AMDCC Annual Report (2011)

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Project Title: Angiogenic Signals in Diabetic Complications
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Abstract: In humans with diabetes, abnormal angiogenesis contributes to the development of end-organ damage. In this regard, "excessive" angiogenesis and increased activity of the vascular endothelial growth factor (VEGF) signaling pathway have been associated with diabetic complications such as retinopathy. In contrast, an inadequate angiogenesis response with a reduced capacity to promote collateral blood vessel growth in cardiac and particularly peripheral skeletal muscle result in more severe manifestations of vascular disease in diabetes. However, the mechanisms responsible for the loss of control of angiogenesis in diabetes and how this dysregulation modulates tissue pathology are not clear. We hypothesize that abnormal signaling in VEGF-associated pathways is a critical factor in the pathogenesis of diabetic complications including peripheral artery disease (PAD) and nephropathy. Furthermore, we posit that distinct properties of individual tissues determine the effects of diabetes on the local angiogenesis response, shaping the resulting pathology. Accordingly, to develop better models of diabetic PAD and nephropathy, we will generate mouse lines with inducible alterations of angiogenic signaling pathways targeted to specific cell lineages in blood vessels, skeletal muscle and kidney. Because both enhanced and diminished VEGF activities have independently been associated with diabetic complications, we will produce models with up- or down-regulated angiogenic signaling. The long-term goals of our studies are: (1) To understand how alterations in angiogenic factors contribute to the development of diabetic complications and (2) To develop mouse models of diabetic PAD and nephropathy that more faithfully reproduce the respective human conditions. To achieve these goals we propose the following specific aims: 1. To develop mouse models with genetic modifications of key signaling pathways linked to angiogenesis. 2. To determine the effects of diabetes on angiogenic signaling in a well-established model of peripheral artery disease. 3. To define the consequences of altered angiogenic signaling on the development of albuminuria and nephropathy in diabetes.

1. Program Accomplishments:

Hypothesis

In humans with diabetes, abnormal angiogenesis, defined as growth and proliferation of blood vessels from existing vascular structures, contributes to the development of end-organ damage. In this regard, "excessive" angiogenesis and increased activity of the vascular endothelial growth factor (VEGF) signaling pathway have been associated with diabetic complications such as retinopathy, and perhaps nephropathy. In contrast, inadequate angiogenesis with a reduced capacity to promote collateral blood vessel growth results in more severe manifestations of coronary and peripheral vascular disease in diabetes. However, the mechanisms responsible for the loss of control of angiogenesis in diabetes and how this dysregulation modulates tissue pathology are not clear. ***We have hypothesized that abnormal signaling in VEGF-associated pathways is a critical factor in the pathogenesis of diabetic complications including nephropathy and peripheral artery occlusive disease (PAOD). Furthermore, we posited that distinct properties of key cellular targets in individual tissues determine the effects of diabetes on the local angiogenesis response, shaping the resulting pathology. We suggest for nephropathy the critical target cell is the podocyte and in PAOD it is skeletal muscle.***

Accordingly, to develop better models of diabetic nephropathy and PAOD, we will generate mouse lines with inducible alterations of angiogenic signaling pathways targeted to podocytes and skeletal muscle. Because both enhanced and diminished angiogenesis responses have independently been associated with diabetic complications, we will use models with up- or down-regulated angiogenic signaling. Some of these models have been generated and are ready to use; we propose others to be generated as a part of the consortium activities. The long-term goals of our studies are: (1) To understand how alterations in angiogenic factors contribute to the development of diabetic complications and (2) To develop mouse models of diabetic nephropathy and PAOD that more faithfully reproduce the respective human conditions.

Progress toward stated aims

SPECIFIC AIM I. To define the role of altered angiogenic signaling in podocytes on the development of albuminuria and nephropathy in diabetes. During the past year, our work in this specific aim has encompassed several areas. One of these was continued evaluation of the effects modulating vascular endothelial growth factor-A (VEGF) on the course of diabetic nephropathy. VEGF is required for endothelial cell differentiation, and survival and its expression is dysregulated in patients with diabetic nephropathy. Deletion or over-expression of VEGF in kidney podocytes leads to glomerular disease in mice. Given the critical role of VEGF in normal glomerular biology, we hypothesized that loss of local VEGF from podocytes of diabetic adult mice would accelerate the course of diabetic nephropathy. To test this hypothesis we used an inducible Cre-loxP gene targeting system to excise the VEGF gene from podocytes of adult mice. Mice were divided into four groups: VEGF KO alone, VEGF KO + diabetes, diabetes alone, and control. Diabetes was induced by streptozotocin (STZ) at 2.5-3 weeks of age. One week after STZ injection, VEGF KO was induced by doxycycline (Dox) in drinking water, to excise the VEGF gene specifically in podocytes. A total of 76 mice were studied. Blood and urine were collected weekly to monitor blood glucose and urine protein concentrations. Mouse kidneys were dissected at 6-10 weeks after STZ to evaluate pathological changes. VEGF KO + DB mice had proteinuria, glomerular sclerosis worse than either DB alone or VEGF KO alone. In addition, the glomeruli of DB mice expressed more VEGF mRNA than the other groups. Therefore, a reduction of local glomerular VEGF during diabetes causes endothelial injury accelerating the progression of diabetic nephropathy. These results suggest that VEGF

inhibitor therapy might be associated with a greater risk of renal toxicity in diabetics and that up-regulation of VEGF in diabetic glomeruli is a protective compensatory response. A manuscript describing these findings is in preparation.

Angiopoietin-1 signaling through the *Tek* receptor is another major regulator of blood vessel development and conventional Angiopoietin-1 knockout mice exhibit embryonic lethality due to vascular defects. It is widely accepted that Angiopoietin-1 is required to stabilize mature vessels, in contrast to the powerful angiogenic effects of VEGF. Using conditional gene targeting, we studied the role of Angiopoietin-1 (ANGPT1). We found that ANGPT1 is critical for regulating both number and diameter of developing vessels but, contrary to current dogma, it is not required for pericyte recruitment. By contrast, deletion of ANGPT1 after embryonic day 13.5 produces no vascular phenotype. This was a surprising finding, but allowed us to use this model to examine the role of ANGPT1 in diabetic kidney injury. The early phase of diabetic kidney disease is characterized by neo-angiogenesis and increased vascular permeability, with leakage of proteins into the urine (albuminuria and proteinuria). Serum levels of both VEGFA and ANGPT2, which inhibits *Tek* in an autocrine fashion, are increased in diabetics and there is an elevation of the ANGPT2:ANGPT1 ratio, which is associated with worse cardiovascular and kidney outcomes. We confirmed upregulation of *Vegfa*, *Angpt2*, and *Tgfb1* in glomerular cell fractions in the STZ model (not shown).

To examine the role of ANGPT1 in diabetes, controls and mice with *Angpt1* deletion starting between E16.5 and P0 (*Angpt1*$^{del/\wedge(E16.5)}$) were used. At the age of 4-6 weeks, they were given injections of STZ to induce diabetes and were then monitored for up to 20 weeks. This regimen typically causes robust hyperglycemia, but has only modest effects on kidney structure and function in wild-type mice, and this was the case for the control mice in our study. By contrast, 20% of the diabetic *Angpt1*$^{del/\wedge(E16.5)}$ mice died before the end of the study, whereas all of the control mice survived ($p<0.05$, Figure 1A). The surviving diabetic *Angpt1*$^{del/\wedge(E16.5)}$ mice

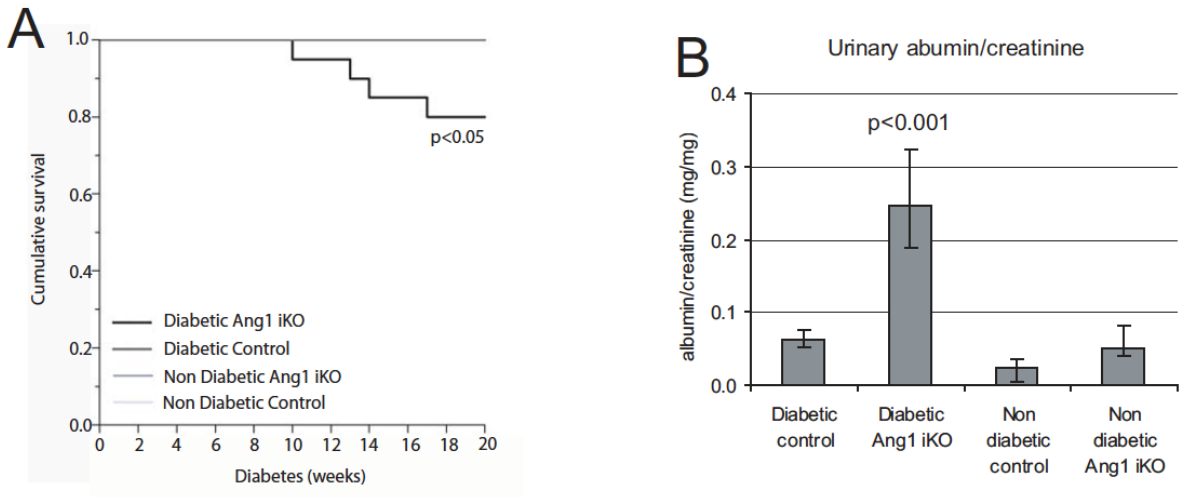


Figure 1. Angiopoietin-1 protects the glomerular vasculature in diabetic nephropathy. *Angpt1*$^{del/\wedge(E16.5)}$ (induced between E16.5 and P0) mice made diabetic shows a significant decrease in survival (A). After 20 weeks of diabetes, *Angpt1*$^{del/\wedge(E16.5)}$ have a significantly higher urinary albumin/creatinine ratio compared to controls and non-diabetic groups (B).

have impaired function of the glomerular filtration barrier (GFB) manifested by significant albuminuria with urinary albumin/creatinine ratios of 0.25 (+0.08,-0.06) mg/mg in diabetic *Angpt1*$^{del/\wedge(E16.5)}$ compared to 0.06 (±0.01) mg/mg in diabetic controls (Figure 1B). On

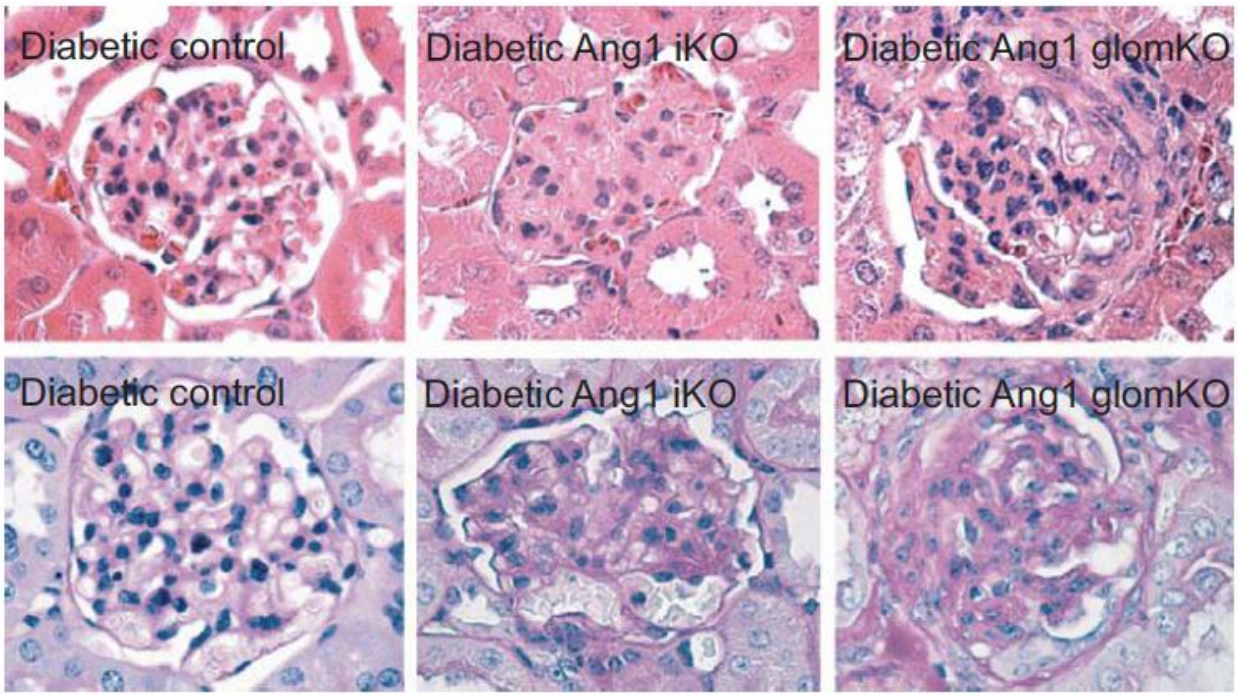


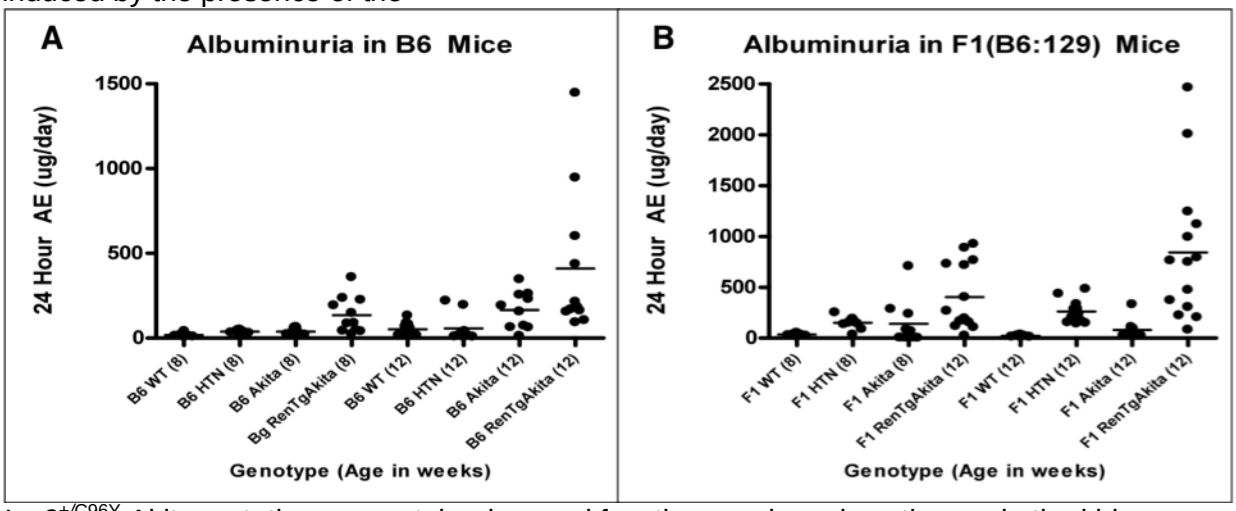
Figure 2. Angiopoietin-1 protects the glomerular structure in diabetic nephropathy. Histology shows an increase in mesangial matrix expansion and sclerosis in diabetic *Angpt1*$^{del/\wedge(E16.5)}$ mice and diabetic *Angpt1*$^{del/\wedge(glom)}$ compared to diabetic controls (C, H&E-upper panel, PAS-lower panel, Bar = 50 µm).

histopathological examination of the kidney, diabetic controls have minimal abnormalities confined to mild mesangial expansion. However, there are marked changes in glomerular histology in the diabetic *Angpt1*$^{del/\wedge(E16.5)}$ mice with dramatic mesangial matrix expansion and glomerulosclerosis (Figure 2); similar pathological features can be seen in humans with advanced diabetic nephropathy. Such changes were never seen in diabetic controls or non-diabetic groups, and have rarely been reported in other studies of diabetic kidney injury in mice. Despite the differences in glomerular pathology, the extent of hyperglycemia achieved is not different between the groups, as reflected by the similar levels of glycosylated hemoglobin (HbA1c) at the end of the study, 0.088±0.003 vs. 0.090±0.003 % for diabetic controls and diabetic *Angpt1*$^{del/\wedge(E16.5)}$, respectively. F4/80 positive macrophages were increased in kidneys from diabeticdiabetes compared to non- diabetic mice, however, there was no difference between controls and *Angpt1*$^{del/\wedge(E16.5)}$ in diabetic or non-diabetic animals (data not shown).

To define the key cellular sources of Angpt1 that protect the glomerulus in diabetes, we generated separate lines of mice with *Angpt1* deleted specifically from podocytes or mesangial cells (*Angpt1*$^{del/\wedge(glom)}$) using podocyte- and mesangial-specific Cre drivers. After STZ-treatment, only compound mutants (i.e. both *Nphs1*-Cre AND *Pdgfrb*-Cre) shows a similar degree of accelerated glomerular damage as the global diabetic *Angpt1*$^{del/\wedge(E16.5)}$ mice indicating that production of Angpt1 by each of these glomerular cell populations provides protection against microvascular injury in diabetes (not shown). Thus, local production of Angpt1 by glomerular cell populations, including the podocyte and mesangial cell, provides significant protection against diabetic kidney injury. A manuscript describing these findings has been submitted to *JCI*.

In our previous studies, we have found potent effects of genetic background on susceptibility to diabetic nephropathy between the C57BL/6 and 129/SvEv strains that are dramatically amplified by RAS activation with a renin transgene. Our preliminary data have suggested that there are also differences in expression of angiogenic factors between the

strains. To further examine the phenotype of the mouse line containing the renin transgene as a genetic sensitizer, we have generated cohorts of mice to examine the effects of diabetes, induced by the presence of the



Ins2$^{+/C96Y}$ Akita mutation, on proteinuria, renal function, angiogenic pathways in the kidney, comparing two strains with relative resistance (C57BL/6) and susceptibility (129/SvEv) to diabetic kidney injury. The studies are carried out in 6 groups of mice: (1) C57BL/6-*Ins2*$^{+/+}$ (WT), (2) C57BL/6- *Ins2*$^{+/C96Y}$ (Akita), (3) 129/SvEv-*Ins2*$^{+/+}$ (WT), (4) 129/SvEv-*Ins2*$^{+/C96Y}$ (Akita), (5) F_1(C57BL/6 x 129/SvEv)-*Ins2*$^{+/+}$ (WT), (4) F_1(C57BL/6 x 129/SvEv. Significant numbers of mice in cohorts (1), (2), (5), and (6) have been generated and phenotyping of these groups is in progress. As can be seen in Figure 1, the relative differences in levels of albuminuria that were seen at 24 weeks are also apparent at earlier time points, indicating that phenotyping for the larger genetic study could be done at 8 or 12 weeks of age. As such, the time and expense for this study would be reduced. While albuminuria levels are higher in the F1 mice, there is some overlap with the parental C57BL/6 line, which is not unexpected. We have generated similar groups of mice on the 129 background and should have that analysis completed in the next few weeks. We anticipate that the levels of albuminuria will be highest in this group, with very little overlap with the C57BL/6 groups. In collaboration with Dr. Christopher Newgard, we also plan to apply targeted metabolic profiling of tissues harvested from the experimental groups to understand the role of perturbation of metabolic pathways in these processes. These studies will provide insights into the heritability of the renal disease susceptibility trait. Taken together, these findings will dictate future strategies for mapping loci associated with kidney disease susceptibility, using albuminuria as the major disease phenotype, with the opportunity to provide an overlay of metabolic pathway analysis. Current approaches for identifying genetic determinants of DN in mice are limited by the relatively modest levels of albumin excretion, even in Akita mice on a susceptible background. Accordingly, the very robust difference in the level of albuminuria between the susceptible and resistant strains with the combination of the Akita mutation and the renin transgene suggest that this model might be very tractable for such an approach. In this case the renin transgene would be used as a genetic sensitizer to amplify the genetic “signal”.

SPECIFIC AIM II. To define the role of altered angiogenic signaling in skeletal muscle in a model of peripheral artery occlusive disease. Diabetes (DM) is a major risk factor for the development of peripheral arterial disease (PAD) and when PAD is present, patients with PAD have poorer clinical outcomes. The effects of controlling blood sugar in patient with PAD and DM is uncertain. Surgical induced hind-limb ischemia (HLI) is an established pre-clinical model

for aspects of PAD and DM has been clearly associated with impaired perfusion recovery and angiogenesis, the growth of new vessels from preexisting vessels, following HLI. Vascular endothelial growth factor (VEGF) and its receptors are among the most extensively studied angiogenic growth factors and impairments in VEGF and its signaling have been linked to adverse outcomes in PAD, but the molecular basis of impairment and the effect of glycemic control with exogenous insulin in poorly understood. We hypothesized that glycemic control would improve perfusion recovery in mice with DM following HLI and these effects would be mediated through the VEGF receptor ligand family. We therefore compared perfusion recovery following HLI in mice with normal glycemia (NG), hyperglycemia (hyperglycemia secondary to beta cell loss or DMI. Ave. Hb1ac 10.5±) and hyperglycemia but insulin treated (ITDMI) with average HbA1c of 6.0 ± . Perfusion recovery was significantly impaired in DMI compared to NG controls (week 5 perfusion ratio = 60.97% SEM 5.8 vs 77.2% SEM 3.6: $P<0.05$, n=13-17) but restored in ITDMI mice (71.02% SEM 4.1 vs 77.2% SEM 3.6 n=17-21, $P>0.05$). Baseline capillary density measurements showed no difference between NG and DMI tissues. To explore the mechanism involved, expression of VEGFA, VEGFR1 and VEGFR2 were analyzed by ELISA and quantitative PCR in skeletal muscle of NG, DMI and ITDMI mice, following experimental PAD. VEGFA expression significantly increased in the NG tissues but not in DMI (76.8±7.8 vs 20.5 ±16.1 pg/mg protein or 3.4 ±0.4 vs 1.6 ±0.9 fold change: $P<0.05$). VEGFR1expression increased similarly in NG and DMI tissues but VEGFR2 expression was impaired (did not reach significance). Insulin treatment significantly increased VEGFR2 expression (fold change DMI vs ITDM1, 1.9±0.8 vs 3.1±0.6 $P<0.05$) but had no effect on VEGFA expression (NG vs ITDMI, 76.8±7.8 vs 25±18 pg/mg $P<0.05$, n=4) and VEGFR1 expression remained unchanged between the groups. These data demonstrate that in experimental PAD, poor perfusion recovery in the setting of hyperglycemia is associated with impaired augmentation of VEGFA and its activating receptor VEGFR2 but insulin treatment restores perfusion recovery and augments VEGFR2 expression.

Plans for the Upcoming Year

During the next year, we will continue our ongoing work assessing the consequences of inducible elimination of VEGF and angiopoietin 1 from podocytes in adult mice on the course of STZ-induced diabetes. We will also carry out appropriate crosses so that similar studies can be done on the Akita background. Based on feedback from the EAC, we will also continue to explore the nature of naturally occurring genetic modifiers that affect the severity of diabetic kidney injury, including any associated metabolic alterations that accomplany these changes. Finally, we will continue our ongoing work characterizing the activity of VEGF-associated signaling pathways in our target tissues of interest, skeletal muscle and the glomerulus, to allow direct comparisons of the extent of angiogenic signal activation in these tissues and to understand how altered angiogenic signaling may contribute to diabetic complications in these tissues.

1. <u>Collaboration:</u>

Within the AMDCC, we have continued our ongoing collaborations with the two groups at UNC (Smithies and Maeda labs). In particular, we have been using the renin transgenic mice produced by the Smithies' lab to augment the severity of diabetic renal disease. In studies going forward, we hope to use this transgene as a genetic sensitizer to characterize strain specific genetic modifiers of kidney injury. In addition, we have interacted with the group at Jackson Laboratories over the past year to successfully transfer the 129/SvEvTac-*Ins2*^{Akita} line. The Jax group will carry out baseline phenotyping of the line. Finally, we have

initiated a collaboration with Dr. Chris Newgard, a non-AMDCC investigator here at Duke. Dr. Newgard is an expert in the area of metabolomics and we plan to carry out an unbiased metabolomic screen of some of our models to look for metabolic signatures that are associated with enhanced renal disease.

2. Address previous EAC comments:

- *Do you have any additional data on the strain comparisons that can be uploaded to the website? Have you uploaded all of your published data on the eNOS models?*

The majority of our strain comparison data have been uploaded, except the data from the current ongoing comparisons of mice in the renin-transgene senistizer study. These will be uploaded as soon as they are complete.

- *Continues to make progress on original aims. The background genetic effects on renal phenotypes continues to be fascinating and completion of the conditional Ang1 deletion should be informative. Expansion into metabolomics analyses may be informative, but will require careful design to generate interpretable results.*

As discussed above, the studies in the conditional Ang 1-deletion mice have indeed been quite informative and we anticipate that they will be published in the *JCI* in the near future. We also agree that the metabolomics studies will require careful design. Accordingly, we will be carrying out simple comparisons as our first step, exclusively employing a well-characterized, targeted metabolomics platform. Finally, our collaborator, Dr. Christopher Newgard, has been one of the pioneers in this area and his laboratory has used this technology to carry out a number of productive and highly informative studies in other models. His advice will be critical for successful execution of this work.

- *Excellent progress is being made in Aim 1 addressing the role of angiogenic factors in diabetic nephropathy. The increase in proteinuria in Ang1 podocyte-specific KO mice in the setting of diabetes, but not under normal physiologic conditions, is a promising finding. We hope that further efforts will define the nature of the podocyte injury, both in vivo (whole kidneys, isolated glomeruli) and perhaps in vitro using cultured (transformed) podocytes exposed to high glucose medium (this may be beyond the scope of the project). Also, while the PI has stated a plan to explore naturally-occurring genetic modifiers, the strategy is lacking.*

We appreciate these positive comments and, as mentioned above, the Ang1 studies have turned out to be quite interesting and will soon be published in a high impact journal. This paper includes more in-depth mechanistic studies along the lines of those suggested by the EAC. Our experimental strategy for further exploring naturally-occurring genetic modifiers in this system employs a renin transgene as a genetic sensitizer and was more fully described in our application for ARRA supplemental funds. Some of these preliminary data are presented above.

- *We found Aim 2, angiogenic signaling in skeletal muscle, very difficult to follow, with many incomplete sentences, poorly explained figures (e.g. in figure 7, the role of high*

fat diet is unclear; P11, unclear if ins2 is the KO gene or something else), and references to past data reports that were not available. The preliminary data in muscle show ~50% reduced VEGFR2 protein (of uncertain significance for disease processes), unaffected by insulin treatment, and unchanged VEGF protein. The proposed studies of downstream consequences of altered VEGFR signaling (Akt, etc.) are of considerable interest and should clarify the biologic significance of the observed changes. Akita mice develop a skeletal myopathy (Krause, J Appl Phyiol 2009) and some effort should be made to relate observed molecular changes to skeletal muscle pathology and if the appropriate methods exist, to relate these to skeletal muscle function as well.

We apologize for the typos in the previous report and we agree that the downstream signaling experiments should be quite informative in understanding the functional significance of the changes in VEGF receptor expression that we have observed. This work is in progress. Furthermore, we have seen similar alterations of VEGFA and its major receptor in a model of T2DM with ischemia, and that these can be corrected with exogenous insulin therapy. Finally, we appreciate the suggestion concerning the myopathy seen in Akita mice and we believe that we can use the data we are generating now comparing VEGF signaling pathways in kidney in muscle of Akita mice on different strain backgrounds to address this issue.

- *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.*

There are a few publications to be added as below and we will work with Dr. McIndoe to assure that all relevant data are uploaded.

1. Influence of genetic background on albuminuria and kidney injury in Ins2(+/C96Y) (Akita) mice.
   Gurley SB, Mach CL, Stegbauer J, Yang J, Snow KP, Hu A, Meyer TW, Coffman TM
   American journal of physiology. Renal physiology, 2010 (298(3)), F788 - F795
2. Mouse Models of Diabetic Nephropathy:A Midstream Analysis from the Animal Models of Diabetic Complications Consortium
   Frank C. Brosius III, Charles E. Alpers, Erwin P. Bottinger, Matthew D. Breyer, Thomas M. Coffman, Susan B. Gurley, Raymond C. Harris, Masao Kakoki, Matthias Kretzler, Edward H. Leiter, Moshe Levi, Richard A. McIndoe, Kumar Sharma, Oliver Smithies, Katalin Susztak, Nobuyuki Takahashi, Takamune Takahashi
   Journal of the American Society of Nephrology : JASN, 2009 (20(12)), 2503 - 2512
3. Vascular endothelial growth factor receptor 2 controls blood pressure by regulating nitric oxide synthase expression.
   Facemire CS, Nixon AB, Griffiths R, Hurwitz H, Coffman TM
   Hypertension, 2009 (54(3)), 652 - 658
4. Myocyte specific overexpression of myoglobin impairs angiogenesis after hind-limb ischemia.
   Hazarika S, Angelo M, Li Y, Aldrich AJ, Odronic SI, Yan Z, Stamler JS, Annex BH
   Arteriosclerosis, thrombosis, and vascular biology, 2008 (28(12)), 2144 – 2150
5. The VEGF receptor Flt-1 spatially modulates Flk-1 signaling and blood vessel branching.
   Kappas NC, Zeng G, Chappell JC, Kearney JB, Hazarika S, Kallianos KG, Patterson C, Annex BH,

Bautch VL
J Cell Biol 2008; 181:847-858. PMID: 18504303

6. High cholesterol feeding in C57/Blc6 mice alters expression within the VEGF receptor-ligand family in corporal tissue.
   Xie D, Hazarika S, Andrich AJ, Padgett ME, Kontos CD, Donatucci CF, Annex BH
   J Sex Meof 2008;5:1137-48. PMID: 18439153
7. Myocyte-specific overexpression of myoglobin impairs angiogenesis after hind-limb ischemia.
   Hazarika S, Angelo M, Li Y, Aldrich AJ, Odronic SI, Yan Z, Stamler JS, Annex BH
   Arterioscler Thromb Vasc Biol. 2008;28:2144-50. PMID: 18818418
8. Impaired angiogenesis after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth factor receptor 1 and soluble vascular endothelial growth factor receptor 1.
   Hazarika S, Dokun AO, Li Y, Popel AS, Kontos CD, Annex BH
   Circ Res 2007;101:948-56. PMID: 17823371
9. Toward a Mouse Model of Diabetic Nephropathy: Is Endothelial Nitric Oxide Synthase the Missing Link?
   Quagin, SE Coffman, TM
   Journal of the American Society of Nephrology : JASN, 2007 (18), 364 - 366
10. Impact of Genetic Background on Nephropathy in Diabetic Mice
    Susan B. Gurley, Sharon E. Clare, Kamie P. Snow, Timothy W. Meyer, and Thomas M. Coffman
    American journal of physiology. Renal physiology, 2006 (290), F214 - F222
11. Diabetic nephropathy: of mice and men.
    Breyer MD, Böttinger E, Brosius FC, Coffman TM, Fogo A, Harris RC, Heilig CW, Sharma K
    Advances in chronic kidney disease, 2005 (12(2)), 128 - 145
12. Mouse Models of Diabetic Nephropathy
    MATTHEW D. BREYER, ERWIN BÖTTINGER, FRANK C. BROSIUS, III, THOMAS M. COFFMAN, RAYMOND C. HARRIS, CHARLES W. HEILIG, AND KUMAR SHARMA (FOR THE AMDCC)
    Journal of the American Society of Nephrology : JASN, 2005 (16), 27 – 45

Additional publications:

Gurley SB, Coffman TM. An IRKO in the podo: Impaired insulin signaling in podocytes and the pathogenesis of diabetic nephropathy. *Cell Metab* 2010; 12(4):311-2.

Jeansson M, Gawlik A, Anderson G, Li C, Kerjaschki D, Henkelman M, Quaggin SE. Angiopoietin-1 is Essential in Vasculature during Development and Disease in Mice. Submitted, *J Clin Invest*.