 2008 (88), 389 – 419
14. Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome
    Bugger H, Abel ED.
    Clinical science, 2008 (114), 195 – 210
15. Cardiac Hypertrophy Caused by Peroxisome Proliferator Activated Receptor-Gamma Agonist Treatment Occurs Independently of Changes in Myocardial Insulin Signaling
    Sandra Sena, Isaac R. Rasmussen, Adam R. Wende, Alfred P. McQueen, Heather A. Theobald, Nicole Wilde, Renata Oliveira Pereira, Sheldon E. Litwin, Joel P. Berger, E. Dale Abel
    Endocrinology, 2007 (148(12)), 6047 – 6053
16. Glucose for the aging heart?
    Abel ED
    Circulation, 2007 (116(8)), 884 – 887
17. Mitochondrial energetics in the heart in obesity related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins
    Sihem Boudina, Sandra Sena, Heather Theobald, Xiaoming Sheng, Jordan J. Wright, Xia Xuan Hu, Salwa Aziz, Josie I. Johnson, Heiko Bugger, Vlad G. Zaha and E. Dale Abel
    Diabetes, 2007 (56(10)), 2457 – 2466
18. Diabetic Cardiomyopathy Revisited
    Sihem Boudina and E. Dale Abel
    Circulation, 2007 (115), 3213 – 3223
19. Recipes for Creating Animal Models of Diabetic Cardiovascular Disease
    Willa Hsueh, E. Dale Abel, Jan L. Breslow, Nobuyo Maeda, Richard C. Davis, Edward A. Fisher, Hayes Dansky, Donald A. McClain, Richard McIndoe, Momtaz K. Wassef, Cristina

Rabadan-Diehl, Ira J. Goldberg
Circulation research, 2007 (100), 1415 – 1427
20. Mitochondrial Uncoupling: A Key Contributor to Reduced Cardiac Efficiency in Diabetes
Sihem Boudina and E. Dale Abel
Physiology (Bethesda, Md.), 2006 (21), 250 – 258
21. Reduced Mitochondrial Oxidative Capacity and Increased Mitochondrial Uncoupling Impairs Myocardial Energetics in Obesity
Sihem Boudina, Sandra Sena, Brian T O'Neill, Prakash Tathireddy, Martin E Young, and E. Dale Abel
Circulation, 2005 (112), 2686 – 2695
22. Contractile dysfunction in hypertrophied hearts with deficient insulin receptor signaling: possible role of reduced capillary density
Alfred P. McQueen, Dongfang Zhang, Ping Hu, LeAnne Swenson, Ying Yang, Vlad G. Zaha, James L. Hoffman, Ui Jeong Yun, Gopa Chakrabarti, Zhengming Wang, Kurt H. Albertine, E. Dale Abel, Sheldon E. Litwin
Journal of molecular and cellular cardiology, 2005 (39), 882 – 892
23. Reduced Cardiac Efficiency and Altered Substrate Metabolism Precedes the Onset of Hyperglycemia and Contractile Dysfunction in Two Mouse Models of Insulin Resistance and Obesity
Jonathan Buchanan, Pradip K. Mazumder, Ping Hu, Gopa Chakrabarti, Matthew W. Roberts, Ui Jeong Yun, Robert C. Cooksey, Sheldon E. Litwin, and E. Dale Abel
Endocrinology, 2005 (146), 5341 – 5349
24. Myocardial Insulin Resistance and Cardiac Complications of Diabetes
E. Dale Abel
Current drug targets. Immune, endocrine and metabolic disorders, 2005 (5), 219 – 226
25. Metabolic perturbations in the diabetic heart: Mechanisms and molecular targets
E. Dale Abel
Drug discovery today, 2005 (2), 115 – 122
26. Optical Mapping of Propagation Changes Induced by Elevated Extracellular Potassium Ion Concentration in Genetically Altered Mouse Hearts
Bonnie B. Punske, Stefano Rossi, Philip Ershler, Isaac Rasmussen and E. Dale Abel
Journal of electrocardiology, 2004 (37), 128 – 134
27. Insulin Signaling in Heart Muscle: - Lessons from Genetically Engineered
E. Dale Abel
Current hypertension reports, 2004 (6), 416 – 423
28. Impaired Cardiac Efficiency and Increased Fatty Acid Oxidation in Insulin-Resistant ob/ob Mouse Hearts.
Mazumder PK, O'Neill BT, Roberts MW, Buchanan J, Yun UJ, Cooksey RC, Boudina S, Abel ED.
Diabetes, 2004 (53), 2366 – 2374
29. Glucose transport in the heart.
Abel ED.
Frontiers in bioscience, 2004 (9), 201 – 215

30. Gender-dependent attenuation of cardiac potassium currents in type 2 diabetic db/db mice.
    Shimoni Y, Chuang M, Abel ED, Severson DL.
    The Journal of physiology, 2003 (555), 345 – 354
31. Minimally invasive aortic banding in mice: Effects of altered cardiomyocyte insulin signaling during pressure-overload.
    Hu P, Zhang D, Swenson L, Chakrabarti G, Abel ED, Litwin SE.
    American journal of physiology. Heart and circulatory physiology, 2003 (285), H1261 - H1269
32. Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression.
    Belke DD, Betuing S, Tuttle MJ, Graveleau C, Young ME, Pham M, Zhang D, Cooksey RC, McClain DA, Litwin SE, Taegtmeyer H, Severson D, Kahn CR, Abel ED.
    The Journal of clinical investigation, 2002 (109), 629 – 639
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