Animal Models of Diabetic Complications Consortium (U01 DK60994)

Annual Report (2004)

"Mouse Models of Diabetic Nephropathy and Neuropathy" University of Michigan and the University of Chicago

Principal Investigator
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1. Project Accomplishments (2004):

The overall goal of our center is to develop improved mouse models of diabetic complications especially for nephropathy and neuropathy. Our main strategy is to enhance diabetic injury by increasing glucose uptake and/or oxidative stress in podocytes in the kidney glomerulus and in peripheral neurons in order to augment diabetic injury. Therefore, we are focusing on models with genetic alterations that should change glucose transporter expression or increase oxidative stress in glomerular podocytes and in peripheral neurons.

Since we are investigating both complications, we have developed 3-4 models for each. Our general impression of the Consortium's findings and our own negative findings from simple models is that at least 2 "hits" are required to enhance diabetic injury. Thus, most of the models we are currently focusing on have a genetic abnormality plus an extra clinically relevant environmental stressor. Some but not all of these are the same for each complication.

Nephropathy models:
1) GLUT4 -/- Akita ± high fat diet
2) GLUT1 tg C57BL/6J db/db high fat diet
3) Nphs2 GLUT1 tg C57BLKSdb/db
4) GCLC +/- C57BL/6J db/db high fat diet

Neuropathy models:
1) GLUT1 tg C57BL/6J db/db high fat diet
2) GCLC +/- C57BL/6J db/db high fat diet
3) C57BLKS db/db
4) Nestin SOD2 knockout STZ diabetic.

Although some of the data below will be from these specific models, most will be from precursor models—often those with only one abnormality.

Major achievements:

1) *GLUT4 -/- C57BL/6J and podocyte specific GLUT4 -/- Streptozotocin mice.* As previously reported, the total body GLUT4 -/- model resulted in a doubling of albuminuria and a significant reduction in podocyte number. These results were somewhat paradoxical given our general hypothesis that enhanced glucose uptake should potentiate diabetic injury. Therefore, in collaboration with Dr. Dale Abel from the University of Utah AMDCC group, we tested the effects of low dose STZ diabetes on podocyte specific GLUT4 knockout mice (Nphs2 Cre//GLUT4 loxP/loxP STZ model). We have finalized our nephropathy data on the total body GLUT4 -/- mice and are near completion of the study on the podocyte-specific GLUT4 knockout mice. Neuropathy data are in progress on the total body GLUT4 -/- and control mice. Initial studies show modest increases in neuropathy (**Fig. 1**). STZ diabetes has produced very little neuropathy in all models tested so far. Therefore we are moving to test the effect of GLUT4 knockout on nephropathy and neuropathy in Akita mice.

As noted before the total body GLUT4 -/- mice had less profound diabetes in response to low-dose STZ, presumably because of compensatory increase of non-insulin dependent glucose transporters in skeletal muscle. Despite less severe diabetes the GLUT4 -/- mice manifested enhanced nephropathy as determined by albuminuria mesangial expansion and podocyte loss (shown in previous meetings and reports). In contrast, the podocyte-specific GLUT4 -/- mice have so far demonstrated no evidence of enhanced nephropathy. If anything, there is some protection against diabetic nephropathy changes in this model as mice with podocyte-specific GLUT4 knockout show reduced albuminuria (not shown) and

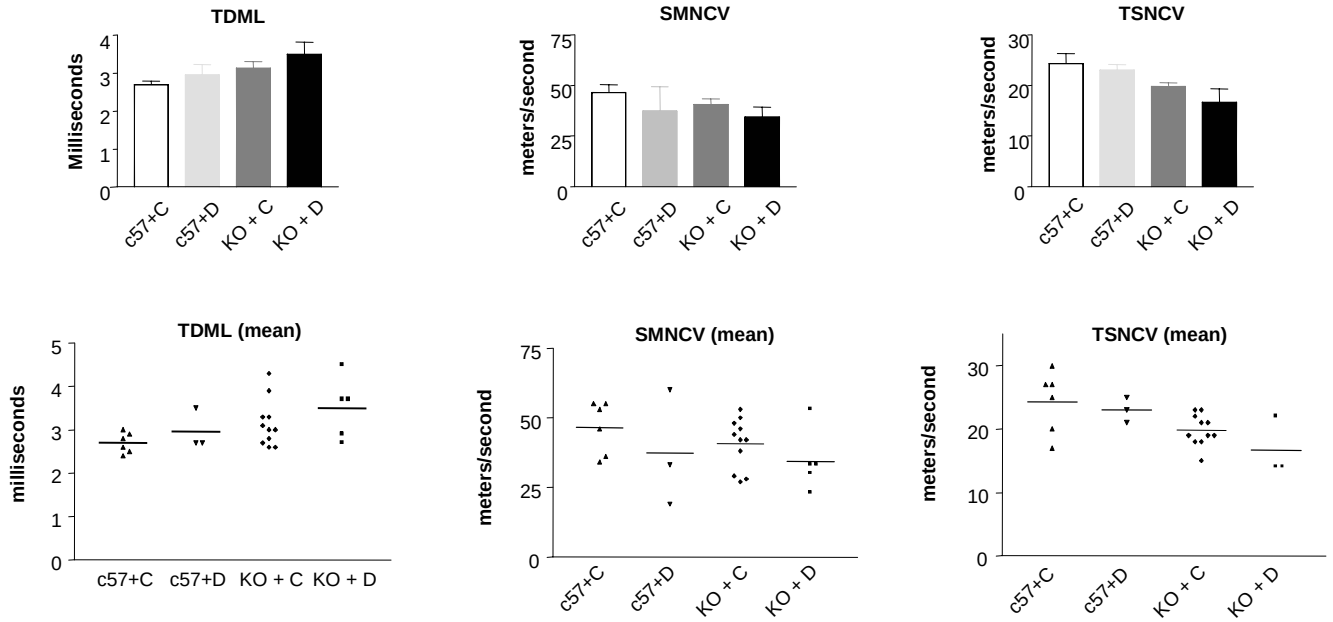

Fig. 1. GLUT4 ko and wild-type (c57) STZ (D) animals show modest decrements in nerve conduction velocities and increased latencies. These data suggest a somewhat more profound neuropathy in the GLUT4 ko animals.

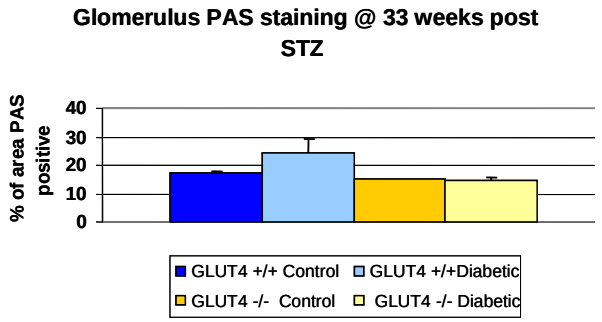

Fig. 2. STZ-diabetic podocyte specific GLUT4 knockout animals develop less profound mesangial expansion than do wild-type STZ mice.

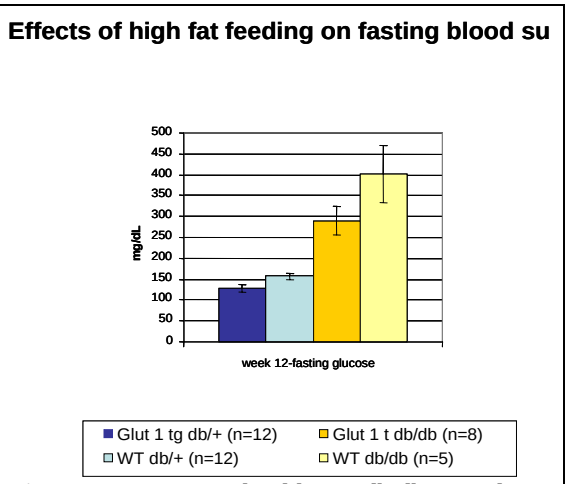

Fig. 3. GLUT1 tg and wild-type db/db animals on high fat diet show sustained fasting hyperglycemia at 12 weeks but FBS in GLUT1 tg animals was lower.

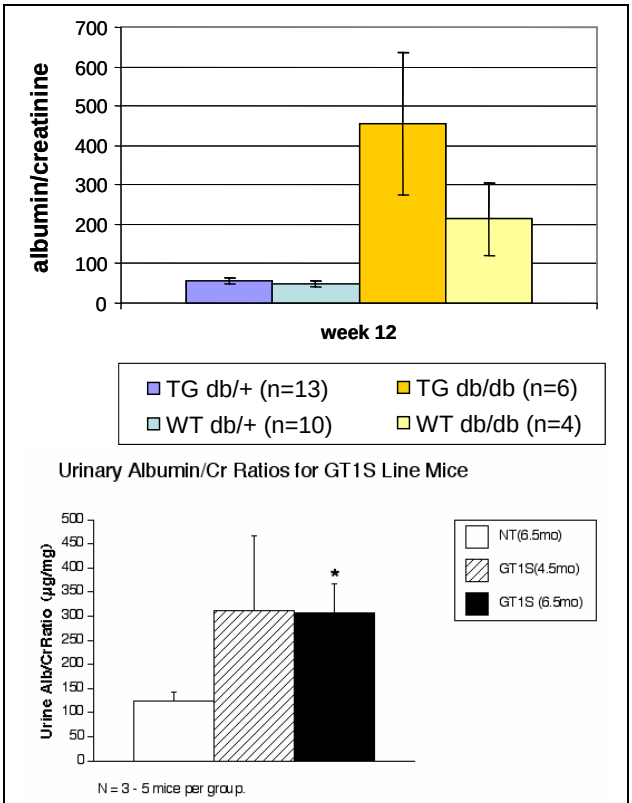

Fig. 4. (upper) GLUT1 tg db/db mice on high fat feeding show greater and substantial albuminuria compared to wild-type db/db. (lower) Non-diabetic GLUT1 tg mice develop substantial albuminuria by 4.5 months of age.

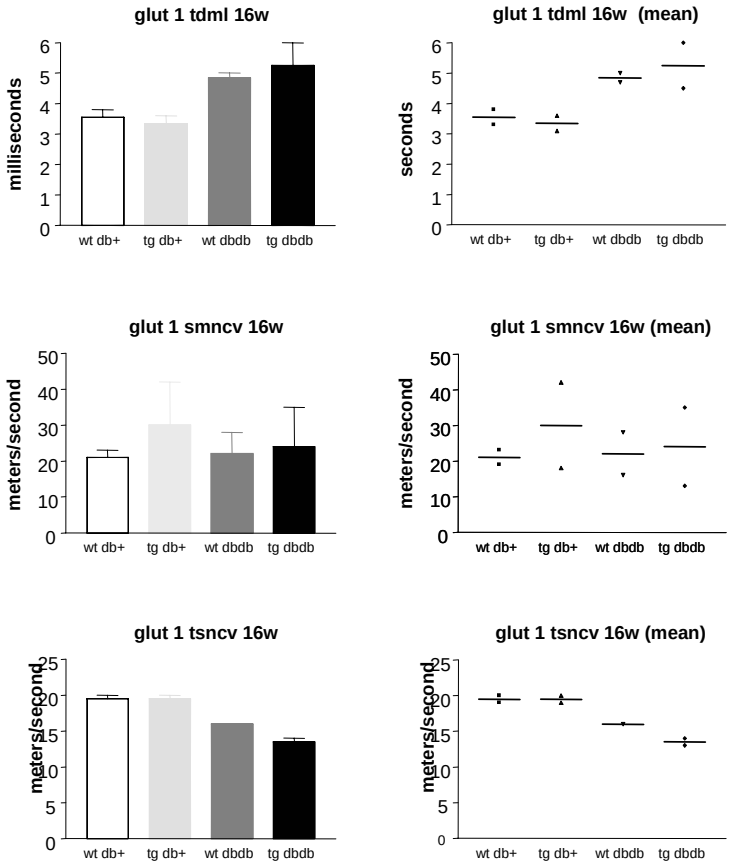

Fig. 5. GLUT1 tg and wild-type (c57) db/db animals show decrements in nerve conduction velocities and increased latencies. These very preliminary data suggest a somewhat more profound neuropathy in the GLUT1 tg animals, but will need to be verified with greater numbers..

mesangial expansion (**Fig. 2**) compared to wild-type diabetic mice. While these results are not yet finalized, they certainly indicate that GLUT4 knockout in podocytes does not predispose to albuminuria or mesangial expansion in diabetic animals.

2. *GLUT1 transgenic db/db C57BL/6J mice on high fat chow.* The original GLUT1 transgenic model was developed by Dr. Heilig at the University of Chicago as part of our AMDCC project. GLUT1 expression is driven by a modified β-actin promoter and is expressed in many tissues including glomerular mesangial cells. These animals were found to be predisposed to albuminuria and mesangial expansion on a wild-type background in the absence of diabetes(**Fig. 4; lower panel**). We have now bred them into a db/+ C57BL/6J background and have placed them on relatively high fat breeder chow (>6.5% fat vs. >4.5% fat in normal chow) to help enhance hyperglycemia. This has resulted in much higher sustained fasting blood sugars and has revealed that the GLUT1 tg db/db mice become less hyperglycemic than wild-type db/db mice (**Fig. 3**), presumably due to enhanced non-insulin responsive glucose uptake in peripheral tissues in the GLUT1 tg mice. Early data suggest significant increases in albuminuria in the diabetic GLUT1 transgenic mice (**Fig. 4; upper panel**). Interestingly, we saw no enhanced albuminuria in the GLUT1 tg db/+ mice.

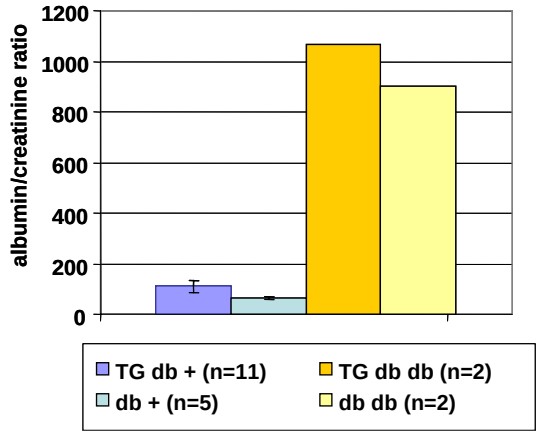

Fig. 6. Preliminary results in 12 week-old podocyte specific GLUT1 tg and wild-type db/db mice on the C57BLKS background show profound albuminuria, greater than previously reported for db/db animals.

Neurologic phenotyping has begun on these same mice on high fat chow. As expected, by 16 weeks of diabetes, both tail flick and hind paw withdrawal latencies were similarly prolonged in the GLUT1tgC57BL/6J db/db mice and the diabetic mice without the GLUT1 transgene (not shown). These same trends occurred in the nerve conduction studies (**Fig. 5**). Tail motor latency was prolonged while sciatic motor nerve and tail sensory nerve conduction velocities were decreased in both sets of diabetic animals regardless of transgene expression. Interestingly, there was a suggestion of a transgene effect when examining the tail sensory nerve conduction velocities. More animals are needed to determine if this is a real effect of increased GLUT1. When examining the control animals, GLUT1tg C57BL/6J db/+ and C57BL/6J db/+ had mildly prolonged latencies when compared to db/+ animals fed normal chow.

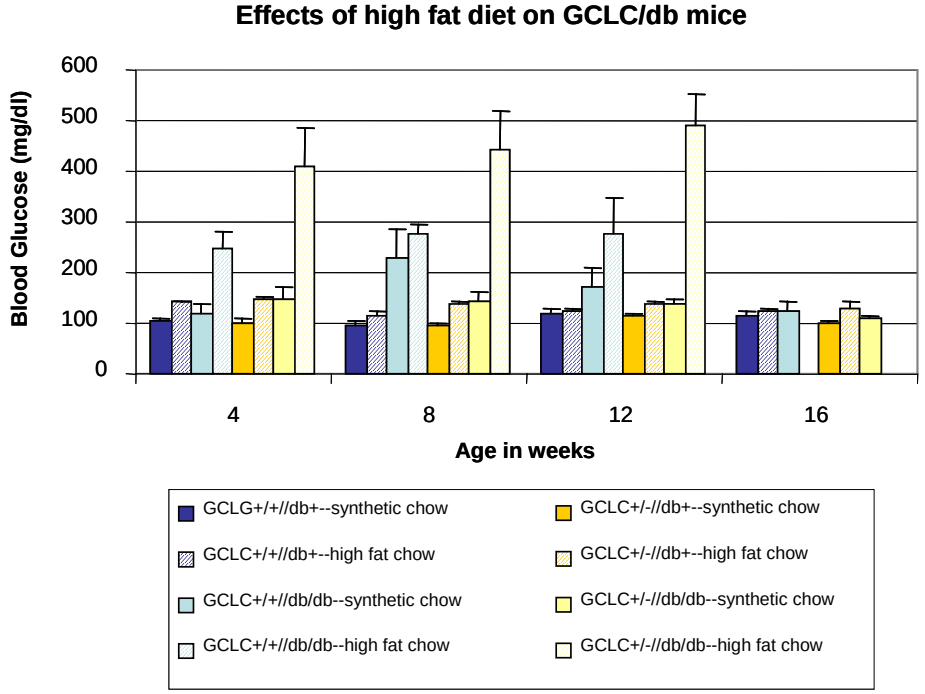

Fig. 7. GCLC +/- and wild-type db/db animals show sustained fasting hyperglycemia on high fat diet but not on a semisynthetic (AIN-76) diet. Fasting blood sugars appeared be higher in the GCLC +/- than in wild-type db/db mice.

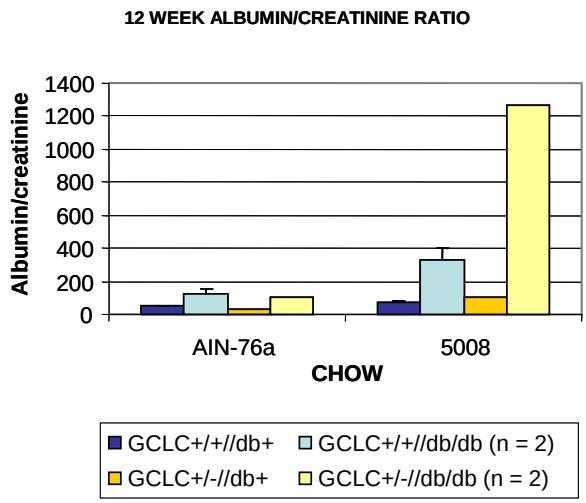

Fig. 8. Albuminuria in GCLC +/- db/db animals appears to be much higher than in the GCLC +/+ db/db mice at 12 wks of age.

3. *Nphs2 GLUT1 tg C57BLKSdb/db mice*. These animals were developed at the University of Michigan and Johns Hopkins University by Dr. Thom Saunders (Transgenic Core Director and AMDCC investigator), Dr. Chuck Heilig (Director of Hopkins AMDCC group; now at University of Chicago) and Dr. Brosius. These tg mice have podocyte specific overexpression of GLUT1 in the C57BLKS db/m background. The db/db mice in this background develop much more profound hyperglycemia (on a normal fat diet) and more severe nephropathy than do db/db animals on a C57BL/6J background. It was our hypothesis that overexpression of GLUT1 in podocytes would increase podocyte injury in this model and augment diabetic nephropathy and induce progressive renal failure. We have obtained 3 strains (one of which was unfortunately lost) that show substantial overexpression of GLUT1. These animals have had early nephropathy phenotyping. Preliminary results show a 10-fold increase in albuminuria in both the transgenic and the wild-type animals (**Fig. 6**). This increase in the wild-type animals is greater than previously reported by other investigators for C57BLKS db/db mice.

4. *GCLC +/- C57BL/6J db/db high fat diet.* Similar to the GLUT1tg on the C57BL/6J background, our initial studies on the GCLC -/- db/db C57BL/6J were hampered by the lack of sustained hyperglycemia. In order to augment hyperglycemia and potentially independently augment nephropathy and neuropathy, we have begun a trial of the same model on high fat diet (breeder chow). This trial is ongoing and only preliminary nephropathy data and no neuropathy data have been obtained. Similar to the GLUT1tg db/db model, high fat feeding substantially augmented hyperglycemia in both db/db groups, but especially in the GCLC -/- animals (**Fig. 7**). In addition, quite preliminary data suggest a substantial increase in albuminuria in both GCLC +/- and wild-type db/db animals, but especially in the GCLC +/- group (**Fig. 8**). These data contrast strikingly with those obtained from the same models on semisynthetic chow.

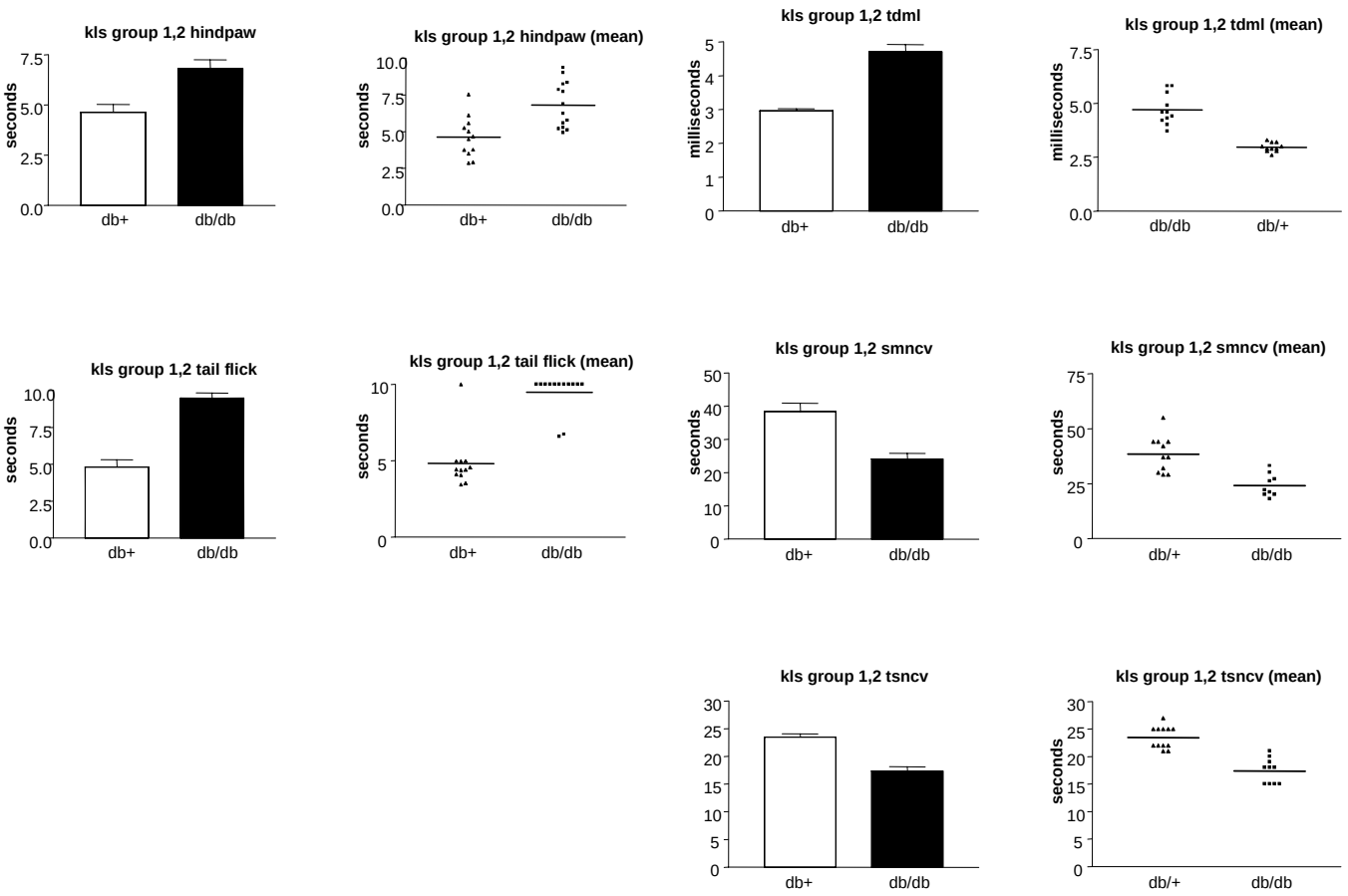

Fig. 9. Tail flick and hindpaw analyses (Left panels) showed substantial increases in C57BLKS db/db mice compared to db/m animals. Similarly, tail distal motor nerve latency (tdml) was enhanced and sural motor nerve conduction velocity (smncv) and tail sensory nerve conduction velocity (tsncv) were significantly and severely depressed in the db/db animals on this background (Right panels).

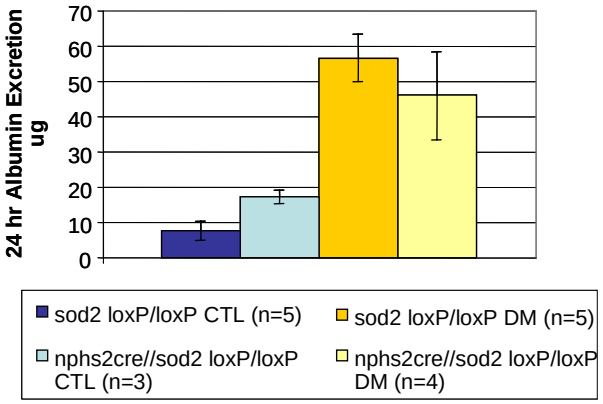

Fig. 10. Albuminuria in both podocyte specific SOD2 knockout (nphs2 cre//sod2 loxP/loxP) and control (sod2 loxP/loxP) diabetic mice was modest and similar.

5. *C57BLKS db/db mice*. This model has been used for neuropathy phentoyping since it has been previously extensively phenotyped for nephropathy by other investigators. Tail flick and hind paw analyses (weeks 12 and 24) and sciatic nerve conduction studies (weeks 12 and 24) confirm significant neuropathy in these animals (**Fig. 9**).

6. *Nphs2 Cre SOD2 loxP/loxP mice*. This model was only assessed for nephropathy since the SOD2 knockout was specific for the podocyte. STZ diabetes in these animals resulted in very little evidence of nephropathy whether or not the SOD2 gene was excised in podocytes (**Fig. 10**). These data were obtained in a STZ diabetic model on normal chow which has routinely been quite resistant to diabetic nephropathy.

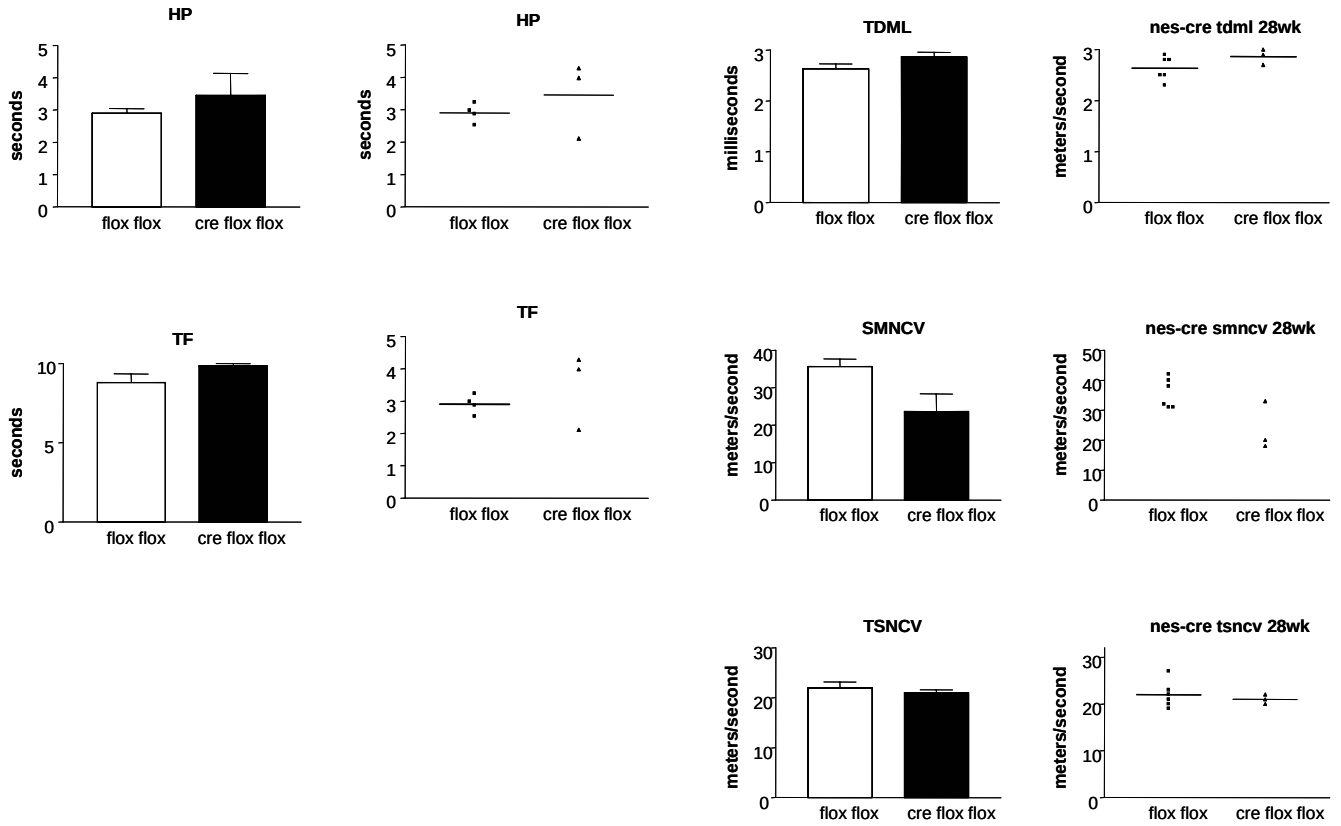

Fig. 11. Tail flick and hindpaw analyses (Left panels) showed some increase in STZ diabetic Nestin SOD2 knockout (cre flox flox) mice compared to STZ wild-type (flox flox) animals. Sciatic motor nerve conduction velocity (smncv) was depressed in the Nestin SOD2 knockout animals, though other nerve conduction changes were not profound (Right panels).

7. *Nestin Cre//SOD2loxP/loxP mice*. This model is being assessed for both neuropathy and nephropathy. Even though the nestin promoter was chosen for its high degree of expression in peripheral neurons, it is also expressed in kidney—proximal tubules and to a lesser extent in glomerular cells. While only 4 control and 3 experimental animals have reached the age of 28 weeks with STZ diabetes, the initial results suggest some neuropathy in the Nestin Cre//SOD2loxP/loxP STZ diabetic mice. The Nestin Cre//SOD2loxP/loxP STZ animals have poorer measures of nerve function than the SOD2 loxP/loxP STZ mice suggesting a more neuropathic phenotype. Hind paw and tail flick latencies are prolonged (**Fig. 11**). Tail motor distal latencies are prolonged while sciatic motor nerve and tail sensory nerve conduction velocities are decreased when compared to the Nestin Cre//SOD2loxP/+ animals. These animals remain alive and will undergo repeat neurologic phenotyping and kidney phenotyping in 4 weeks.

2. <u>Collaboration within your group:</u>

We have coordinated all trials and models with the neuropathy group. All AMDCC personnel at the University of Michigan meet monthly to discuss models, timing, data and new approaches. All decisions are made as a group. The mouse colony manager works closely with both nephropathy and neuropathy phenotyping personnel to coordinate phenotyping and tissue harvest. Dr. Heilig's group from the University of Chicago has been instrumental in developing the initial generalized and podocyte specific GLUT1tg animals and performed initial and ongoing assessments of these models.

3. Collaboration with other AMDCC groups:

Since our center incorporates the Neuropathy Core as part of its operations, all eligible animals are phenotyped for neuropathy. In addition, at tissue harvest we routinely harvest eyes and bladders for the Retinopathy and Uropathy Cores. In the past year, Dr. Daneshgari brought his staff to the Neuropathy Phenotyping Core to measure bladder elasticity of selected animal models. Dr. Danshgari and his staff have established the feasibility of performing this test on a recovery basis and that this technique is an important addition to overall model characterization. Future experiments will include testing of bladder function prior to euthanasia and tissue harvest.

The Neuropathy Core has been especially active in phenotyping models from the other AMDCC investigators. A number of these collaborations have been highlighted in the Nephropathy Core report and will not be repeated here.

Other collaborations have been made with several of the AMDCC groups. These include: 1) the generation and phenotyping of the Nphs2 GLUT4 knockout model with Dr. Dale Abel (University of Utah); 2) Nphs2 SOD2 knockout model with Drs. Harris and Breyer (Vanderbilt University). 3) Total body GLUT4 -/- knockout mice with Dr. Charron (Albert Einstein College of Medicine) and Dr. Erwin Bottinger (Mt. Sinai School of Medicine); and 4) Nphs2 PPARγ knockout project with Drs. Hsueh and Nichols (UCLA). Finally, any models used for terminal neuropathy phenotyping that have not had kidney phenotyping will have spot urine albumin/creatinine ratios determined and the kidneys will be assessed for morphological changes.

4. Pertinent non-AMDCC Collaboration:

1) JDRF Center of Excellence in the study of diabetic complications. This center encompasses a number of collaborative projects exploring the role of oxidative stress in diabetic complications. It also includes a clinical project testing antioxidants and other agents in the treatment of diabetic complications. The Center Director is Dr. Feldman. Drs. Russell and Stevens have projects in this center. Dr. Brosius' project was recently renewed as a separate JDRF grant that will be performed in coordination with the Center.

2) Dr. Feldman is PI for several collaborative NIH grants investigating the etiology, pathogenesis and treatment of diabetic polyneuropathy. Dr. Russell is a co-investigators on several of these grants.

3) Dr. Feldman is an investigator in neuropathy aspects of the multi-institutional Epidemiology of Diabetes Interventions and Complications (EDIC) study.

4) Dr. Brosius is PI on two NIH grants investigating the role of glucose transporters in vascular disease. Collaborators include several vascular biologists.

5) Drs. Feldman and Brosius are Co-Is on a grant proposal with Dr. Michael Uhler (University of Michigan) in a collaborative informatics project attempting to define a common set of transcriptional events that occur early in diabetic neurons and podocytes.

6) Dr. Heilig is the PI on several funded and pending NIH proposals for the study of GLUT1 in diabetic and nondiabetic nephropathy and diabetic embryopathy as well as GLUT1 haplodeficiency syndromes. Dr. Brosius is a collaborator and consultant on these proposals.

7) Dr. Stevens is the PI on several collaborative grants investigating myocardial aspects of diabetic autonomic neuropathy.

8) Dr. Russell is the PI on a NIH project investigating the role of IGF-1 in oxidative stress and apoptosis in diabetic neuropathy; collaborators on this project include Drs. Feldman and Michael Brownlee (Albert Einstein College of Medicine). Dr. Russell is also the PI on a VA grant studying IGF-1 and Schwann Cells in neuropathy.

9) Dr. Holzman has collaborative projects and grants on glomerular podocyte cell biology and pathology with multiple glomerular disease investigators worldwide.

5. Address previous EAC comments:

1. *Please upload to the website the protocol for podocyte counting (from Roger Wiggins?).*

We have recently reassessed the protocol for podocyte counting in concert with Dr. Wiggins. This revised protocol will be given to Dr. McIndoe to post on the website by the date of the Steering Committee meeting.

2. *Encouraged to publish (at least on the website) your negative findings from the SOD mice.*

This paper has been submitted for publication.

6. Publications:

1. Breyer MD, Bottinger E, Brosius FC 3rd, Coffman TM, Harris RC, Heilig CW, Sharma K. Mouse Models of Diabetic Nephropathy. J Am Soc Nephrol. 2005;16:27-45.
2. Heilig CW, Brosius FC III. Roles for glucose transporters in diabetic kidney disease. Recent Res Devel Physiol, in press.
3. Brosius FC III, Heilig CW. Glucose transporters in diabetic nephropathy. Ped Nephrol, in press
4. Sullivan KA, Vincent AM, Schin ML; Backus C; Hayes JM, McLean LL, Burke KL, Russell JW, Brosius FC, Feldman EL. Phenotypic characterization of new mouse models of diabetic complications, submitted.
5. Siu BB, Saha J, Smoyer WE, Sullivan KA, Stevens MJ, Brosius FC III. Podocyte Loss in Early Streptozotocin Diabetes: Prevention by Lipoic Acid Treatment, in revision.

Action Items

1. Please email a copy of your final 2004 Annual Report to me no later than *February 28, 2005*.

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2. Tom Coffman will compile a "general response" to the previous EAC comments that will be included at the front of the Annual Report booklet.

Tom – please send me this document by *February 28, 2005* for inclusion into the booklet.

3. Please email updated "Feldman tables" for the 3 animal models that you are focusing on to Rick McIndoe by *February 14, 2005*.

Rick – please compile these tables into a single "master Feldman table" and send this to me by *February 28, 2005*.