Animal Models of Diabetic Complications Consortium
(U01 DK076134)

Annual Report
(2010)

Novel mouse models of diabetic nephropathy: Role of FXR
University of Colorado Denver

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Part A:

Principal Investigator's Summary

1. Program Accomplishments:

Hypothesis:

The hypothesis of our proposal was that FXR deficiency especially in nephropathy susceptible genetic backgrounds will result in accelerated nephropathy including diabetic nephropathy.

Progress toward stated milestones:

Our previous studies had determined the consequences of FXR deficiency in 6 months old FXR null mice on C57BL/6 background fed a western (high fat and cholesterol) diet and STZ induced hyperglycemia.

We have now completed an additional study to determine if FXR deficiency per se would accelerate diabetic kidney disease using **FXR null mice on C57BL/6 background**, *which is a diabetic nephropathy resistant strain*. 2 month old wild type control and FXR null mice on the C57Bl/6 genetic background were made diabetic with STZ injection and were fed a regular chow. The animals were followed for 12 weeks after they achieved hyperglycemia. The metabolic data are summarized in **Table 1**.

We have seen that the induction of hyperglycemia with STZ results in a significant increase in proteinuria (**Figure 1**) which is associated with mesangial expansion (**Figure 2**), tubulointerstitial fibrosis (**Figure 3**), and podocyte damage (**Figure 4**). In addition diabetic FXR KO mice have increased expression of extracellular matrix protein fibronectin (**Figure 5**), increased kidney lipid accumulation (**Figure 6**), and macrophage infiltration (**Figure 7**).

Table 1

	WT	FXR KO	WT+STZ	FXR KO+STZ
Body weight (g)	31.5±0.29	26.8±0.70[a]	26.5±0.43[a]	23.3±0.36[bc]
Plasma glucose (mg/dl)	208±10	193±23	521±29[a]	453±34[c]
Plasma TG (mg/dl)	71.6±10.7	59.9±14.8	85.7±13.2	26.5±8.23
Plasma TC (mg/dl)	109±6	138±28	92±3	421±67[bc]
Food intake (g/kg)	117±19	109±11	208±23[a]	225±34[bc]

Data are means ± SE (n=4-6 mice in each group): [a] $p < 0.05$ vs. WT, [b] $p < 0.05$ vs. WT+STZ, [c] $p < 0.05$ vs. FXR KO

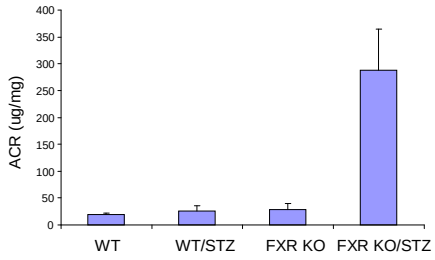

Figure 1: Diabetic FXR KO mice have pronounced albuminuria.

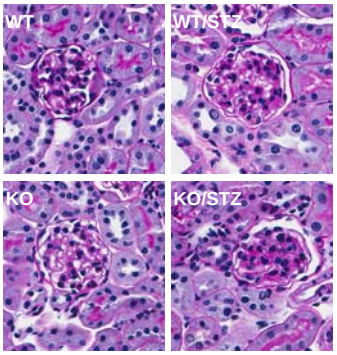

Figure 2: Diabetic FXR KO mice show severe mesangial expansion by PAS staining.

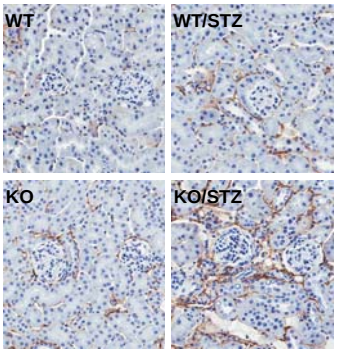

Figure 3: Diabetic FXR KO mice have increased tubulo-interstitial fibrosis shown by collagen III staining.

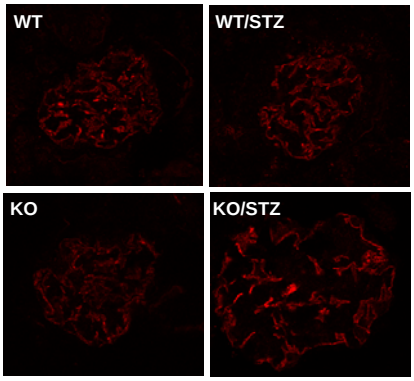

Figure 4: Diabetic FXR KO mice have less density of podocytes per glomerulus as shown by synaptopodin staining.

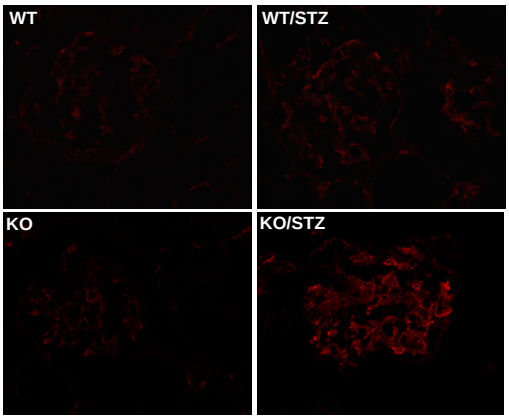

Figure 5: Diabetic FXR KO mice have increased expression of fibronectin in glomerulus.

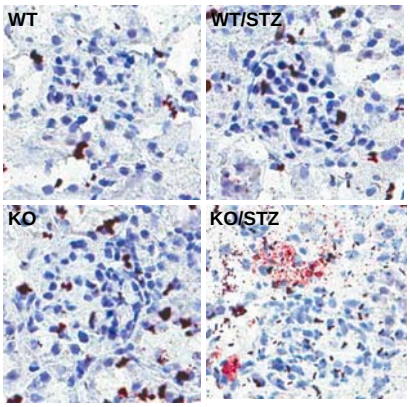

Figure 6: Diabetic FXR KO mice have increased renal lipid accumulation by oil-red-O staining.

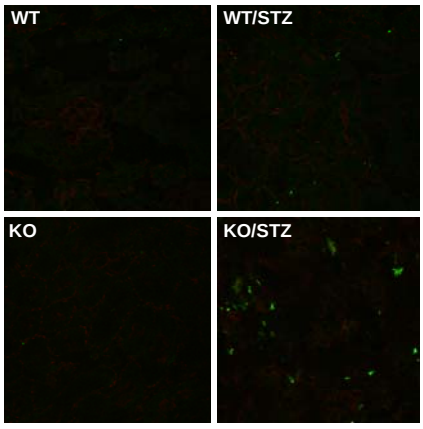

Figure 7: Diabetic FXR KO mice kidneys have increased macrophage infiltration shown by CD68 staining (green).

In additional studies with **FXR null mice on FVB/N genetic background** we performed a preliminary phenotyping to determine if FXR deficiency has adverse effects in the kidney. In 6-8 month old FXR KO mice on FVB/N genetic background, both male and female mice show severe glomerulosclerosis (**Figure 8**) even without hyperglycemia. However, the FXR KO mice on FVB background made hyperglycemic by introducing the Akita mutation caused additional damage to the kidney including more than10-fold increase of proteinuria (**Figure 9**), severe tubule atrophy and fibrosis (**Figure 10**), increased extracellular matrix protein level (**Figure 11**), lipid accumulation (**Figure 12**), and macrophage infiltration (**Figure 13**).

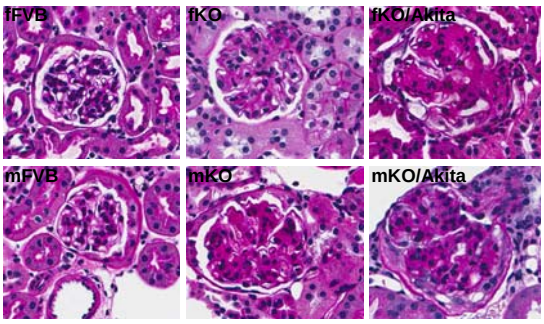

Figure 8: FXR KO mice on FVB background show severe mesangial expansion by PAS staining (f: female; m: male).

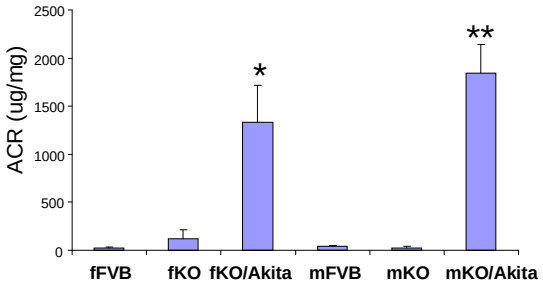

Figure 9: Diabetic FXR KO mice on FVB background develop dramatic albuminuria (f: female; m: male).

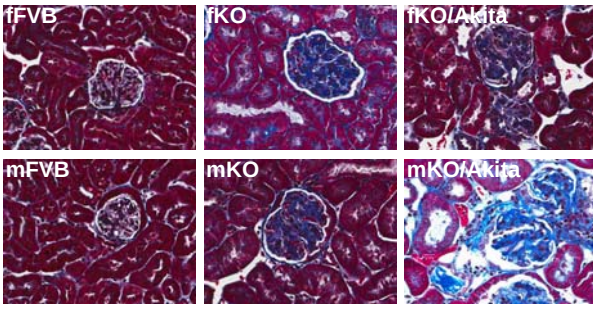

Figure 10: Diabetic FXR KO mice on FVB background have increased tubulointerstitial fibrosis especially for males as shown by Masson's trichrome staining.

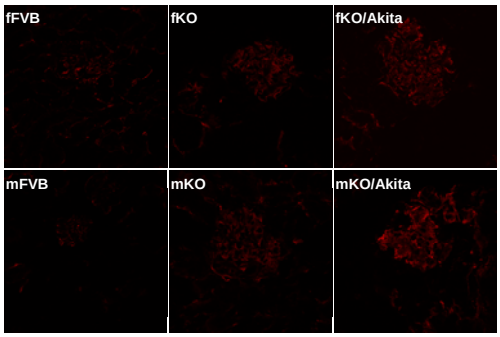

Figure 11: Diabetic FXR KO mice on FVB background have increased expression of fibronectin in glomerulus.

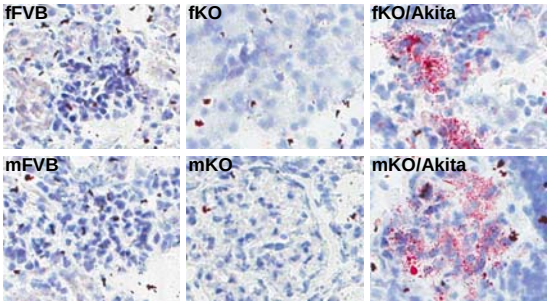

Figure 12: Diabetic FXR KO mice on FVB background have increased renal lipid accumulation by oil-red-O staining.

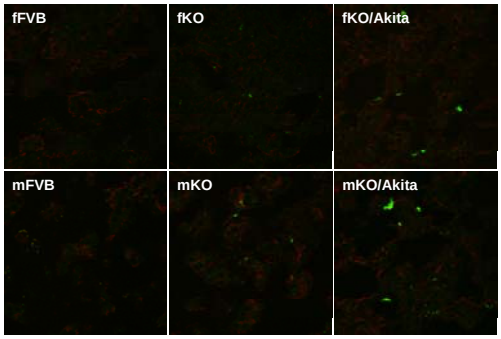

Figure 13: Kidneys from diabetic FXR KO mice on FVB background have increased macrophage infiltration shown by CD68 staining (green).

In summary, the study using 2 month old diabetic FXR KO mice on C57BL/6 background fed a chow diet demonstrated that generalized FXR deletion worsens kidney disease even without the added lipid burden by western diet in our previous study in 6 month old mice.

The phenotype of FXR KO mice on FVB/N background further emphasizes the importance of genetics in the role of FXR in diabetic nephropathy. It further provides the rationale, along with the beneficial effects of FXR activating ligands in prevention of diabetic nephropathy, for generating the new animal model with inducible constitutive FXR expression in podocytes proposed in Specific Aim 2.

Plans for the Upcoming Year

We are transferring the breeding of FXR KO mice on FVB/J background or on DBA/2J background from Jackson Labs to our own facility.

We are also breeding the corresponding Akita mice and we plan to cross breed them with the FXR KO mice.

Within next 3-6 months we will finish the phenotyping of both FXR KO mice on FVB/J background and DBA/2J background.

We are now studying cohorts of 1) Generalized FXR transgenic mice, 2) Inducible podocyte-specific FXR transgenic mice to test the effects of FXR overexpression in diabetic nephropathy.

In addition in 9 months we will have cohorts of 3) podocyte-specific and 4) proximal tubule-specific FXR KO mice available for the study.

2. Collaboration:

With other AMDCC PIs: With Ray Harris at Vanderbilt we are studying the effects of FXR ligands in eNOS KO x db-db mice.

With JAX: We are generating above strains of FXR mice.

With the MMPCs: We are using the Seattle MMPC for renal pathology.

With other non-AMDCC PIs: In collaboration with Jeffrey Kopp at NIH we are generating podocyte and proximal tubule specific FXR transgenic mice.

3. Address previous EAC comments:

EAC comments attached

- **Remain somewhat skeptical about FXR null mouse as model of human disease; fat deposition in kidney tissue not a characteristic of human DN. We hope the difference in phenotypes obtained at JAX (nephropathy not dependent on DM status) and Colorado (nephropathy in DM mice) will continue to be discussed by the Consortium.**

We agree that complete FXR deficiency, as well as complete eNOS or bradykinin receptor deficiency, may not be a common or prominent feature of human diabetic or non-diabetic kidney disease. We are however going to determine FXR expression and FXR activity in diabetic human samples.

However fat (lipid) deposition has been reported in diabetic human samples since the 1850s since the first description by Virchow. We are enclosing some of the references including the classical reference by Kimmelstiel and Wilson.

Kimmelstiel P, Wilson C: Intercapillary lesions in the glomeruli of the kidney. *Am J Pathol* 12:83-98, 1936

Newburger RA, Peters JP: Intercapillary glomerulosclerosis: A syndrome of diabetes, hypertension and albuminuria. *Arch Int Med* 64:1252-1264, 1939

Wilens SL, Elster SK: The role of lipid deposition in renal arteriolar sclerosis. *Am J Med Sci* 219: 183-196, 1951

Lee HS, Lee JS, Koh HI, Ko KW: Intraglomerular lipid deposition in routine biopsies. *Clin Nephrol* 36: 67–75, 1991

As a practicing nephrologist I have seen very commonly lipid deposits in the kidneys of subjects with type 1 diabetes mellitus and type 2 diabetes mellitus, even in the absence of abnormalities in serum lipids, when we have asked the pathologist to perform oil red o staining in fresh frozen sections. We are now writing an IRB to repeat this survey across many centers to perform either

oil red o staining in frozen human kidney biopsies or adipophilin (lipid droplet marker) immunohistochemistry in paraffin sections. At the same time we also plan to examine FXR and SREBP expression and activity in these biopsy samples.

- **The data in the report does not contain assessment of typical risk factors: blood sugar, weight/food intake and blood pressure.**

We have provided the body weight, food intake, and serum glucose and lipids in our manuscripts and we are now also providing the data in Table format in this report (**Table 1**).

We have recently acquired two blood pressure measurement systems: A) Tail-cuff and B) Telemetry and we will perform BP measurements with the next batch of FXR KO mice.

4. Publications:

Please list

Breusegem SY, Takahashi H, Giral-Arnal H, Wang X, Jiang T, Verlander JW, Wilson P, Miyazaki S, Sutherland E, Caldas Y, Blaine JT, Segawa H, Miyamoto K, Barry NP, and **Levi M**. Differential Regulation of the Renal Sodium/Phosphate Co-Transporters NaPi-IIa, NaPi-IIc and PiT-2 in Dietary Potassium Deficiency. *Am J Physiol Renal Physiol. 2009 Aug; 297(2):F350-61*. PMID: 19493963

Giral-Arnal H, Caldas Y, Sutherland E, Wilson P, Breusegem SY, Barry NP, Blaine JT, Jiang T, Wang XX, **Levi M**: Regulation of the Rat Intestinal Na-dependent Phosphate Transporters by Dietary Phosphate. *Am J Physiol Renal Physiol. 2009 Nov;297(5):F1466-75.* PMID: 19675183

Choudhury D, Tuncel M, **Levi M**. Disorders of lipid metabolism and chronic kidney disease in the elderly. *Semin Nephrol. 2009 Nov;29(6):610-20*.PMID: 20006793

Wang XX, Jiang T, Shen Y, Adorini L, Pruzanski M, Gonzalez FJ, Lewis L, Miyazaki-Anzai M, **Levi M**. Farnesoid X receptor modulates renal lipid metabolism and diet-induced renal inflammation, fibrosis and proteinuria. *Am J Physiol Renal Physiol. 2009 Dec; 297(6):F1587-96.* PMID: 19776172

Blaine J, Okamura K, Giral H, Breusegem S, Caldas Y, Millard A, Barry N, **Levi M**. PTH-induced internalization of apical membrane NaPi2a: role of actin and myosin VI. *Am J Physiol Cell Physiol. 2009 Dec;297(6):C1339-46.* PMID: 19776390

Lim RS, Kratzer A, Barry NP, Miyazaki-Anzai S, Miyazaki M, Mantulin WW, **Levi M**, Potma EO, Tromberg BJ. Multimodal CARS microscopy determination of the impact of diet on macrophage infiltration and lipid accumulation on plaque formation in ApoE-deficient mice. *J Lipid Res. 2010 Mar 5.* [Epub ahead of print]PMID: 20208058

Wang XX, Jiang T, **Levi M**. Nuclear hormone receptors in diabetic nephropathy. *Nat Rev Nephrol. 2010 Apr 27.* [Epub ahead of print] PMID: 20421884

Miyazaki-Anzai S, **Levi M**, Kratzer A, Ting TC, Lewis LB, Miyazaki M. Farnesoid X Receptor Activation Prevents the Development of Vascular Calcification in ApoE-/- Mice With Chronic Kidney Disease. *Circ Res. 2010 Apr 29.* [Epub ahead of print]PMID: 20431060

Wang XX, Jiang T, Shen Y, Caldas Y, Miyazaki-Anzai S, Santamaria H, Scherzer P, Lewis L, Gonzalez FJ, Adorini L, Pruzanski M, Kopp JB, Verlander JW, **Levi M**. Diabetic Nephropathy is Accelerated by Farnesoid X Receptor Deficiency and Inhibited by Farnesoid X Receptor Activation in a Type 1 Diabetes Model. *Diabetes* (in revision)