Animal Models of Diabetic Complications Consortium
(U01 DK076133)

Annual Report
(2008)

"Adiponectin and Nox4 in Diabetic Kidney Disease"
University of California at San Diego

Principal Investigator
Kumar Sharma

Address: **Center for Renal Translational Medicine**
9500 Gilman Drive, Stein Building Suite 405-7
UCSD/VASDHS
La Jolla, CA 92093-0711
Phone: **858-822-7484**
E-mail: **kusharma@ucsd.edu**

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Project 3 (if applicable): "Title" **Responsible Investigator: Name**	**X**

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Part A:

Principal Investigator's Summary

Our group at UCSD has made significant progress towards the goals of the AMDCC. During the preceding year we have published our work on the adiponectin KO mice and identified the podocyte as a key target cell that responds to adiponectin. The podocyte also has strong baseline AMPK activity that is reduced with high glucose and regulates the pro-inflammatory enzyme Nox4. These studies relevant to both type 1 and type 2 diabetes and has spurred work towards agents that mimic adipoectin and AMPK action for kidney disease as well as spurring work towards inhibiting Nox4 for kidney disease. The interactions within the AMDCC group of investigators, the Jackson Lab, program staff and the EAC have all played a major role in enhancing the pace of our work.

1. Program Accomplishments:

Hypothesis: Our overall hypothesis is that Adiponectin and Nox4 play key roles in diabetic kidney disease. More specifically, we are testing the hypothesis that adiponectin is the critical link between obesity and kidney disease and that Nox4 is the key inflammatory enzyme involved in mediating renal involvement with diabetes.

Recent Progress and Major Accomplishments

Adiponectin Project. The results of our study with the adiponectin KO mouse has proven to be of great interest in the nephrology and diabetes communities. The addition of streptozotocin-induced diabetes exacerbated the albuminuria and hydrogen peroxide generation in the adiponectin KO mouse. Lack of adiponectin further inhibited glucose-induced inhibition of AMPK activity and upregulated Nox4 expression in podocytes. The initial study was published in the JCI with an editorial commentary. Additional invited reviews on this work has been requested by Kidney International and Journal of the American Society of Nephrology. There has also been outside reviews on this work published in several journals. Several implications of this work are being pursued:

1. Role of adiponectin as a susceptibility factor for diabetic kidney disease. Based on clinical and our basic science studies, low adiponectin levels pre-dispose podocyte dysfunction in response to the stress of hyperglycemia. Our initial studies were with streptozotocin-induced diabetes at an early stage. We are completing our long-term studies with strep-induced diabetes at a late stage (12 mo) and with Akita induced diabetes. Chronic diabetes in the adiponectin KO mouse is associated with enhanced glomerular enlargement, kidney hypertrophy and mesangial matrix expansion. Surprisingly, the albuminuria did not progress and even diminished with age in both the wild type and adiponectin KO diabetic mice. The basis for the improvement in albuminuria is unclear although Dr. Leiter suggested the there may be islet cell regeneration with increased insulin levels in the low –dose strep groups. The data will be presented at the ASN annual meeting this year and a manuscript is in preparation.

2. Adiponectin regulates AMPK in podocytes. The role of AMPK in podocytes is likely to be a highly important area. As AMPK is the critical energy sensor for all cells, it is likely critical to mediate the response of cells to states of caloric imbalance. Our JCI study found that AMPK activity was predominant in podocytes of wild type mice and decreased with adiponectin deficiency. The question as to why podocytes have strong AMPK activity and how AMPK is regulated by high glucose are important questions. We have received funding from a VA Merit award to pursue these studies. Another implication of reduced AMPK activity is the potential down-regulation of the key mitochondrial biogenesis transcription factor peroxisome proliferator-activated receptor gamma coactivator-1 (PGC1a). We have found that PGC1a is down-regulated in the diabetic kidney and specifically in podocytes. This finding has the important implication that mitochondrial synthesis is reduced in the diabetic kidney and thus may be involved in the progressive disease of diabetic kidney disease. We are pursuing this question in the coming year and we are in agreement with the EAC to expand our collaborations to assess mitochondrial function in the diabetic kidney.

3. The effect of diet vs obesity vs hyperglycemia on adiponectin and kidney effects. In agreement the EAC and other members of the AMDCC, we have become interested in the effects of diet on renal function. Specifically we have found that the high fat diet increases albuminuria more so in the adiponectin KO mouse than in the wild type mouse. We have recently begun to explore this question by comparing normal rodent diets with high fat and low fat diets in wild type mice. We will be expanding these studies to the adiponectin KO mouse with and without Akita diabetes.

4. A major interconnecting pathway was identified by our demonstration that adiponectin, via AMPK activation, inhibits Nox4 protein levels. The mechanisms of regulation of Nox4 by adiponectin and AMPK will be followed up in subsequent studies. As Nox4 was found to be upregulated in podocytes of adiponectin KO and diabetic mice, a major question is the functional role of Nox4 in podocytes. The studies in aim 2 will directly address this question.

Nox4 project. We have made significant progress with the creation of an inducible, podocyte specific Nox4 transgenic mouse. Dr. Young You in our group has prepared and tested the construct. The construct was submitted to the Jackson Labs for creation of the mice. We then received 10 different lines of mice by June-July of 2009! Due to efficiency of the Jackson Labs group and Racheal Wallace in particular we are moving ahead of schedule and have already begun mating the tetOn-Nox4 tg mice with the podocin-rtTA mice. Thus far we have several litters that we have screened for bi-transgenic expression. There appears to be Mendelian inheritance thus far in the litters we have screened. The litters are undergoing administration of doxycycline to determine if there is inducible and cell specific expression of the Nox4 transgene. We will hopefully identify at least three separate lines that will demonstrate reproducible mild, moderate and high inducible over expression of Nox4 in podocytes. The mice will be an ideal model to test the role of the key renal inflammatory enzyme, Nox4, with and without diabetes in planned studies.

In studies performed with additional funds, we recently reported that the anti-fibrotic drug pirfenidone had beneficial effects in the db/db mouse model. In addition, a systems biology approach with proteomic data identified the mRNA translation pathway to be a new target of pirfenidone. Similar approaches will be pursued with our various animal models to better define the disease phenotype under various genetic manipulations.

Plans for the Upcoming Year

In the following year we will complete our studies examining long term diabetes in the adiponectin KO mice. Due to the transfer of institutions (from TJU to UCSD) we have been delayed in generating Akita diabetic adiponectin KO mice. These studies are now underway. As part of the phenotype analysis, we will examine GFR, blood pressure and degree of glomerular enlargement at various periods after the onset of hyperglycemia. These studies will determine whether adiponectin deficiency is sufficient for glomerular volume increases and if there is an additive role for hyperglycemia. In addition, we will examine the regulation of AMPK and mitochondrial function in these models. The further evaluation of glomerular enlargement and mitochondrial function have been encouraged by the EAC. Studies with the adiponectin receptor KO mice will be pursued as part of a separately funded project.

For our second project we will complete the characterization of the various lines of double transgenic mice for doxycycline inducible podocyte Nox4 upregulation and activity. The phenotype of the transgenic mice will be assessed to determine the specific role of Nox4 in podocytes independent of systemic diabetes. We will in parallel initiate studies to cross mice with Akita mice in the same background strain (FVB). Depending on the phenotypes in early and late stages of diabetes, further studies will be planned in Bl6 mice or in DBA mice.

Preliminary Milestones for 2010 and Beyond

By 2010 we plan to have published our studies with the strep-induced adiponectin KO mice. In addition, the mice will be studied with development of diabetes by crossing with Akita mice. The podocyte specific Nox4 inducible mouse has been generated and its phenotype will be characterized with and without diabetes. Additional studies with high fat feeding will determine the role of the adiponectin axis and Nox4 in development of obesity related complications. Mechanistic studies will be pursued with independent funds.

2. Collaboration:

With other PIs

During the past year we completed a sub-contract with Dr. Bottinger and the Mt. Sinai group. Specifically we studied the double KO decorin/LDL receptor mouse with diabetes. Surprisingly this mouse did not develop a severe renal phenotype. We are presently analyzing the data with high fat feeding in these mice. The negative data in this model was included in the AMDCC nephropathy review article coordinated and led by Frank Brosius.

We will initiate studies with Dr. Moshe Levi with respect to fat feeding studies in future studies, possibly with the adiponectin KO mouse and Akita mice.

Additional studies to characterize our mice with respect to cardiovascular paprameters is a major goal of our projects. We plan to send cardiac and aortic tissues to Drs. Ira Tabas and Dale Adel.

We have begun a collaboration with Dr. Smithies group at UNC. We have planned to collaborate by employing mitochondrial assays, proteomics and metabolomics. We are seeking additional funding for these projects. One project that we have greatly expanded is a collaborative project with Robert Naviaux at UCSD. Dr. Naviaux is a mitochondrial expert and has taken a major interest in the response of mitochondria to glucose excess in vivo. The ongoing studies are of great interest and will be presented at a future AMDCC meeting.

We are in process of generating the Akita adiponectin KO mice. Upon renal phenotyping we will work with Dr. Feldman to characterize the nerve findings. The mice will be available for additional cardiovascular and bladder phenotyping as well.

With Jax We have had great success with Dr. Ed Leiter and Racheal Wallace in the generation of the inducible tetOn human Nox4 transgenic mouse (HAHNox4tg). After receiving 10 founder lines, we are in process of mating the mice with the podocin rtTA mice and screening

the litters for doxycycline induced podocyte Nox4. Once sufficient lines have been well characterized, the mice will be studied for other diabetes related cardiovascular phenotypes. We hope to work with Drs. Abel and Tabas on these future studies.

With the MMPCs We have sent representative slides from our decorin KO and adiponectin KO diabetic mice to the University of Washington MMPC group for renal histology assessment. We have developed our core here at UCSD to perform semi-quantitative assessment of mesangial matrix expansion. It would be of great value to have the MMPC group standradrize and provide measurements of podocyte numbers in each of our diabetic groups.

With other non-AMDCC PIs Drs. Bary Goldstein and Dr. Kevin Williams of TJU have been co-investigators in our proposal. They have both left TJU within the past year and the collaboration within the scope of the AMDCC has been discontinued. We have developed new collaborators within UCSD, including Volker Vallon (renal GFR phenotyping), Robert Naviaux (mitochondrial measurements), Laura Dugan (in vivo imaging), Nigel Calcutt (neuropathy) and Daniel O' Connor (blood pressure).

3. Address previous EAC comments:

"The focus of the Sharma lab is the role of vascular wall oxidative stress in pathogenesis of diabetic nephropathy. A key finding was published in the JCI earlier this year, demonstrating that adiponectin downregulated Nox 4 activity by activating AMPK. Proposed work will focus on post-natal changes in Nox 4 expression/activity to promote vascular wall oxidant stress. Progress has been good; data high quality and interesting, with potential translational significance.

The adiponectin studies are interesting. The plan to study hemodynamics seems worthwhile. Is there a plan to pursue the differences in glomerular size?

The proposed collaboration regarding mitochondrial function also seems potentially informative."

Our present and proposed studies will address the comments of the EAC. Our JCI study was focused on podocytes and we will pursue the role of podocyte Nox4 in our ongoing studies. Further studies in collaboration with other groups will focus on cardiovascular and nerve roles of Nox4. We are also pursuing hemodynamic and glomerular measurements in the Akita adiponectin KO mouse. Mitochondrial studies have been very informative and mitochondrial function will be further characterized in our ongoing studies.

4. Publications in 2008-9 (relevant to AMDCC):

Please list

1. **Sharma, K.** RamachandraRao, S., Qiu, G., Kataoka Usui, H, Zhu, Y., Dunn, S.R., Ouedraogo, R., Hough. K., McCue, P., Chan, L., Falkner, B., Goldstein, B. Adipokines and Albuminuria: A Direct Communication between Adiponectin and Podocytes. ***Journal of Clinical Investigation***, **118**(5): 1645-1656 (2008). doi:10.1172/JCI32691.

2. Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, **Sharma K**, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le Couteur D, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de Cabo R. Resveratrol Delays Age-Related Deterioration and Mimics Transcriptional Aspects of Dietary Restriction without Extending Life Span. ***Cell Metab.*** 2008 Aug;8(2):157-68. Epub 2008 Jul 3.

3. Deelman L, **Sharma K.** Mechanisms of kidney fibrosis and the role of antifibrotic therapies. ***Curr Opin Nephrol Hypertens***. 2009 Jan;18(1):85-90.

4. Sanjoy Ghosh, Majid Khazaei, Farzad Moien Afshari, Lisa S. Ang, David J. Granville, Bruce Verchere, Stephen R. Dunn, Peter McCue, Andrew Mizisin, **Kumar Sharma**, and Ismail Laher. Moderate exercise attenuates caspase-3 activity, oxidative stress, and inhibits progression of diabetic renal disease in db/db mice ***Am J Physiol Renal Physiol*** (January 14, 2009). doi:10.1152/ajprenal.90548.2008

5. Sanchez AP, **Sharma K**. Transcription factors in the pathogenesis of diabetic nephropathy. ***Expert Rev Mol Med.*** 2009 Apr 28;11:e13.

6. Ramachandrarao SP, Zhu Y, Ravasi T, McGowan TA, Toh I, Dunn SR, Okada S, Shaw MA, Sharma K. Pirfenidone Is Renoprotective in Diabetic Kidney Disease. ***J Am Soc Nephrol***. 2009 Aug;20(8):1765-75. Epub 2009 Jul 2.

7. **Sharma K.** The link between obesity and albuminuria: adiponectin and podocyte dysfunction. ***Kidney Int.*** 2009 Jul;76(2):145-8. Epub 2009 Apr 29.

8. Frank C. Brosius III, Charles E. Alpers, Erwin Bottinger, Matthew D. Breyer, Thomas M. Coffman, Matthias Kretzler, Susan B. Gurley, Raymond C. Harris, Edward H. Leiter, Moshe Levi, Richard McIndoe, **Kumar Sharma,** Oliver Smithies, Katalin Susztak, Noboyuki Takahashi, Takamune Takahashi, for the Animal Models of Diabetic Complications Consortium. Mouse Models of Diabetic Nephropathy. ***J Am Soc Nephrol.*** 2009 in press.

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Part B:

Update by Individual Project Leaders
(not applicable)

Project 1 (if applicable): "Title"

Responsible Investigator: Name

1. Project Accomplishments:

Hypothesis

Recent Progress and Major Accomplishments

Plans for the Upcoming Year

Preliminary Milestones for 2009 and Beyond

2. Collaboration:

With other AMDCC PIs

With Jax

With the MMPCs

With other non-AMDCC PIs

3. Publications:

Please list

Project 2 (if applicable): "Title"

Responsible Investigator: Name

1. Project Accomplishments:

Hypothesis

Recent Progress and Major Accomplishments

Plans for the Upcoming Year

Preliminary Milestones for 2009 and Beyond

2. Collaboration:

With other AMDCC PIs

With Jax

With the MMPCs

With other non-AMDCC PIs

3. Publications:

Please list

Project 3 (if applicable): "Title"

Responsible Investigator: Name

1. Project Accomplishments:

Hypothesis

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2. Collaboration:

With other AMDCC PIs

With Jax

With the MMPCs

With other non-AMDCC PIs

3. Publications:

Please list