AMDCC Annual Report (2011)

PI: SHARMA, KUMAR
Project Title: Adiponectin and Nox 4 in Diabetic Kidney Disease
Grant Number: U01 DK076133

Abstract: During the first phase of the AMDCC our group at TJU studied the decorin KO diabetic mouse. Our hypothesis was that decorin acts as an endogenous protective factor by inhibiting active TGF-b. We found that decorin is indeed protective, as decorin KO mice had accelerated kidney disease and surprisingly increased mortality. Based on our results, the AMDCC investigators as a group chose the decorin KO diabetic mouse as a leading success during the 1st funding period. Of major mechanistic interest, we found that decorin KO diabetic mice that died exhibited evidence of renal insufficiency and low plasma adiponectin levels, months prior to mortality. This is similar to the human clinical condition. Low adiponectin levels are a powerful biomarker of increased cardiovascular morbidity and mortality in patients with kidney disease. Additionally, the decorin KO mice had increased NADPH oxidase (Nox4) expression in the kidney which may contribute to more severe nephropathy. In the next phase of the AMDCC, we propose test the following hypotheses. 1. Deficiency of adiponectin in combination with lack of decorin leads to enhanced lethality and diabetic nephropathy and 2. Increased Nox4 in vascular smooth muscle cells enhances diabetic nephropathy and the vascular complications of diabetic kidney disease. We propose to generate new diabetic mouse models by crossing adiponectin KO mice with decorin KO mice and by generating mice transgenic for smooth muscle Nox4 using the SM22 promoter. Diabetes will be induced by crossing with Akita mice. These mice will be characterized for diabetic nephropathy and cardiovascular disease. Both new models will be fully characterized for diabetic nephropathy. More importantly, we propose a series of interventional studies to test the causal role of TGF-b, adiponectin, and Nox4 in the pathogenesis of accelerated diabetic nephropathy. Key features we will focus on include matrix accumulation, renal function, autoregulation, and mortality. The proposed studies are directly related to the human condition, in which TGF-(, adiponectin, and Nox4 are key biomarkers for worse disease, but their pathogenetic role is unproven. Our findings will advance our mechanistic understanding of diabetic nephropathy and vascular disease and may lead to better biomarkers and novel treatments.

1. Program Accomplishments:

Hypothesis

Our original proposal sought to determine the role for adiponectin and Nox4 in diabetic kidney disease. **Our hypothesis was that adiponectin and Nox4 are key modifier genes for diabetic nephropathy and diabetic cardiovascular complications.** The animal models that we originally proposed remain the same.

a. diabetic adiponectin KO mouse
b. an inducible tissue specific Nox4 transgenic diabetic mouse

Progress toward stated aims

Recent Progress and Major Accomplishments
Project a. In the annual report of 2010 we reported on the effect of adiponectin deficiency with chronic diabetes. We had hoped to submit the manuscript last year but due to a delay in accumulating all of the data the manuscript will be submitted this year. Essentially, the data demonstrated that there was a modest worsening of diabetic kidney disease in the adiponectin KO mouse with chronic diabetes with low dose STZ. We had planned to add Akita induced diabetes to the *Apn* KO mouse, however due to loss of the colony we had to obtain more mice from Jackson Labs. These were backordered and it took some time to expand the colony. These studies are underway and should be completed in the next few months. A major outcome of our studies with the adiponectin KO mouse was the recognition that adiponectin played a beneficial role on podocytes via stimulating AMPK. We have expanded this concept and have studied the effect of AMPK regulation on diabetes and mice fed a high fat diet.

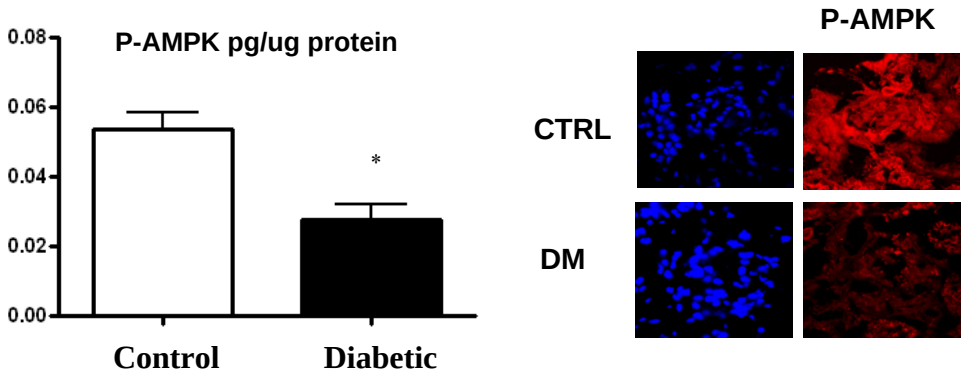

Figure 1. AMPK is reduced in diabetic mice. Wild type C57Bl6J male mice were made diabetic with the low dose STZ regimen and mice were examined after 6-8 weeks of diabetes. There is a reduction in overall AMPK activity as measured by an AMPK specific ELISA on renal tissue (left panel). By immunostaining there is a tubular and glomerular reduction of AMPK activity (right panel).

As AMPK was reduced in the diabetic kidney we postulated that there would be a consequent reduction of mitochondrial biogenesis as PGC1a the master regulator of mitochondrial

biogenesis is stimulated by AMPK. Indeed we found a reduction of PGC1a at the gene expression level and by immunostaining in glomerular podocytes (Figures 2 and 3).

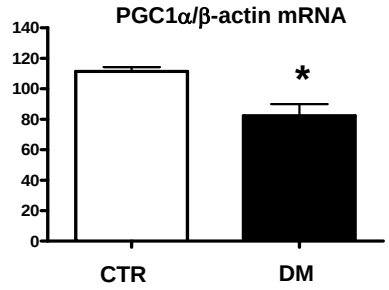

Figure 2. PGC1α is reduced in diabetic kidneys. PGC1a gene expression was measured in kidney cortex in control mice (CTR) and C57Bl6 mice made diabetic (DM) with low dose STZ after 6 weeks of hyperglycemia by real time PCR analysis (n=6 per group, *p< 0.05).

To determine the functional role of AMPK reduction in the diabetic mouse, both STZ-induced and Akita induced diabetic mice were treated with the AMPK activator, AICAR for a period of 10 days and after two months of diabetes.

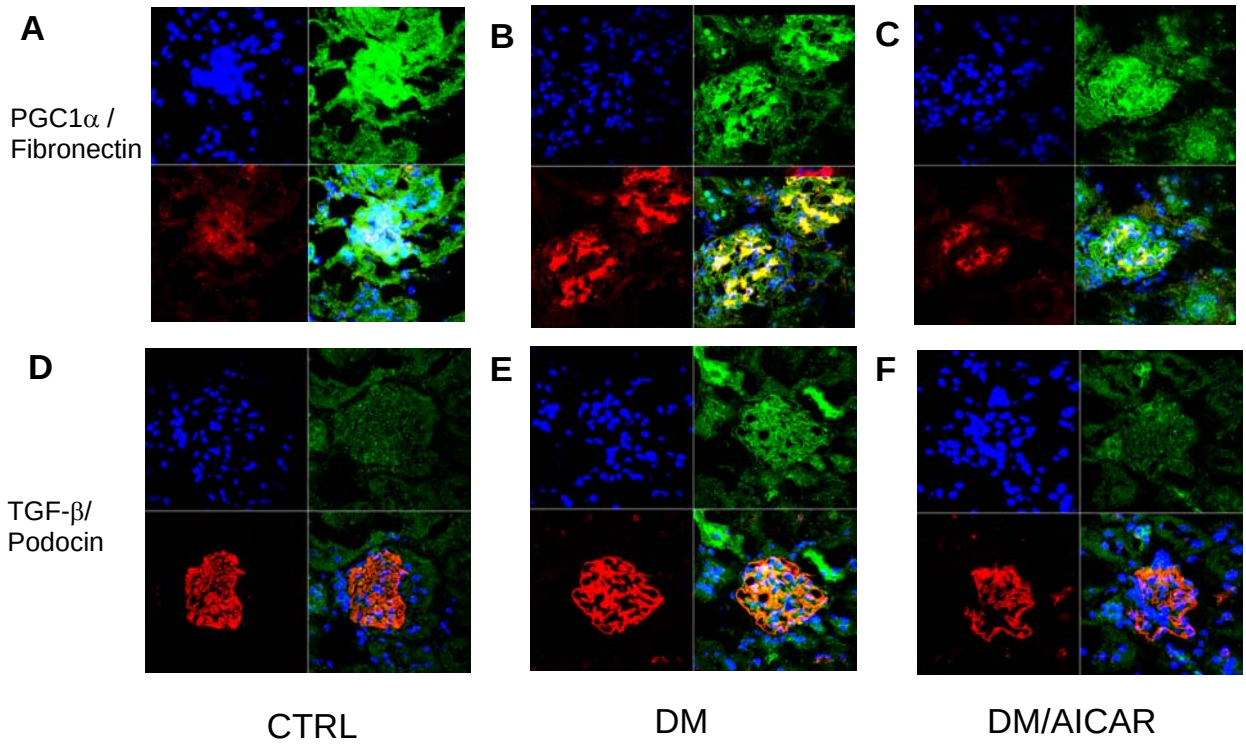

Figure 3. AMPK activation increases PGC1α and reduces markers of diabetic kidney disease. (A, B, C), AICAR treatment increased PGC1α (green) in the diabetic kidney

concurrently with a reduction in glomerular fibronectin (red). **(D, E, F),** AICAR treatment reduced glomerular TGF-β (green) in diabetic kidneys (podocin staining in red).

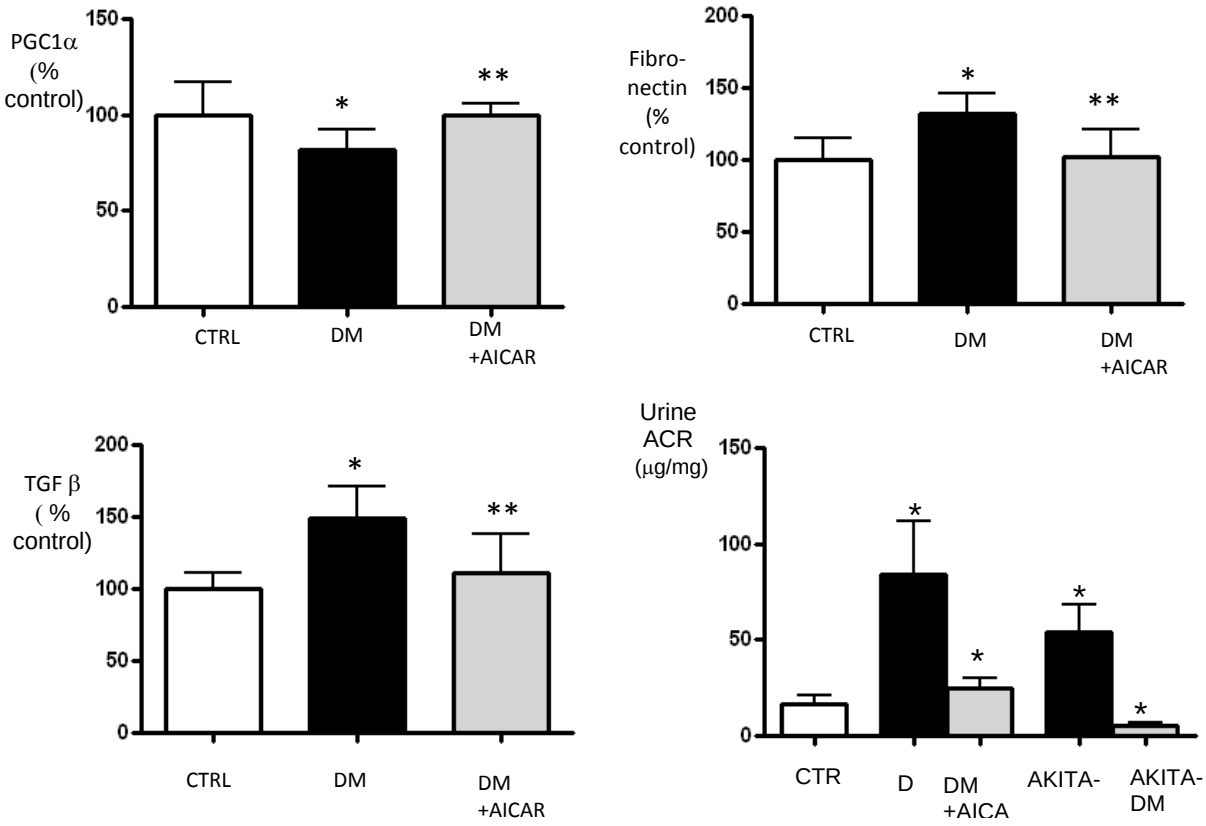

Figure 4. AMPK activation increases PGC1a, reduces fibronectin and TGF-b, and reduces albuminuria. Semi-quantitative data of immunostaning of PGC1α, fibronectin and TGF-β in glomeruli. ($n \geq 6$ per group, *$p<0.05$ vs control, **$p<0.05$ vs corresponding diabetic group)**.** AICAR reduced the urine albumin/creatinine ratio in the diabetic mice. ($n \geq 8$ per group, *$p<0.05$ vs control, **$p<0.05$ vs corresponding diabetic group).

These studies demonstrate for the first time that PGC1a protein levels are reduced in diabetic mouse kidneys and is regulated by AMPK activity. There is an associated reduction of fibronectin and TGF-b in glomeruli with AMPK activation. A manuscript is in final preparation to report our results. The role of AMPK will be studied in AMPK KO mice and the mechanisms of AMPK regulation of PGC1a and TGF-b will be pursued as part of an RO1 application. Recent studies have also identified PGC1a reduction in human models of obesity and type 2 diabetes suggesting that diabetes-related PGC1a regulation may be of primary importance to understand multiple organ pathologies associated with type 1 and type 2 diabetes.

Based on our studies with adiponectin deficiency and the known association of adiponectin with obesity and insulin resistance we have also performed studies to evaluate the role of the adiponectin-AMPK pathway with high fat feeding. In studies with wild type C57Bl6J mice fed a 60% high fat diet (HFD) we find that albuminuria begins to increase after 4 weeks whereas there is an earlier increase in urine hydrogen peroxide that is noted at 1 week after onset of the HFD.

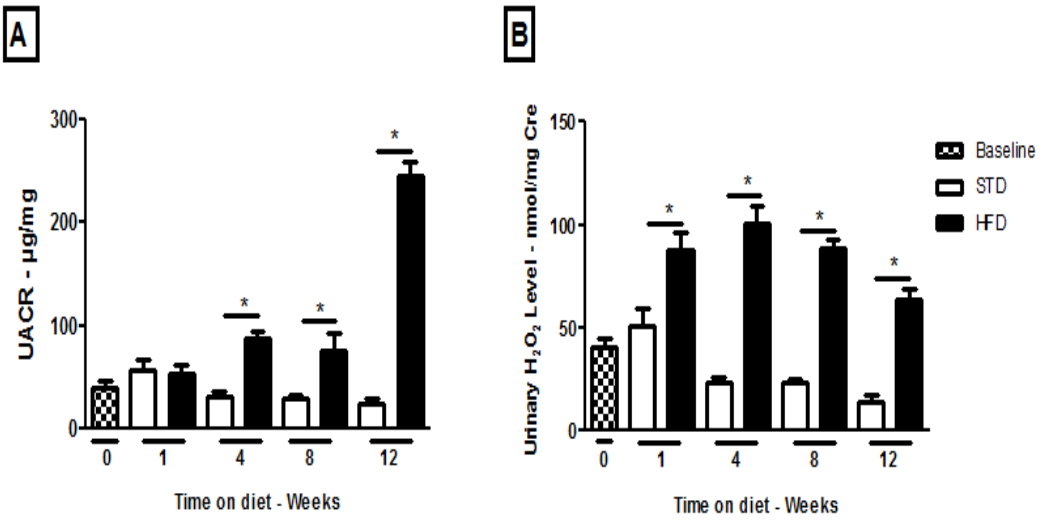

Figure 5. Natural history of urine markers in mice on HFD. Urine albumin/creatinine ratio (UACR) indicates elevation in UACR with HFD at 4, 8 and 12 weeks (A). Urine hydrogen peroxide (H_2O_2)/creatinine level in mice on high-fat diet (HFD) for increases by 1 week and remains elevated during the 12 week period (B). Urine MCP-1/creatinine level is increased in mice fed a HFD for 1 week. Values are means ± SEM. N=5 in each group. Statistical analyses were performed by one-way ANOVA followed by Newman-Keuls (A and B) *$p \leq 0.05$ *versus* mice on STD at corresponding timepoint.

At 12 weeks of the HFD there was a marked increase in glomerular pathology and a marked stimulation of inflammatory and pro-fibrotic gene expression.

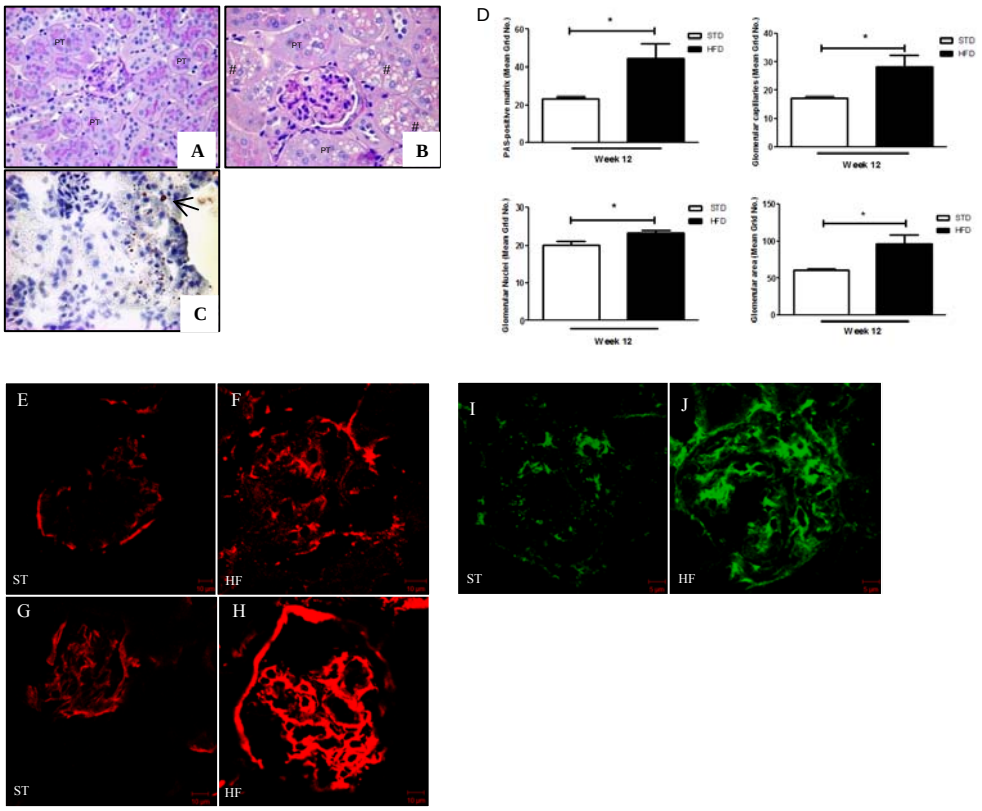

Figure 6. Renal cortical features from mice on STD and HFD. Mice fed a HFD displayed large glomeruli and vacuolated cytoplasm (#) symbol (A-B). Oil-Red O Staining on kidney sections on a HFD at 12 weeks: black arrow shows the accumulated neutral lipid (C). Quantitative analysis of glomeruli demonstrates increased mesangial matrix expansion, capillary filtration area, nuclei and overall surface area (D). Immunofluorescence microscopy of cortical sections demonstrates increased collagen type I (E-F), collagen type IV (G-H) and fibronectin (I-J) in mice fed a HFD (F, H & J) vs STD (E, G, I) for 12 weeks. Values are means ± SEM. N=5 in each group. Statistical analyses were performed by unpaired t-test *p ≤ 0.05 *versus* mice on STD.

Interestingly, at both 1 and 12 weeks of the HFD there was a reduction in AMPK activity in the kidney. Adiponectin was found to be reduced at 1 week but not at 12 weeks of the HFD. To determine the role of AMPK we administered AICAR to the HFD mice for 1 week. The reduced AMPK activity was increased by AICAR (Figure 7).

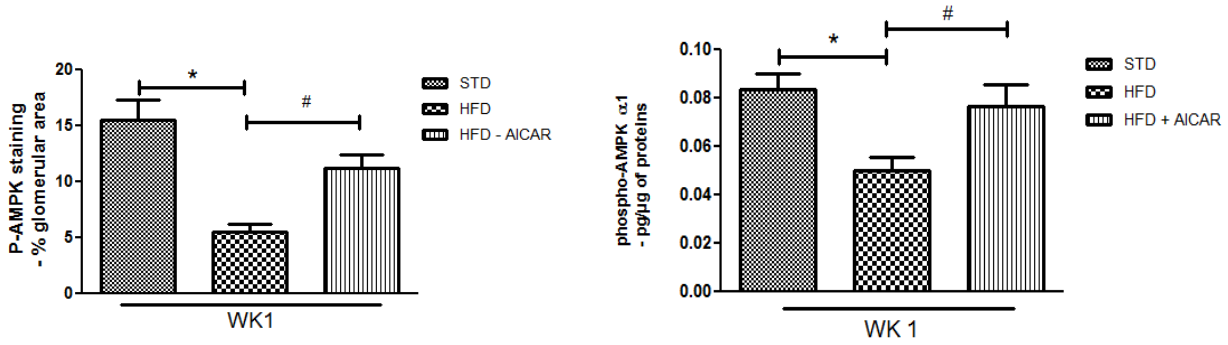

Figure 7. **Renal P-AMPK is reduced with HFD at 1 week and increased with AICAR.** Representative photomicrographs of p-AMPK (green) staining in glomeruli from mice on a STD (A), HFD (B) or HFD + AICAR (C) for 1 week. Nuclei were stained using DAPI (blue color). Semi-quantitative analysis of p-AMPK-positive staining per glomerular area (D), and quantitative analysis of p-AMPK level in the protein extractions from renal cortex (E) at week 1. Values are means ± SEM. N=6 in each group. Statistical analyses were performed by one-way ANOVA followed by Newman-Keuls *p ≤ 0.05 *versus* mice on STD; #p ≤ 0.05 *versus* mice on HFD.

In addition, the early markers of inflammation (urine hydrogen peroxide and urine MCP1) were significantly reduced by AICAR (Figure 8).

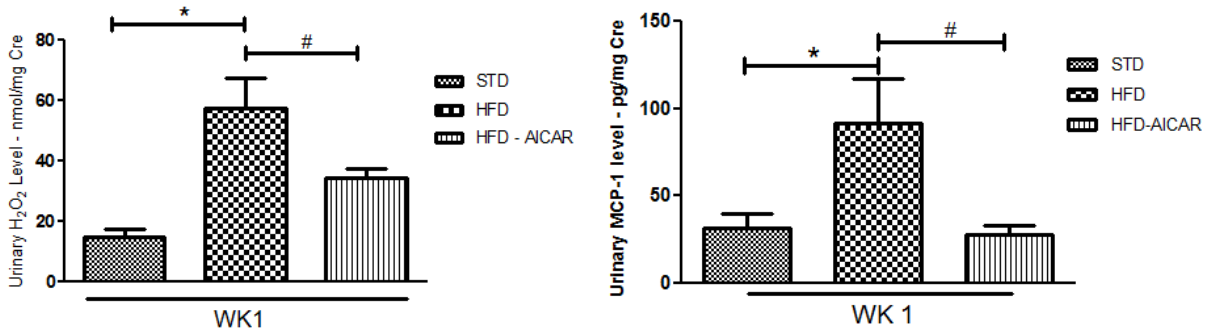

Figure 8. Urine markers of inflammation with HFD are reduced by AMPK activation. Urine hydrogen peroxide (H_2O_2)/creatinine level (A) and Urine MCP-1/creatinine level (B) in mice fed a STD, HFD or HFD + AICAR for 1 week. Values are means ± SEM. N=6 in each group. Statistical analyses were performed by one-way ANOVA followed by Newman-Keuls *p ≤ 0.05 *versus* mice on STD; #p ≤ 0.05 *versus* mice on HFD.

These studies show for the first time that AMPK activity plays a critical early role in HFD induced kidney disease in C57Bl6 mice. This study is under review at a high impact nephrology journal. Ongoing studies are examining the role of chronic AMPK activation and the role of adiponectin by administering the HFD to adiponectin deficient mice.

Project b. In the annual report of 2010 we reported on our preliminary results with the Nox2 KO mice with diabetes. We found that the degree of hyperglycemia was the same in the Nox2 KO diabetic mice as the wild type mice. Despite the lack of Nox2 there was no improvement in renal hypertrophy, albuminuria, urine hydrogen peroxide, or histologic PAS accumulation. To understand the role of NAPDH oxidase in diabetic kidney disease, we evaluated the gene expression of the various Nox isoforms and their membrane and cytosolic partners with multi-low dose STZ in Bl6J mice. Gene expression in wild type and Nox2 KO kidneys revealed that Nox2 and Nox4 are the most highly expressed in the normal kidney (Figure 9).

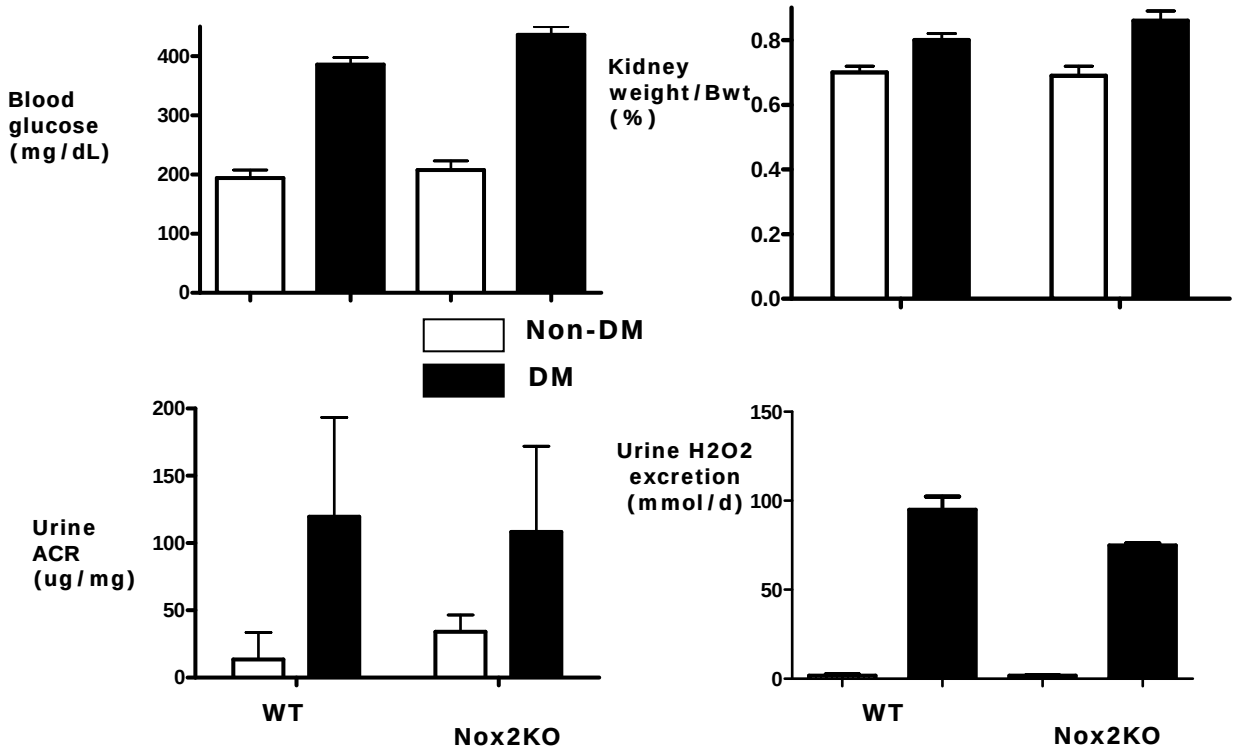

Figure 9. Wild type and Nox2 KO (gp91 KO) mice were made diabetic with multi low dose STZ protocol. There is a similar increase in blood glucose (upper left), kidney hypertrophy (upper right). Urine albumin (left bottom) and urine hydrogen peroxide levels were increased with diabetes but similar in WT and Nox2 KO mice.

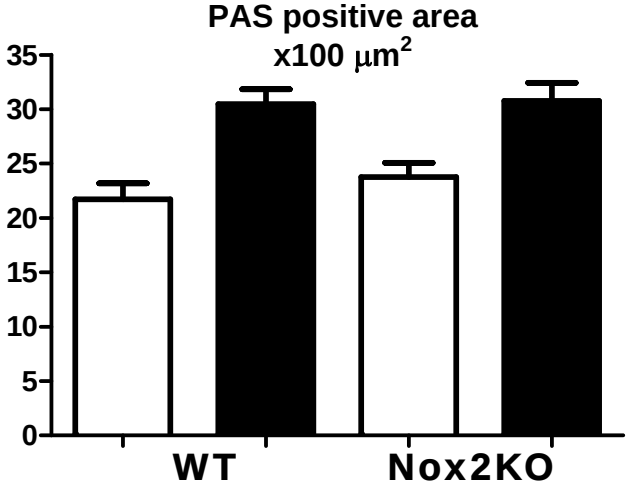

Figure 10. No effect of Nox2 on glomerular mesangial matric expansion. Mesangial matrix area is defined as the PAS-positive area (red area) in the tuft area. The mesangial matrix index represents the ratio of mesangial matrix area divided by tuft area.

In collaboration with Dr. Nigel Calcutt we also examined the degree of diabetic neuropathy in the wild type and Nox2 KO diabetic mice. With the multi-low dose regimen (50 mg/kg/d x 5d) there was an increase in paw thermal latency in the wild type diabetic mouse but not observed in the Nox2 KO diabetic mouse (data not shown). However the wild type diabetic mice did not show any reduction in nerve motor conduction velocity. Therefore the experiment was repeated with a higher dose STZ regimen (90 mg/kg/d x 2d) and mice were studied at 7 weeks of diabetes (Figure 10).

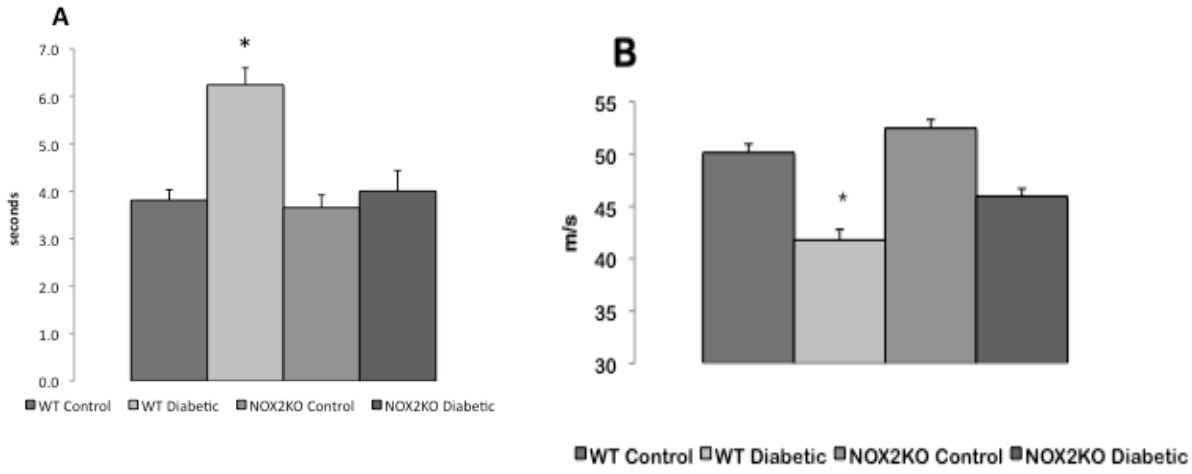

Figure 11. Paw thermal response latency (A) and sciatic nerve motor conduction velocity (B) in wild type and NOX-2 KO mice ± 7 weeks of diabetes induced by daily injection of 90 mg/kg STZ on 2 consecutive days. Data are group mean ± SEM. * = $p<0.05$ vs other groups by ANOVA with Dunnett's post-hoc test.

Interestingly, we found a greater degree of Nox2 vs Nox4 gene expression in dorsal nerve. Thus there appears to be a greater dependency of neuropathy with Nox2 than with nephropathy. Indeed, there was a marked stimulation of glomerular Nox4 in the Nox2 KO diabetic mice may have compensated for the reduction in Nox2 (Figure 12).

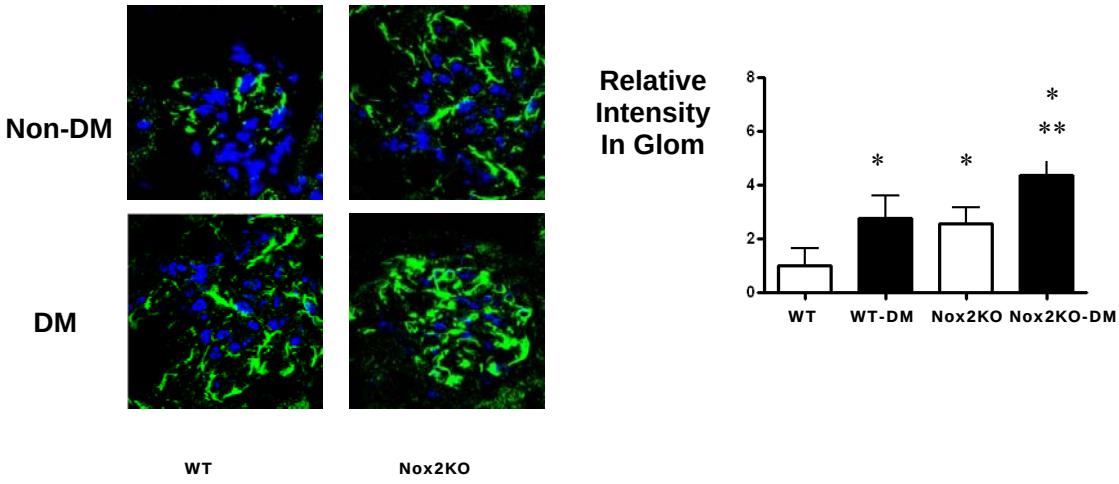

Figure 12. Nox4 expression was increased in the glomeruli of diabetic Nox2KO mice. Representative immunofluorescence images demonstrating expression of Nox4 in the glomeruli of wt (n=4) and Nox2KO mice (n=4) with semiquantitative analysis for Nox4. Sections were visualized by confocal microscopy. Original magnification was X100 objective. Quantitative shown on the right panel. *p<0.05 vs WT, **p<0.05 vs WT-DM.

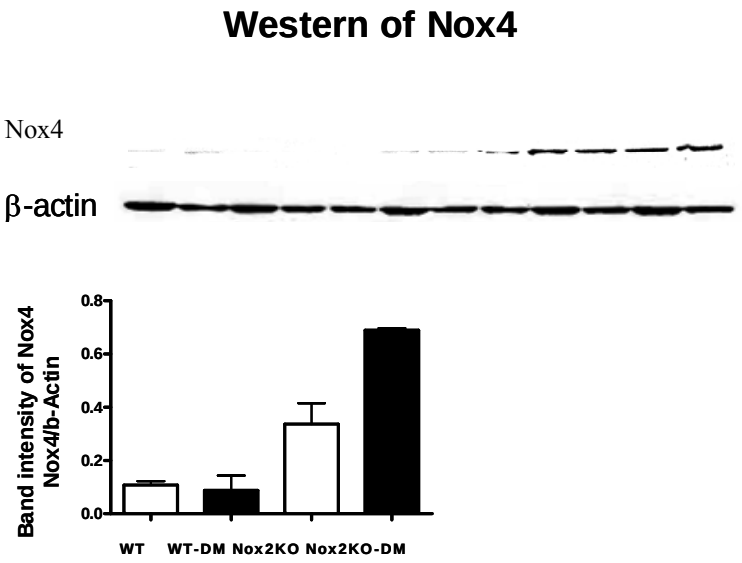

Figure 13. Immunoblot analysis of Nox4 in WT and Nox2 control and diabetic kidneys. Cortical lysates from control and diabetic kidneys (2m of diabetes) were immunoblotted with antibody for Nox4 and b-actin. Quantitative data presented below.

To evaluate the functional role of Nox4 we are taking two approaches. We recently performed a pharmacologic study evaluating the role of a Nox4 inhibitor on the F1 model of diabetic kidney disease. This data is presently being analyzed. In addition, as previously described in last years annual report we have successfully generated an inducible podocyte specific Nox4 tg mouse. We have begun the characterization of several lines that have robust transmission. We have identified 3 lines (46-high expressors, 56-moderate expressor, and 69-low expressors) that we are expanding.

We have previously reported that the Nox4 tg mouse with doxycycline exhibited increased glomerular size and GBM thickening. Although this pattern is clearly similar to early changes seen with diabetic kidney disease, the changes may not be specific for diabetic kidney disease. Ongoing studies will evaluate podocyte counts via WT1 staining and other markers of podocyte stress.

Initial studies with the high and moderate expressor mice demonstrate stimulation of the HA-tagged human Nox4 in the glomerular podocytes after 10 days of doxycycline administration (Figure 13). Furthermore, there appears to be subtle alterations of the podocyte foot processes and glomerular enlargement (data not shown). Thus, a single gene stimulation in podocytes recapitulates many of the cardinal features of diabetic nephropathy, without diabetes. Ongoing studies will evaluate the effect of diabetes on the Nox4 tg mouse. We expect that with persistent

upregulation of Nox4 coupled with hyperglycemia there will be a rapid progression of podocyte dysfunction leading to marked albuminuria, GBM thickening, and mesangial matrix expansion.

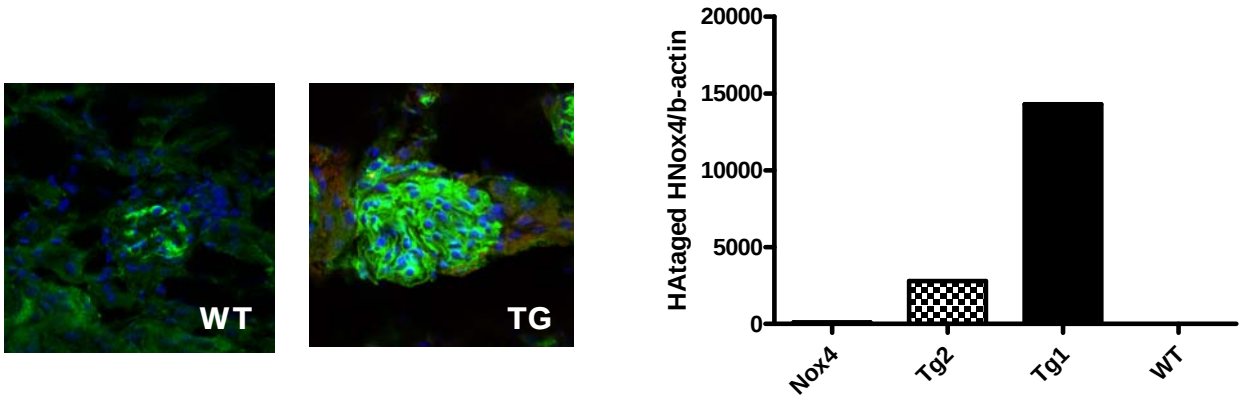

Figure 14. HA Tagged Human Nox4 transgene expression in glomerulus (A), Gene expression analysis of HA transgene by QRT-PCR in isolated GM from kidney by Dynabeads (B) 2 weeks after Doxycycline treatment in wild-type and RT/HAHNox4 bi-TG mice.

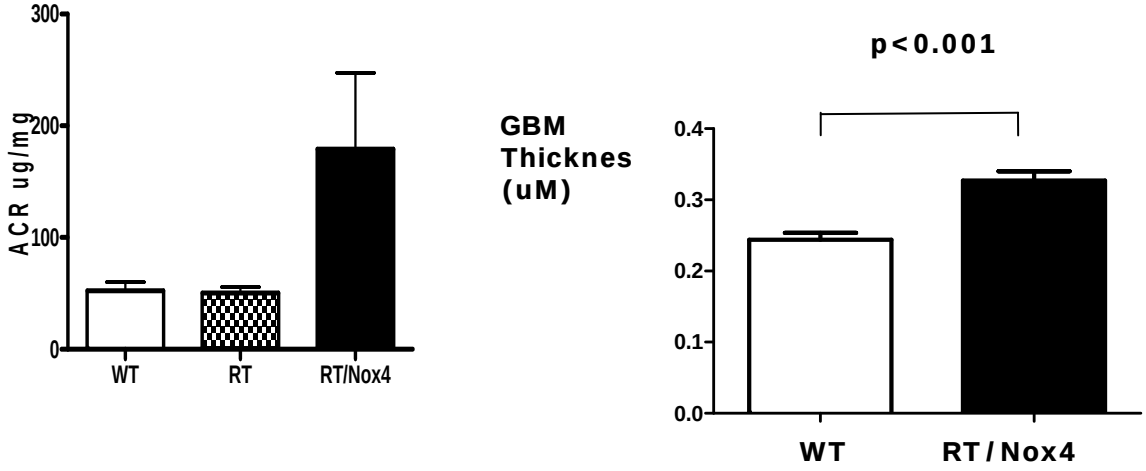

Figure 15. Two lines of high and moderate expressors for podocyte Nox4 tg mouse demonstrate an increase in urine ACR and GBM thickening. The urine ACR was measured in 2 mice per line and averaged together after 10 days of doxycycline. The GBM thickness was measured in coordination with EM analysis of glomeruli that was performed in collaboration with Dr. Marilyn Farquhar (UCSD).

We had planned to generate Akita mice carrying the transgene. However the FVB Akita mice have been backordered and due to the long duration it will require to generate adequate numbers of mice carrying the two transgenes and Akita on the FVB background, we have started to conduct the diabetic studies with the low dose STZ regimen. Further mouse lines will be

evaluated and it is expected that we will be able to study 3 separate lines of podocyte inducible Nox4 tg mice with diabetes by end of June. These studies are ongoing.

Plans for the year and completion of the project

The manuscript describing the chronic diabetic findings on kidney disease in the *Apn* KO mouse is being finalized and will be submitted shortly. We will complete the early characterization of the Akita diabetic *Apn* KO mouse. Both sexes will be studied as it is possible that adiponectin deficiency will lead to a more robust diabetes in females as well as male Akitas. Studies describing the effects of HFD on the kidney will be submitted. In addition the manuscript describing the role of AMPK in diabetic kidney disease will also be submitted.

The Nox2 KO diabetes manuscript will be submitted shortly. For the tgNox4 mice, we have discussed further plans with Racheal Wallace and have set up the following strategy. The 3 lines on the FVB background and with varying degrees of Nox4 expression will be expanded and made diabetic with the low dose STZ regimen. The data with Nox4 overexpressing mice and with a Nox4 inhibitor will be submitted in the next few months. The 3 lines will be placed onto the Bl6 background in order to study its role in this strain and determine if the strain is protective. Additional studies with the inducible Nox4 tg mice can be studied in other organs and cell types with cell specific promoters on the Bl6 background. By end of funding period, we will have completed the characterization of diabetes and HFD with adiponectin deficiency.

2. Collaborations:

With other AMDCC PIs

We have worked closely with other members of the AMDCC for an updated review article published last year. We will make available several new methods for phenotyping diabetic mice as they become accepted for publications. These methods will include mtDNA analysis and urine metabolomic analysis. We are continuing to run HPLC creatinines for consortial and non-consortial members. The protocols will be added to the website.

With Jax

We are working closely with Racheal Wallace to identify the best lines to expand of our Nox4 tg lines and to backcross the tg to the Bl6 background. This collaboration has been excellent and there are now three lines available at JAX in the B6 and FVB background.

With the MMPCs

We will be submitting the samples from our latest Nox4 tg mice to Dr. Alpers for validation of our findings with light and EM analysis.

With other non-AMDCC PIs

We have performed neuropathy studies with Dr. Nigel Calcutt and found that Nox2 plays a key role in diabetic neuropathy. We have begun to evaluate diabetic retinopathy with Dr. Kang Zhang at UCSD who is an expert in diabetic retinopathy. These studies are in the F1 B6/DBA mouse with Akita induced diabetes at 6 months of age.

3. **Address previous EAC comments:**

Please address each comment

- Has all of the adiponectin, Nox and decorin data been uploaded to the website?

We have uploaded almost all of the data from the diabetic adiponectin, Nox and decorin studies to the website. This will be completed by end of April.

- A compensatory increase in NOX4 is mentioned, but data is not shown. The reason for choosing NOX4 for transgenic amplification rather than NOX is not made clear. The studies of fat in the diet are interesting but do not seem in line with the other discussion or future plans. Productivity is difficult to judge with mixture of relevant and irrelevant publications.

The data showing upregulation of Nox4 is now shown in the diabetic glomeruli by immunostaining and in the cortex by western analysis. The data using the Nox2 KO mice clearly demonstrates that Nox2 is not necessary for diabetic renal pathology. The high fat studies are clearly relevant as adiponectin deficiency was first identified in the model of HFD in mice and adiponectin levels are reduced in humans with insulin resistance and obesity. In addition, we find an early stimulation of urine hydrogen peroxide with the HFD. This may well be due to Nox activation. As the mouse glomerular pathology is much more severe with the HFD in the Bl6 mouse this is a very useful model to examine the relevant pathways. Studying HFD in addition to diabetes is clearly relevant from the public health standpoint as this approach is used to examine the role of obesity in mouse models.

- Continues to pursue characterization of AMPK activity in DN pathogenesis and has generated Nox4tg animals with interesting glomerular phenotypes. Presented reasonable plans for upcoming year to complete ongoing projects.

The data with AMPK regulation and diabetic and HFD-related kidney disease demonstrates a convincing role for this central energy sensing pathway in mediating renal pathology. Future studies will examine the role of specific AMPK isoforms and pathways downstream of AMPK that relate to kidney disease. The Nox4tg studies are showing interesting results and we anticipate even more intriguing studies with the diabetic Nox4tg mice.

- Two projects are on-going. First, progress has been excellent on the adiponectin KO mouse, which manifests important features of diabetic nephropathy (albuminuria, elevated serum creatinine, modest glomerulomegaly, mesangial expansion). Plans call for studies of the Akita adiponectin KO mouse. Second, initial work with Nox2 KO and an inducible, podocyte-specific Nox4 transgenic mouse is promising, with altered H2O2 excretion (Nox2 KO) and GBM thickening and glomerulomegaly in the absence of diabetes (Nox4 Tg). Progress has been excellent. One concern: caution is warranted in the interpretation that GBM thickening in the Nox4 mouse in the absence of diabetes informs us about diabetic GBM thickening; a Pubmed search suggests some human conditions and many mouse conditions associated with GBM thickening. Unfortunately, in the absence of GBM thickening in another mouse model, the more direct confirmation that knock-down of Nox4 reverses GBM thickening is not available.

As noted we have had a delay in generating the Akita Apn KO mice but we expect to have further data with these mice in the next few months. The Nox4tg studies are progressing well. We agree with the EAC regarding the caution in relation to GBM thickening. We have recently performed studies with a specific Nox4 inhibitor and find benefit in glomerular pathology. Subsequent studies with EM analysis will be performed in the diabetic mice with the Nox4 inhibitor if it can reduce GBM thickening. Upon generation of a floxed Nox4 mouse (in future studies), we would be able to generate podocyte specific Nox4 deletion to determine if Nox4 is necessary for diabetic GBM thickening.

- 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.

We are actively working with Dr. McIndoe to upload all relevant data and identify all publications relevant to AMDCC. Some of the references below have been marked to be deleted and an updated list has been provided for 2010-11 publications.

4. Publications 2009-10:

1. Meijering, B. Els A. van der Wouden, Vincent Pelgröm, Robert H. Henning, **Kumar Sharma,** and Leo E. Deelman. TGF-ß inhibits Ang II-induced MAPK signaling in vascular smooth muscle cells through downregulation of Ang II type 1 receptors. ***Journal of Vascular Research***, 10:459-468, 2009

2. Burnt-out diabetes: impact of chronic kidney disease progression on the natural course of diabetes mellitus. Kalantar-Zadeh K, Derose SF, Nicholas S, Benner D, **Sharma K**, Kovesdy CP. ***J Ren Nutr***. 2009 Jan;19(1):33-7 **(to be deleted from list)**

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

4. Bakris GL, Fonseca VA, **Sharma K**, Wright EM. Renal sodium-glucose transport: role in diabetes mellitus and potential clinical implications. ***Kidney Int.*** 2009 Jun;75(12):1272-7. (to be deleted from list)

5. 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*** 2009 Apr;296(4):F700-8.

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

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. 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 Jul 2. [Epub ahead of print]

9. Brosius FC 3rd, Alpers CE, Bottinger EP, Breyer MD, Coffman TM, Gurley SB, Harris RC, Kakoki M, Kretzler M, Leiter EH, Levi M, McIndoe RA, **Sharma K,** Smithies O, Susztak K, Takahashi N, Takahashi T; for the Animal Models of Diabetic Complications Consortium. Mouse Models of Diabetic Nephropathy. ***J Am Soc Nephrol*** 2009 Sept 3 [Epub ahead of print]

10. Ix JH, Sharma K. Mechanisms Linking Obesity, Chronic Kidney Disease, and Fatty Liver Disease: The Roles of Fetuin-A, Adiponectin, and AMPK. ***J Am Soc Nephrol*** 2010 [Epub ahead of print]

11. Rossini M, Naito T, Yang H, Freeman M, Donnert E, Ma LJ, Dunn SR, Sharma K, Fogo AB.

Sulodexide ameliorates early but not late kidney disease in models of radiation nephropathy and diabetic nephropathy.. **Nephrol Dial Transplant.** 2010 Jan 7. [Epub ahead of print]

Additional publications to be added for 2010-11
(partly or completely supported by U01):

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