**AMDCC Annual Report (2011)**

**PI:** GOLDBERG, IRA
**Project Title:** Creating Glucose Responsive Cardiovascular Complications
**Grant Number:** U01 HL087945

**Abstract:** The PIs of this application have developed techniques for producing and studying atherosclerosis using funds from the first AMDCC program. Specifically, we have found that streptozotocin-treated mice develop increased atherosclerosis in the presence of a transgene for human aldose reductase (hAR). We have also noted that hearts from these mice have areas of cardiac apoptosis. In addition, we have developed novel methods to study atherosclerosis regression that can be applied to studies of lesions in control and diabetic mice. Aim 1 To create new mouse models of diabetic cardiovascular disease: We propose to create two new genetically altered mice. Aim 1a is to use the tet on system to allow expression of hAR in a time dependent manner. This system will allow us to test whether hAR expression in established lesions alters plaque morphology. These animals can also be used to produce tissue specific expression of hAR. Aim 1b is to produce mice with expression of hAR in cardiomyocytes. These mice, we hypothesize, will develop cardiomyopathy with diabetes. Aim 2 To study the development of vascular lesions in diabetic mice: Mild diabetes due to deficiency of Pdx1 or high fat diets did not alter atherosclerosis in Ldlr-/- mice. In addition, Pdx1 did not affect regression after transplant of arteries containing atherosclerosis. We will use two additional methods to generate hyperglycemia, Akita and high fat diets on the FVB background, in Ldlr-/- mice q hAR. Increased vascular disease in STZ-treated hAR mice could result from greater monocyte/macrophage accumulation in lesions, or could be secondary to a defect in lesion regression. Both processes will be studied in vivo and mechanistic information obtained by studying gene and protein expression.

## 1. Project Accomplishments (2011)

**Introduction**: This Project has led to a continued intellectual and experimental collaboration between the Co-PIs and their laboratories. This has included meetings approximately every 6 weeks and weekly phone communications. Both major projects are proceeding and being critiqued by the PIs and an outside reviewer, Dr. Jan Breslow (Rockefeller University). In addition, we have collaborated with Dr. Abel (U Utah) to study models of cardiomyopathy

The Project includes methods to produce and evaluate mouse models of two major cardiovascular complications of diabetes: atherosclerosis and heart failure. We have focused on the effects of diabetes on atherosclerosis using a novel model to assess atherosclerosis regression. This allowed us to illustrate changes in vascular biology exclusive of alterations in plasma lipoproteins; lipoproteins levels are markedly reduced in control and diabetic mice in this model. In addition, we have pursued studies of the effects of aldose reductase (AR) the initial enzyme in the polyol pathway and showed that this enzyme increased atherosclerosis in the apoE knockout background. Finally, we have collaborated with Dr. Abel to characterize a rescued lipotoxic heart model. Lipotoxicity is thought to be one major causes of diabetic heart failure.

**Hypothesis:** Overexpression of AR increases the aberrant metabolism of glucose leading to vascular and cardiac toxicity.

**Progress toward stated aims**

**New model development:** We proposed to create models in which to study the effects of the enzyme aldose reductase (AR) in two complication of diabetes: atherosclerosis and heart failure. In addition, we planned to develop an animal model to assess how diabetes affects atherosclerosis regression, defined as the loss of CD68+ cells from atherosclerotic plaques after reduction of circulating cholesterol.

**AR and atherosclerosis:** We have completed studies of the effects of generalized expression of human AR (hAR) on atherosclerosis in the mouse. The animals were crossed onto the apoE knockout (apoE0) background. We used this model because diabetic vascular disease can be studied while the mice are consuming a chow rather than high fat or high cholesterol diet. hAR/apoE0 diabetic mice developed a marked increased in atherosclerosis lesion area compared to diabetic apoE0 mice; non-diabetic mice show no effects due to expression of the hAR transgene. These studies were performed in collaboration with R. Ramasamy (Columbia).

**Creation of methods to alter hAR in various cells:** We created and sent to Jackson Labs two vectors with a tet-responsive hAR and a tet-on promoter element associated with a major histocompatibility gene, the same promoter used to create the hAR transgenic line that we have used in the past. These vectors were used by the Jackson Labs to create mice and we were able to show doxycycline induction of hAR expression in cells from the mice. Unfortunately, Jackson Labs co-injected both constructs into the same mice making it impossible to use these animals to alter hAR expression in a single tissue, but these mice are

still useful to breed to LDLR-/- mice to look at effects of global induction of hAR expression on pre-existing plaques.

**MHC-hAR mice:** A line of mice in which hAR was expressed in cardiomyocytes was studied. These mice develop heart dysfunction, i.e. reduced ejection fraction, at greater than 10 months. In addition, with Dr. Ramasamy we have shown that these mice have defective recovery from ischemia/reperfusion injury. We hypothesized that in the setting of greater glucose uptake and reduced use of fatty acids the mice would be especially compromised. To test this, we crossed the MHC-hAR transgene onto the PPARalpha knockout background. These mice are being analyzed currently, however, they do not appear to have greater heart dysfunction.

**Plans for the year and completion of the project:** We have completed studies of the effects of hAR overexpression on atherosclerosis in apoE knockout mice and the manuscript is under review. For the MHC-hAR mice, we will study gene expression and lipid accumulation of mice. In addition, we will complete the studies of ischemia/reperfusion and will determine whether in his model increased heart dysfunction is associated with greater ROS generation, lipid accumulation, or activation of inflammatory pathways.

**Hypothesis: Hyperglycemia causes increased monocyte/macrophage inflammation which inhibits atherosclerosis regression**

**Progress toward stated aims:**

**Development of generalizable models of plaque regression:** We have developed several models of regression that do not employ surgical transplant by either inducing deletion of the apoB-lipoprotein assembly protein microsomal triglyceride transfer protein (MTTP) - Reversa mice) - or by addition of a LDL receptor expressing adenovirus into LDL receptor knockout mice. The virus aspect was conducted in collaboration with Dr. Lawrence Chan, Baylor.

We have completed a study of atherosclerosis regression in the setting of streptozotocin diabetes and this paper was accepted for publication by the journal Diabetes. Regression, defined as loss of CD36+ cells from the artery, was partially blocked by diabetes. Laser capture microdissection studies showed that the artery CD68+ cells tended to be more inflammatory under diabetic conditions.

**Effects of glucose reduction on plasma lipids and circulating monocytes:** We have studied the effects of hyperglycemia on plasma lipid levels in the LDL receptor knockout model. Diabetes causes a marked increase in circulating triglyceride and cholesterol levels in this model. By reducing plasma glucose using an inhibitor of the renal sodium glucose transporter (SGLT2) we have been able to reduce plasma glucose almost to normal levels in streptozotocin diabetic mice. After several weeks, this was associated with a reduction in both triglyceride and cholesterol in these mice. Why is this? The changes in lipoprotein metabolism that are associated with reduction of glucose, but no change in insulin, are a new topic for investigation.

To further investigate the reasons for defective regression in diabetic mice, we have begun to examine the effects of hyperglycemia and glucose reduction on circulating white blood cells. Streptozotocin and Akita diabetes is associated with an increase in circulating monocytes. This appears to be corrected by reduction of glucose using SGTL2 inhibition. The biology of monocyte production and turnover and the effects of glucose on this process are currently under investigation.

**Plans for the year and completion of the project:** We will complete studies of the effects of streptozotocin and Akita diabetes on monocyte number and gene expression. In addition, we will assess bone marrow to determine whether diabetes and glucose reduction alter bone marrow monocyte precursors.

**Hypothesis:** Lipid accumulation in the heart, as occurs with diabetes, is a cause of cardiac dysfunction.

**Progress toward stated aims:**

**Correction of lipotoxic cardiomyopathy:** We hypothesized that cardiac dysfunction associated with increased lipid accumulation in a mouse that overexpresses the fatty acid metabolism regulating transcription factor PPARgamma would be corrected by crossing this transgene onto a background of PPARalpha deficiency. This turned out to be true. However, the reasons for this were different than we had expected. Surprisingly, without PPARalpha, the PPARgamma transgene caused greater induction of downstream genes that regulate lipid uptake and oxidation. The heart levels of lipids were not reduced. However, the lipids were stored in larger droplets. With Dr. Abel, we found that mitochondria function was not altered but in line with the gene expression data, fatty acid oxidation by isolated perfused hearts was increased. These data show that in this model of diabetic cardiomyopathy, neither triglyceride storage pools nor fatty acid oxidation is toxic.

**Plans for the year and completion of the project:** Along with colleagues at Columbia we are studying the cause of premature death in these PPARgamma mice. This appears to be increased ventricular fibrillation and we will determine the pathways that might invoke this. In addition, we will test whether the anti-diabetic drug pioglitazone alters heart function and rhythm of wild type and MHC-PPARgamma mice.

## 2. Collaborations:

**Within the AMDCC:** The Goldberg/Fisher Project has developed an on-going collaboration with Dr. Abel to assist with the evaluation of cardiomyopathic mice (see above).

***With Jax*:** Inducible hAR mice were made with Jackson Laboratories.

**Outside the AMDCC:** An ongoing collaboration has continued with Dr. Breslow, Rockefeller U. Dr. Fisher has established a collaboration with Dr. L. Chan (Baylor) to utilize helper dependent

adenoviral infection to reverse hypercholesterolemia and atherosclerosis in Ldlr-/- mice. Drs. Goldberg and Fisher have collaborated with R. Ramasamy (NYU) to study the effects of hAR in atherosclerosis and in regression.

### 3. Address previous EAC comments:
### Comments of EAC are in bold

1. **hAR-Tg mice were crossed onto the apoE(-/-) background. When diabetes is induced, these mice have accelerated atherosclerosis development compared to the diabetic apoE(-/-) animals. The hypothesis that aldose reductase plays an important role in cardiovascular complications continues to be investigated. Since no manuscript is listed, it is assumed that it is in preparation or there are ongoing studies investigating mechanism. In addition, tetracycline-inducible hAR vectors have been sent to JAX and founder lines have been produced. These mice are being bred to the LDLr(-/-) mouse and should be a significant aid to the study of atherosclerosis in general and specifically to diabetes-related vascular disease.** The EAC is correct. A manuscript showing the effects of generalized and endothelial cell specific hAR expression on atherosclerosis is currently in revision (ATVB). Also, the breeding of the mice from Jackson to *Ldlr*$^{-/-}$ mice has begun, after deciding with JAX the best founder line to try.
2. **MHC-hAR mice are being studied for myocardial phenotypes in conjunction with Dr. Abel. Are studies planned to investigate the effects of diabetes and/or insulin resistance?** This is a good suggestion. We are studying the effects of the MHC-hAR on genes and lipids both on the wildtype and PPAR alpha knockout background. Depending on the outcome of those studies, we might also study the heart insulin signaling pathway in more detail.
3. **Two models of plaque regression are described with at least one demonstrating inhibition by diabetes. LCM studies indicate increased expression of inflammatory markers and reduced expression of markers of M2 macrophages. What studies are planned to investigate this interesting finding?** As noted, we are currently investigating two aspects of this study. 1. The effects of hyperglycemia per se on circulating monocytes, number and expression of inflammatory markers. 2. The effects of glucose reduction on regression itself.
4. **The model of reversible hypercholesterolemia is interesting as is the exacerbation of some injury using the hAR. Investigation of injury in other target tissues in addition to large vessels seems an economy that the AMDCC provides. The plans for the next year should have been more explicit. Productivity seems modest.** We agree. However, the AMDCC MMPC arrangement would require this project to fund these investigations, e.g. evaluate the renal effects of reduced cholesterol and reversal of hyperglycemia.
5. **The goal of this project is to study atherosclerosis and heart failure in diabetes, with the idea of separating the effects of diabetes from the effects of hyperlipidemia. The investigators have made good progress during the last year; two manuscripts are being prepared for submission, although it is unclear of the timeframe of one of them (one is in revision, one in preparation). The investigators have established collaborations with Dr. Abel (U of Utah) for cardiomyopathy studies, and have sent vectors to JAX for production of hAR mice. The investigators continue to work to separate the effects of glucose from hyperlipidemia on these cardiovascular parameters. The PIs are encouraged to continue the STZ-Reversa mouse studies of macrophage function.** We agree and are continuing these studies. The paper on the effects of diabetes on lesion regression is in press.
6. **Has all of the relevant data from these publications been uploaded to the website?** Our publications have a supplementary data. For the cardiomyopathy study, gene array and lipidomics data are included. All the relevant data in the atherosclerosis regression study are included in the manuscript.

## 4. Publications (10-11):

Ravichandran R, IJ Goldberg. Aldose reductase and cardiovascular diseases, creating human-like diabetic complications in an experimental model. Circ Res, 2010 106:1449-58. PMID: 20466987

PPARγ-induced cardiolipotoxicity in mice is ameliorated by PPARα deficiency despite increases in fatty acid oxidation. Son NH, Yu S, Tuinei J, Arai K, Hamai H, Homma S, Shulman GI, Abel ED, Goldberg IJ. J Clin Invest. 2010 120:3443-54. PMID: 20852389

Parathath S, L Grauer, L-S Huang, M Sanson, IJ Goldberg, EA Fisher. Diabetes mellitus inhibits macrophage depletion during atherosclerotic plaque regression in mice. Diabetes, accepted.

**Submitted**

Vedantham S, H-L Noh, R Ananthakrishnan, N Son, T Ravindranath, K Hallam, Q Li, Y Hu, S Yu, R Rosario, Y Lu, N Reiniger, X Shen, K Drosatos, LA Huggins, AM Schmidt, IJ Goldberg, R Ramasamy. Human Aldose Reductase Expression Accelerates Atherosclerosis in Diabetic ApoE-/- Mice