Animal Models of Diabetic Complications Consortium
(U01 DK076134)

Annual Report
(2008)

"Novel Mouse Models of Diabetic Nephropathy"
University of Colorado Health Sciences Center

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Part A:

Principal Investigator's Summary

1. Program Accomplishments:

Hypothesis

FXR is an important modulator of diabetic kidney disease: FXR activating ligands and/or FXR overexpression will prevent or attenuate diabetic kidney disease and FXR deficiency will worsen or accentuate diabetic kidney disease.

Progress toward stated milestones

I) In previous studies we have shown that treatment of db-db mice with obesity, hyperglycemia, and hyperlipidemia with FXR agonists significantly decreases urinary albumin excretion, glomerulosclerosis, and tubulointerstitial fibrosis by decreasing lipid accumulation, expression of profibrotic growth factors, proinflammatory cytokines, and oxidative stress.

II) In recent studies we have also found a similar effect of FXR activating ligands to decrease urinary albumin excretion, lipid accumulation, expression of profibrotic growth factors, proinflammatory cytokines, and oxidative stress in a model of diet induced obesity and insulin resistance in DBA/2J mice fed a high-fat and high-cholesterol (western) diet.

III) Since FXR expression per se is decreased in the kidney of mice with diet induced obesity and diabetes, we wanted to determine if FXR deficiency per se would accelerate diabetic kidney disease. For this purpose we obtained generalized FXR KO mice on the C57Bl/6 genetic background from Frank Gonzalez at NCI/NIH and we developed our colony at the University of Colorado. We performed experiments in the following 4 groups of mice:

1) C57Bl/6 mice fed a low fat diet and treated with streptozotocin

2) FXR KO mice fed a low fat diet and treated with streptozotocin

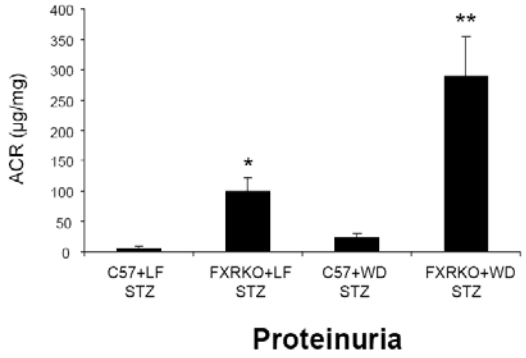
3) C57Bl/6 mice fed a western diet and treated with streptozotocin

4) FXR KO mice fed a western diet and treated with streptozotocin

The animals were followed for 12 weeks. Urine collection was obtained for measurement of urinary albumin and creatinine (ELISA). The animals were then anesthetized, blood was drawn and the kidneys were processed for **a)** histology, **b)** immunofluorescence microscopy, **c)** protein extraction and western blots, **d)** RNA extraction and QPCR, and **e)** lipid extraction and lipid composition analysis.

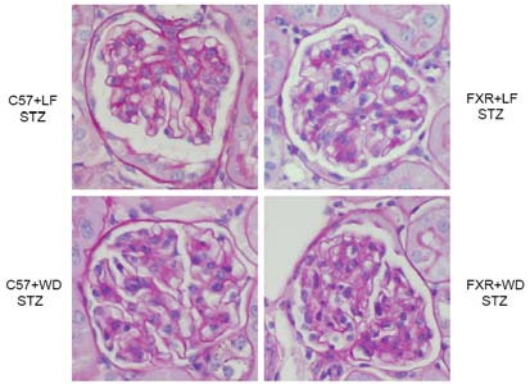
The results indicate that induction of diabetes in FXR KO mice leads to a marked 15-fold increase in albumin/creatinine ratio (ACR) when compared to similarly treated C57Bl/6 mice. Interestingly C57Bl/6 mice fed a western diet and treated with STZ had a 3-4 fold increase in

albuminuria. Urinary albumin excretion was further amplified in FXR KO mice fed a western diet and treated with STZ (**Figure 1**).

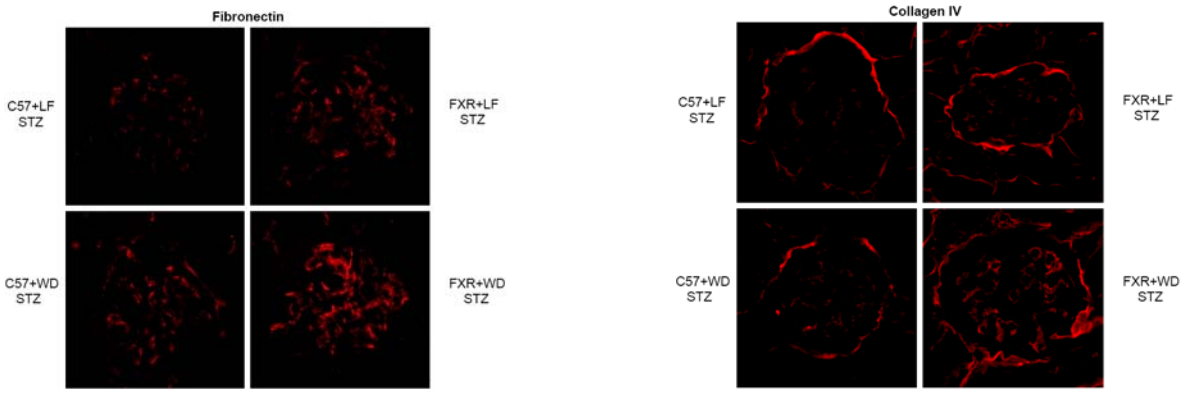


We have now started to determine the mechanisms for the markedly enhanced proteinuria in the FXR KO mice.

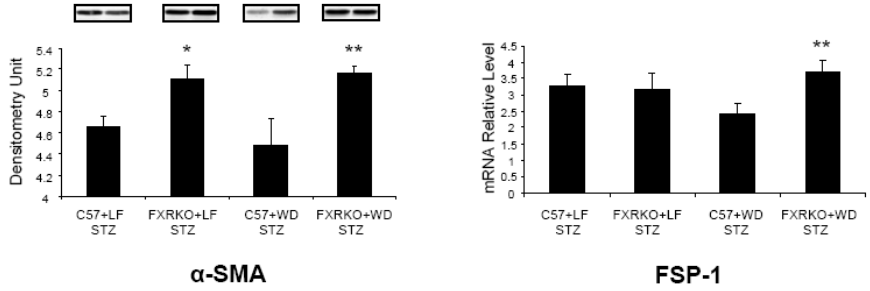
PAS staining shows that there is increased mesangial expansion in FXR KO mice (**Figure 2**).



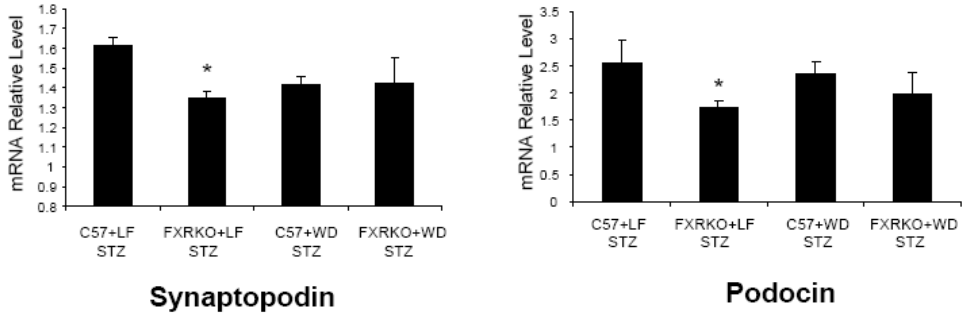
Immunofluorescence microscopy for extracellular matrix proteins shows increased accumulation of fibronectin (**Figure 3**) and type IV collagen (**Figure 4**).



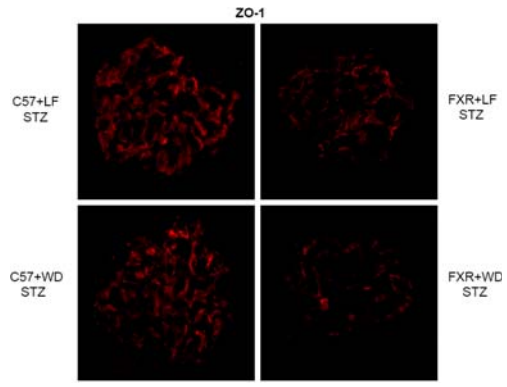
There are also increases in α-smooth muscle actin (α-SMA) protein and fibroblast specific protein-1 (FSP-1) mRNA expression, markers of EMT (**Figure 5**).



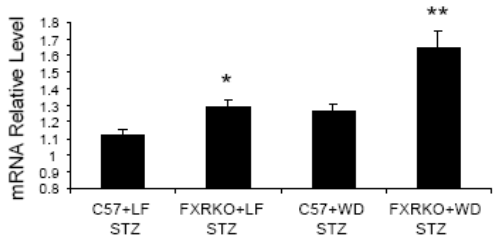
Unfortunately we did not process the kidney samples for Electron Microscopy (which we plan to do when we repeat the studies shortly) but we have probed for podocyte markers by QPCR, western blotting, and immunofluorescence microscopy. In RNA isolated from the cortex we have found decreases in synaptopodin and podocin mRNA levels in FXR KO mice treated with STZ (**Figure 6**).



In addition in kidney sections using immunofluorescence microscopy we have found a decrease in ZO-1 expression (**Figure 7**).

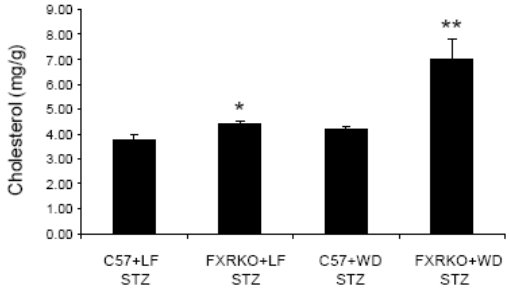


We have also found increased TGF-β mRNA expression in the kidneys of FXR KO mice which parallels the urinary albumin data (**Figure 8**).



TGF-β1

In addition we have also found a significant increase in kidney cholesterol content in the kidneys of FXR KO mice (**Figure 9**).



Kidney Cholesterol

These studies are ongoing in our laboratory and we will have more data during the next month and then we hope to submit these results for publication by the end of July 2008.

Plans for the Upcoming Year

We are going to repeat these studies in FXR KO mice on the **C57Bl/6** genetic background and at that time we will set up kidney samples for analysis by electron microscopy (EM) and we will also going to perform the studies in glomeruli and tubules isolated from the kidney.

Jackson Labs is now generating the FXR KO mice in the **FVB**, **DBA/2J**, and perhaps **BLKS** genetic backgrounds and as soon as they become available we will repeat the studies in these mice as well.

We are discussing with Matthias Kretzler and Frank Brosius about sending our samples from FXR KO mice for genome-wide transcriptional profiles.

2. Collaboration:

With other AMDCC PIs

Dr. Nobuyuki Takahashi for measurement of urine and serum creatinine by LC-MS

Dr. Matthias Kretzler and Dr. Frank Brosius for measurement of genome-wide transcriptional profiles of FXR KO mice

With JAX

Generation of FXR KO mice in FVB and DBA/2J genetic backgrounds

With the MMPCs

Seattle MMPC for kidney histopathology

With other non-AMDCC PIs

None yet

3. Address previous EAC comments:

a. Dr. Levi has phenotyped diabetic animals with FXR phenotypes and the appropriate strains of mice are being made at JAX (for crossing) to fulfill his aims. Lots of data suggests that the FXR knockouts on the diabetic backgrounds will yield promising models. Perhaps you should consider BKS instead of DBA since there are quite a few data on this background in the consortium already?

We have brought this to the attention of Jackson Labs

b. You mentioned development of a mesangial specific cre? This would be a very useful tool for the consortium.

Actually Dr. Sue Quaggin from U of Toronto is working on this

c. Thank you for agreeing to assist in a consortium wide review of our established nephropathy protocols (http://www.amdcc.org/shared/protocols.aspx).

I am working on this with Dr. Chris Ketchum and the rest of the AMDCC Nephropathy PIs.

4. Publications:

1. Proctor G, Jiang T, Iwahashi M, Wang Z, Li J and **Levi M**: Regulation of Renal Lipid Metabolism, Lipid Accumulation, and Fibrosis in Akita and OVE26 Mice with Type 1 Diabetes. Diabetes 55: 2502-2509, 2006

2. **Levi M**: Do statins have a beneficial effect on the kidney? Nature Clinical Practice Nephrology: 2: 666-667, 2006

3. Jiang T, Wang XX, Scherzer P, Wilson P, Tallman J, Takahashi H, Li J, Iwahashi M, Sutherland E, Arend L, and **Levi M**: FXR Modulates Renal Lipid Metabolism and Fibrosis and Diabetic Nephropathy: Diabetes 56: 2485-2493, 2007

4. Villa-Bellosta R, Bogaert YE, **Levi M**, Sorribas V: Characterization of Phosphate transport in rat vascular smooth muscle cells: Implications for vascular calcification: ATVB 27: 1030-6, 2007

5. Wang C, Sorribas V, Sharma G, **Levi M**, Draznin B: Insulin attenuates vascular smooth muscle calcification but increases vascular smooth muscle cell phosphate transport: Atherosclerosis 195: e65-75, 2007

6. Bauer T, Reusch J, **Levi M**, Regensteiner J: Skeletal Muscle Deoxygenation Following the onset of moderate exercise suggests slowed microvascular blood flow kinetics in type 2 Diabetes: Diabetes Care 30: 2880-2885, 2007