

A PROPOSAL TO THE BAKER FUND COMMITTEE

TITLE OF PROJECT: Examining the role of STAT5 in diabetic kidney damage

NAME OF APPLICANT: Karen Coschigano

STATUS: Asst. Prof. Assoc. Prof. Prof. Full-Time Admin.

CAMPUS ADDRESS: ARC 302d

E-MAIL ADDRESS: coschigk@ohio.edu

RE-SUBMISSION: YES (Original Submission Date _____)
 NO

BUDGET: Total Request \$12,000
(May not exceed \$12,000)

IRB AND IACUC APPROVAL:

To ensure that the University is in compliance with all federal regulations, complete the checklist below. Note: your proposal can be approved prior to IRB or IACUC approval, but funding will be withheld until notification of approval or exemption.

| Yes | No | Office of Research Compliance | Policy # |
|-----|----|--|----------|
| | | Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date: | 19.052 |
| X | | Animal Species: Mus musculus Institutional Animal Care & Use Committee (IACUC) Approval #: H04-12 Expiration Date: 7/12/13 | 19.049 |

SIGNATURES

| Applicant's Signature | | Chair/Director's Signature | |
|-----------------------|----------------------------|----------------------------|------------------------------|
| Signature | <i>Karen T. Coschigano</i> | Signature | <i>Andreas R. Biknevicus</i> |
| Name | Karen T. Coschigano | Name | Andreas R. Biknevicus |
| Dept/School | Biomedical Sciences | Unit | Biomedical Sciences |
| Date | 10/11/12 | Date | 10/11/12 |
| Dean's Signature | | | |
| Name | <i>Jack Blazyk</i> | Signature | <i>Jack Blazyk nfg</i> |
| College | HCOM | Date | 10/11/12 |

Optional: If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises. (Sign below)

Signature: _____ Date: _____

BAKER FUND APPLICATION CHECKLIST

Applicants **must** complete and sign the checklist. The checklist should be included as the second page of the application (following the cover page).

- | | |
|--|---|
| <input checked="" type="checkbox"/> Cover page | use Baker form |
| <input checked="" type="checkbox"/> Checklist | use Baker form |
| <input checked="" type="checkbox"/> Abstract* | 1 double-spaced page |
| <input type="checkbox"/> Introduction (<i>For Continuations or Re-submissions Only</i>)* | 1 double-spaced page |
| <input checked="" type="checkbox"/> Discussion | 10 double-spaced pages |
| <input type="checkbox"/> Glossary/Definition of Terms* (<i>Not required</i>) | 2 double-spaced pages |
| <input checked="" type="checkbox"/> Bibliography (<i>Not required</i>) | 3 pages |
| <input checked="" type="checkbox"/> Biographical information (<i>Applicant(s) and key personnel</i>) | 3 pages per person |
| <input checked="" type="checkbox"/> Other Support (<i>Applicant(s) and key personnel</i>) | 1 page per person |
| <input checked="" type="checkbox"/> Budget and Justification | no limit specified |
| <input checked="" type="checkbox"/> Appended Materials | 10 pages |
| <input type="checkbox"/> Appended Electronic Materials | No more than 10 minutes of footage |
| <input checked="" type="checkbox"/> Recommended Reviewers | 5 required |
| <input checked="" type="checkbox"/> Electronic copy of proposal | Single Acrobat file, containing entire proposal and required signatures |

* These sections should be written in language understandable by an informed layperson to assist the committee in its review.

Please Note: Proposals that do not conform to these format and section requirements will be returned without review by the committee.

Applicant signature: Karen J. Coschugar

ABSTRACT

The long term goals of my research are to learn how diabetes damages the kidney with the hope of discovering ways of halting or even preventing the damage. We use mice to model the human disease. We work with a particular strain of mice that cannot express the protein Signal Transducer and Activator of Transcription 5, or STAT5 knockout. Based on other people's work, we expected that mice lacking this protein would be protected from kidney damage due to diabetes. But when we made the mice diabetic, we found the opposite result, the kidneys of the STAT5 knockout mice appeared to be more damaged than normal mice. This suggested that the STAT5 protein may be protective rather than detrimental. We have spent the last few years trying to document the damage that we observe so as to publish our results. Our first manuscript submission was rejected, but we received several helpful comments. This proposal is seeking funding so that we can perform the experiments that were suggested by the reviewers. Three undergraduate and one graduate student will help me with the experiments, along with the help of a colleague. All of the experiments require techniques that are routinely used in the lab. As we collect our data, we plan to present our findings at appropriate meetings, allowing everyone to polish their presentation skills. Once we have adequately addressed the concerns of the reviewers through our data collection and analyses, we plan to rewrite and resubmit the manuscript for review.

DISCUSSION

A. Specific Aims

The long-term goal of my research is to elucidate the role that STAT5 proteins play in diabetic nephropathy while training the next generation of research scientists. Investigations in my lab utilizing a STAT5 knockout mouse line have indicated that these mice, when made diabetic by injection with streptozotocin, exhibit more pronounced characteristics of kidney damage than diabetic mice with an intact STAT5 gene. These results suggest that STAT5 may be playing a protective role in the kidney, which is contrary to what we expected. Thus we have spent considerable time characterizing these diabetic STAT5 knockout mice. Our initial attempt to publish our findings was met with several concerns and a rejection of the manuscript. Thus, **the goal of this proposal is to address the reviewers' comments and resubmit the manuscript.**

To achieve this goal, we need to complete the following specific aims: *1) assess additional morphological and physiological parameters characteristic of diabetic nephropathy to more fully support our claim of increased kidney damage; 2) assess protein levels to establish whether they correlate with the RNA expression that we reported for several genes; 3) assess the activation status of several other STAT family members that have been shown to compensate in situations of STAT deficiency; 4) assess the RNA levels of several targets of STAT5 regulation to demonstrate STAT5 deficiency; and finally 5) edit and resubmit the manuscript for review.*

B. Significance

Contribution to the Research Goals of the Applicant. By collecting the additional data requested by the reviewers, we will strengthen our claims regarding the effect of STAT5 deletion in the

diabetic kidney. This should aid our efforts to publish our novel findings and provide the basis for future research regarding STAT5 and diabetic nephropathy. One future goal would be to seek external funding to support our research efforts. A publication establishing our credibility in this area is practically a requirement for a successful grant application. This proposal would also engage and train students, mainly undergraduates, in basic research. This is a high priority for the PI, who has established a strong track record of involving students in her research for more than twenty years.

Contribution to the Discipline. Little is known regarding the role of STAT5 in diabetic nephropathy. In vitro, it has been shown that activity of three STAT family members, STAT1, STAT3, and STAT5, was enhanced when rat glomerular mesangial cells were grown in the presence of high glucose (1). In vivo, activation of the same three STAT proteins was substantially increased in glomeruli of streptozotocin-treated diabetic rats, and this increase was dramatically attenuated by treatment of the rats with an angiotensin receptor antagonist; the inhibitor also prevented a streptozotocin-induced increase in urinary protein excretion, another hallmark of diabetic kidney damage (2). Increasing expression of phosphorylated (i.e. activated) STAT5 protein was also seen in kidney biopsies of patients with early to advanced stage diabetic nephropathy (3). While observation of activated STAT proteins in these pathological settings suggests a role in diabetic nephropathy, it is not clear exactly what this role is. As a first step toward elucidating this role for the STAT5 proteins, we induced diabetes in a STAT5 gene-disrupted (knockout or SKO) mouse line using streptozotocin. We are the first to assess the effect of diabetes in the STAT5 knockout mice. Surprisingly, indicators of diabetic nephropathy increased rather than decreased in the diabetic SKO mice, suggesting a protective role for the

STAT5 proteins in diabetic kidneys (see Preliminary Studies). The knowledge gained from these studies will help establish, or clarify, the role of STAT5 in the diabetic kidney. This knowledge may someday be translated into a therapeutic drug that could help treat or prevent diabetic nephropathy.

Dissemination. In addition to the anticipated publication, the results of this study will be presented at local and national/international scientific meetings when appropriate. The PI's students will be encouraged to present their work in group meetings, seminars, and Ohio University's annual Research and Creative Activities Expo in the spring, just as former students have. The PI will likely present the findings at an annual meeting of either the American Society of Nephrologists or the Endocrine Society. Each of these forums will serve to publicize the research results, increase presentation skills, and augment the reputation of the researchers and Ohio University.

C. Preliminary Studies

Initial manuscript. In our first manuscript submitted for peer review, we reported the results of a number of physiological, morphological and molecular analyses that we felt demonstrated an increase in kidney damage in the diabetic STAT5 knockout mice as compared to nondiabetic and nontransgenic (i.e. STAT5 intact) controls. We first demonstrated that the STAT5 knockout mice responded to the diabetes in a similar manner to non-transgenic controls in terms of body weight gain or loss (Figure 1A), kidney wet weight (Figure 1B) and degree of hyperglycemia (data not shown). One difference between the STAT5 knockout and the non-transgenic controls was the change in glomerular volume. A significantly increased glomerular volume, a

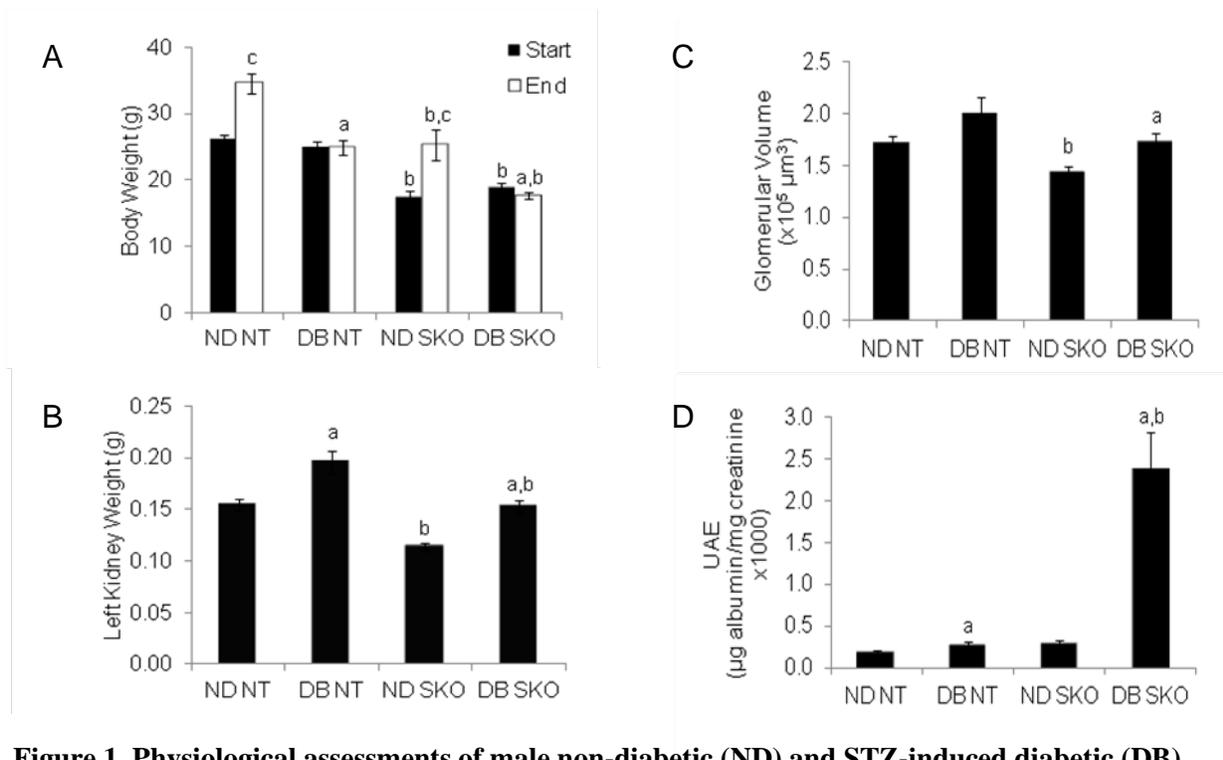


Figure 1. Physiological assessments of male non-diabetic (ND) and STZ-induced diabetic (DB) nontransgenic (NT) and STAT5a/b knock-out (SKO) mice after 11 weeks of STZ-induced diabetes. Start and end body weight; left kidney wet weight; glomerular volume; and urinary albumin excretion calculated as a ratio of albumin and creatinine. Means \pm SE of 10 mice for each group are presented. a: significantly different ($P < 0.05$) from corresponding ND. b: significantly different ($P < 0.05$) from corresponding NT. c: significantly different ($P < 0.05$) from corresponding start weight.

suggestion that the kidney is working harder than usual, was seen in the diabetic STAT5 knockouts in comparison to the non-diabetic STAT5 knockouts; a significant increase was not detected for the non-transgenic diabetic mice (Figure 1C). The most striking difference of the physiological assessments, however, was an approximately 10-fold increase in urinary albumin excretion over all other mouse groups (Figure 1D). This is a hallmark of diabetic nephropathy suggesting damage to the glomerulus, or blood filtering unit of the kidney (Figure 1D).

We also assessed RNA levels of a number of different genes which indicated increased kidney damage in the diabetic STAT5 knockout mice. For example, RNA levels of two extracellular

matrix genes, fibronectin and collagen IV, were highest in the diabetic STAT5 knockout mice (Figure 2). Accumulation of extracellular matrix proteins leads to diabetic glomerular fibrosis, which is strongly linked to the progressive decline of kidney function (4). In addition, since expression of fibronectin and collagen IV had previously been shown to be regulated by two other proteins, transforming growth factor (TGF) β -1 and monocyte chemoattractant protein (MCP)-1, in mesangial cells (from the glomerular filtering unit) cultured under high glucose mimicking diabetic conditions (5), we assessed the RNA expression of these two genes as well. As we predicted, both were also significantly elevated in the diabetic STAT5 kidneys, most closely resembling the results seen for fibronectin (data not shown). Finally, the increased levels of MCP-1 suggested recruitment to the kidney of a special type of immune cell called macrophages, which have also been linked to diabetic kidney damage (6). Exactly as seen for the previous genes, the RNA expression of two different macrophage markers, F4/80 and CD68, was increased to the greatest extent in the diabetic SKO kidneys (data not shown). Considering all of our results, STAT5 knockout mice exhibited increased rather than decreased diabetic

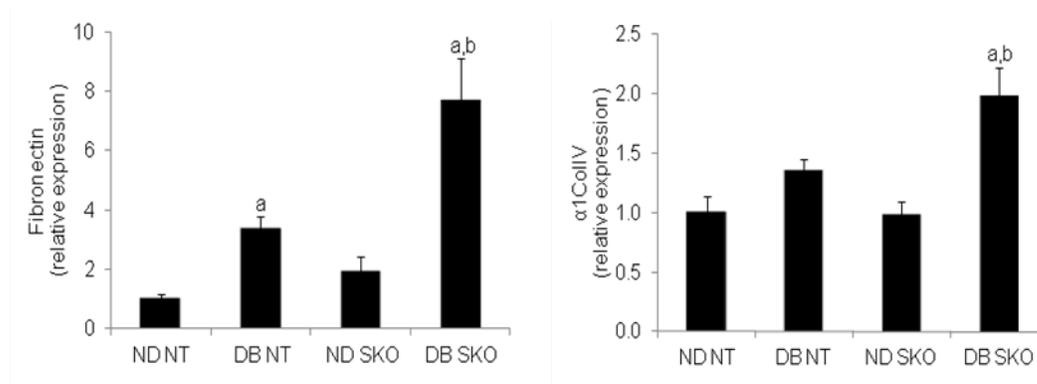


Figure 2. Real-Time Reverse Transcription Polymerase Chain Reaction (RT-RT/PCR) analysis of mRNA levels in kidneys of non-diabetic (ND) and STZ-induced diabetic (DB) male nontransgenic (NT) and STAT5a/b knock-out (SKO) mice after 11 weeks of STZ-induced diabetes. Expression was normalized using the geometric mean of γ -actin and GAPDH expression. Means \pm SE of 10 mice for each group are presented relative to the ND NT males. CollIV, collagen IV. a: significantly different ($P < 0.05$) from corresponding ND. b: significantly different ($P < 0.05$) from corresponding NT.

nephropathy, implying a protective role for STAT5 in the kidney.

Recent progress. The comments of the reviewers of our manuscript focused on four major points. First, increased kidney weight, glomerular volume and urinary albumin excretion were not sufficient to support our claim of increased kidney damage. They wanted to see measures of additional morphological and physiological parameters characteristic of diabetic nephropathy, such as mesangial area or glomerular basement membrane thickness. Both are typically increased in diabetic nephropathy. We measured each. We saw no differences in mesangial area among the four groups, and the only significant difference we found in membrane thickness was a decrease, not increase, in the diabetic STAT5 knockouts (data not shown). Neither support our claim of increased kidney damage in the diabetic STAT5 knockouts, but suggest that we may be examining an early stage of kidney damage before these changes are apparent. This is supported by the fact that we terminated our study after 11 weeks of diabetes, whereas other studies using a similar diabetes induction method to ours often go much longer. Another factor may be the genetic background of our mice. It has been demonstrated that different genetic backgrounds respond differently to diabetes (7). Yet a third explanation is presented below.

The second major comment was that we needed to assess protein levels to establish whether they correlate with the RNA expression that we reported for several genes. Using immunohistochemical methods to assess F4/80 quantity and location of expression within the kidney have been the most successful. We found that macrophage infiltration was significantly greater in the diabetic STAT5 knockout mice, and further, that the infiltration was limited to the tubulointerstitial region; no staining was seen in the glomeruli (data not shown). This suggests

that the kidney damage is predominantly outside of the glomeruli, which might explain why we are not seeing changes in the glomerular parameters of increased mesangial area and membrane thickness. In light of the macrophage results, we also examined expression of vascular cellular adhesion molecule 1 (VCAM1), which helps macrophage infiltrate tissues and is a marker for dysfunctional endothelial cells. We had already shown that VCAM1 RNA expression was increased in the diabetic STAT5 knockout mice. In correlation with the RNA levels and the macrophage markers, we saw increased VCAM1 staining in the diabetic STAT5 knockout mice (data not shown).

The third major comment was that we should assess the activation status of several other STAT family members that have been shown to compensate in situations of STAT5 deficiency, in particular STAT1 and STAT3 (8). Assessments of each at the RNA level have not demonstrated significant increases in the diabetic STAT5 knockout mice. Initial attempts at measuring the phosphorylated, or active, forms of STAT1 and STAT3 have been inconclusive. These studies need to be pursued further.

The final major comment suggested looking at RNA levels of targets of STAT5 regulation. Two genes mentioned were SOCS2 and SOCS3. We have determined that these genes are probably not regulated exclusively by STAT5 and thus are not reliable indicators of STAT5 activity. In fact, STAT1 and STAT3 may contribute to regulation of SOCS3 regulation of SOCS3 (9). Thus, different target genes are needed.

D. Methods

Our preliminary studies attempted to establish a role for STAT5 in diabetic nephropathy by assessing several parameters of kidney damage in diabetic STAT5 knockout mice. We also have started to address the concerns of the reviewers of our initial manuscript reporting our findings. However, significant issues remain to be tested before we can resubmit our manuscript. This proposal seeks funding for the following specific aims and studies:

1) assess additional morphological and physiological parameters characteristic of diabetic nephropathy to more fully support our claim of increased kidney damage. Since we observed macrophages in the tubulointerstitial and not glomerular region of the kidney, we plan to assess tubulointerstitial parameters of kidney damage. We will measure capillary dilation in the four groups of mice, using the VCAM1 stained slides mentioned in the Preliminary Results. VCAM1 strongly stains the endothelial cells of the capillaries. This feature will make it easy to outline cross-sections of the capillaries and calculate the area of each. My students will do this under supervision of my collaborator, Dr. Ramiro Malgor, a pathologist whose specialty is morphological and immunohistochemical methods. The methods used will be similar to those used for measuring glomerular volume presented earlier. We expect to see a larger cross-sectional area of capillaries in diabetic STAT5 knockout mice.

2) assess protein levels to establish whether they correlate with the RNA expression of specific genes. We plan to assess protein levels in several ways. First, we will continue to try western blot analysis as these can be fairly easily quantified. We will also perform immunohistochemistry to determine amount and location within the kidney. Finally, we will use the Millipore Milliplex Luminex Bioanalyzing System at Konneker Research Labs here at Ohio

University to quantify soluble proteins like MCP1 and TGF β -1. This last method offers the advantages of assaying multiple proteins in a single kidney sample and assaying up to 40 kidney samples simultaneously. We already have samples prepared for this analysis and a dedicated technician will perform the assays for us.

3) assess the activation status of several other STAT family members that have been shown to compensate in situations of STAT deficiency. It has been demonstrated that STAT1 and STAT3 proteins are upregulated when there is a deficiency of STAT5 (8). Further, it has been suggested that STAT3 may play a damaging role in the kidney (10). Thus, it is imperative to determine whether STAT1 or STAT3 activity increases in the absence of STAT5 in the kidney. While we have been trying to determine phosphorylated levels of the STAT proteins by western blot analysis, use of the Milliplex system promises to be faster, easier and more quantitative; this is the method that we will pursue in the future. Millipore has a kit that can detect the phosphorylated forms of those three STATs together in one sample. They also have kits that can detect the total (phosphorylated and nonphosphorylated) STATs in a separate reaction. The total STAT measurements could be used to normalize the phosphorylated STAT results between kidney samples. Determining levels of phosphorylated STAT1 and STAT3 could help establish whether the kidney damage seen in the diabetic STAT5 knockouts is due to loss of STAT5 or gain of STAT1 or STAT3.

4) assess the RNA levels of several targets of STAT5 regulation to demonstrate STAT5 deficiency. Through literature searches, we have identified two genes that appear to be regulated predominantly by STAT5, insulin-like growth factor 1 (IGF1) (11) and cytokine-inducible SH2-

containing protein (CISH) (9). We plan to use real-time RT/PCR to assess their RNA levels in the four groups of mice. We expect to see decreased levels of each in the STAT5 knockout mice, reflecting the absence of STAT5 protein.

5) edit and resubmit the manuscript for review. Once we have adequately addressed the four major concerns of the reviewers, we will edit and resubmit the manuscript for review. With three undergraduate and one graduate student performing most of the experiments, we expect to complete the tasks within a year. During the school year, their time in the lab will be understandably limited, but we should make significant progress over the summer and breaks.

E. Collaborations

Dr. Ramiro Malgor, MD, Associate Professor of Pathology in the Department of Biomedical Sciences at Ohio University will continue to assist with morphological and immunohistochemical assessments as needed, as these are his areas of expertise (see letter of collaboration in Appendix). Three undergraduate and one graduate student will assist with all aspects of this project, just as they have for most of my research endeavors. Two of the students are already proficient in all of the needed techniques and will help to train the newer students.

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11. D. J. Chia *et al.*, "Characterization of distinct Stat5b binding sites that mediate growth hormone-stimulated IGF-I gene transcription," *J. Biol. Chem.* **281**, 3190 (2006).

BIOGRAPHICAL INFORMATION

Karen T. Coschigano, PhD
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Heritage College of Osteopathic Medicine
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Publications (last 5 years out of 50 total)

(†, corresponding author; *undergraduate; **medical student)

Coschigano KT†, Wetzel AN, Obichere N**, Sharma A*, Lee S*, Rasch R, Guigneaux MM, Flyvbjerg A, Wood TG, Kopchick JJ (2010) Identification of Differentially Expressed Genes in the Kidneys of Growth Hormone Transgenic Mice. *Growth Hormone & IGF Research* 20, 345-55.

Stevenson AE, Evans BAJ, Gevers EF, Elford C, McLeod RWJ, Perry MJ, El-Kasti MM, **Coschigano KT**, Kopchick JJ, Evans SL, and Wells T (2009) Does adiposity status influence femoral cortical strength in rodent models of growth hormone deficiency? *Am J Physiol Endocrinol Metab*, Jan 2009; 296: E147 - E156.

Smid JR, Rowland JE, Young WG, **Coschigano KT**, Kopchick JJ, Waters MJ (2007) Mouse molar dentin size/shape is dependent on growth hormone status. *J Dent Res* 86, 463-8.

Davies JS, Gevers EF, Stevenson AE, **Coschigano KT**, El-Kasti MM, Bull MJ, Elford C, Evans BA, Kopchick JJ, Wells T (2007) Adiposity profile in the dwarf rat: an unusually lean model of profound growth hormone deficiency. *Am J Physiol Endocrinol Metab* 292, E1483-94.

Venken K, Moverare-Skrtic S, Kopchick JJ, **Coschigano KT**, Ohlsson C, Boonen S, Bouillon R, Vanderschueren D (2007) Impact of Androgens, Growth Hormone and IGF-I on Bone and Muscle in Male Mice during Puberty. *J Bone Miner Res* 22, 72-82.

Zhang X, Mehta RG, Lantvit DD, **Coschigano KT**, Kopchick JJ, Green JE, Hedayat S, Christov KT, Unterman TG, Swanson SM (2007) Inhibition of estrogen independent mammary carcinogenesis by disruption of growth hormone signaling. *Carcinogenesis* 28, 143-150.

Papers Presented (last 5 years out of 49 total)

(†, presenting author; *undergraduate; **medical student; ***graduate student)

Magali Araujo†, Nancy Koles, **Karen T. Coschigano**, Edward O. List, Sonia Q. Doi. Markers of podocyte injury at the initial stages of CKD. Powerpoint presentation for the CIN2011 6th Congress of Nephrology in Internet.

Magali Araujo†, Audrey Lee*, Cecilia Courreges, Nancy Koles, **Karen T. Coschigano** and Sonia Q Doi. Aberrant expression of CD68 in podocytes precedes inflammatory markers in STZ-induced diabetic nephropathy. Oral presentation at the American Diabetes Association's 2010 annual meeting in Orlando, FL in June.

Sulalita Chaki†***, Audrey Lee*, and **Karen T Coschigano**. Characterization of mesangial cell lines established from nontransgenic (NT) and growth hormone receptor knockout (GKO) mice. Poster presentation at the 2010 OU Student Research and Creative Activity Expo in May.

Amanda Harwood†*, Chad Keller, Audrey Lee*, Brett Buller**, Erika Swanson* and **Karen T Coschigano**. Differential expression of inflammation-related genes in kidneys of diabetic STAT5a/b mice. Poster presentation at the 2010 OU Student Research and Creative Activity Expo in May.

Katie Schreiber†*, Chad Keller, Audrey Lee*, and **Karen T Coschigano**. Real-time RT/PCR validation of microarray data for GHR KO protection based on inflammatory gene expression in the diabetic mouse kidney. Poster presentation at the 2010 OU Student Research and Creative Activity Expo in May.

Karen T. Coschigano†, Erika Swanson*, Chad Keller, Audrey Lee*, Amanda Harwood*, Maria C Courreges, and Ramiro Malgor. STAT5a/b Knockout Alters Expression of Inflammation-Related Genes in Kidneys of Diabetic Mice. Poster presented at the American Society of Nephrology's 2009 annual meeting in San Diego in October.

Magali Araujo†, Audrey Lee*, Cecilia Courreges, Nancy Koles, **Karen Coschigano** and Sonia Q Doi. Early up-regulation of glomerular class A scavenger receptor (SRA) is dissociated from CD68 in STZ-diabetic mice. Abstract submitted for the American Society of Nephrology's 2009 annual meeting in San Diego in October.

Sulalita Chaki†***, Chad M Keller, Audrey Lee*, **Karen T Coschigano**. The influence of diabetes and reduced growth hormone signaling on inflammation-related RNA expression in mouse kidney. Poster presentation at the 2009 OU Student Research and Creative Activity Expo in May and at the ENDO 2009 Annual meeting in Washington, DC in June.

Audrey Lee†*, Chad M Keller, Sulalita Chaki***, Ramiro Malgor, **Karen T Coschigano**. Sialoporphin and Complement Component 3 Expression Correlate with Diabetic Kidney Damage in Mice. Poster presentation at the 2009 OU Student Research and Creative Activity Expo in May and at the ENDO 2009 Annual meeting in Washington, DC in June.

Erika Swanson†*, Chad M. Keller, Audrey Lee*, Ramiro Malgor, and **Karen T Coschigano**. Deletion of STAT5a/b Increases Diabetic Damage in Mouse Kidney. Poster presentation at the 2009 OU Student Research and Creative Activity Expo in May and at the ENDO 2009 Annual meeting in Washington, DC in June.

Elahu S Gosney†, Andy Dittenhofer, Markus E Riders, **Karen T Coschigano**, John J Kopchick. Discovery and Characterization of Two Isoforms of Sec24-D mRNA Expressed in Dwarf Growth Hormone Receptor Gene-Disrupted Mice. Poster presentation at the ENDO 2009 Annual meeting in Washington, DC in June.

Sonia Q. Doi†, Chad M. Keller, Audrey A.C. Lee*, Nancy Koles, Daniela Gusmao and **Karen T. Coschigano**. (2008) Up-regulation of class A scavenger receptor in diabetic nephropathy is growth-hormone dependent. The American Society of Nephrology Annual Meeting, Philadelphia, PA. Poster.

Coschigano, K.T.†, Malgor, R., Keller, C.M. (2007) Assessing physiological and molecular indicators of kidney damage in diabetic mice expressing a kidney androgen regulated protein (KAP) transgene. The American Society of Nephrology Annual Meeting, San Francisco, CA. Poster.

BIOGRAPHICAL INFORMATION

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Associate Professor of Pathology
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Publications (last 5 years)

“Wnt5a is Elevated in Advanced Atherosclerotic Lesions and Serum from Patients with Clinical Cardiovascular Disease” **Ramiro Malgor**, Pooja M Bhatt, Beth Gregory, Denise L. House, Mitchell J. Silver, Kelly D. McCall, Douglas J. Goetz. *Under review Atherosclerosis*

1. Pooja M. Bhatt, Christopher J. Lewis , Denise L. House , Chad M. Keller, Leonard Kohn, Mitchell J. Silver, Kelly D. McCall, Douglas J. Goetz and **Ramiro Malgor** Increased Wnt5a mRNA expression in advanced atherosclerotic lesions, and oxidized LDL- treated human monocyte-derived macrophages. *The Open Circulation and Vascular Journal*, 2012, 5, 1-7.
2. K. McCall, F. Benencia, L. Kohn, **R. Malgor**, A. Schwartz and F. Shwartz. Toll-like Receptors as Novel Therapeutic Targets for the Treatment of Pancreatic Cancer. Book Chapter accepted “Pancreatic Cancer / Book 2” ISBN 979-953-307-701-6. Editor Sanjay Srivastava.
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5. Chinna Malakondaiah Kummitha, Kristine M. Mayle, Mark A. Christman II, Sudhir P. Deosarkar, Anthony L. Schwartz, Kelly D. McCall, Leonard D. Kohn, **Ramiro Malgor**, Douglas J. Goetz. A sandwich ELISA for the detection of Wnt5a *J. Immunol Methods* 352(1-2):38-44. 2010.
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8. Sudhir P. Deosarkar, **Ramiro Malgor**, Jie Fu, Leonard D. Kohn, Justin Hanes, Douglas J. Goetz. Polymeric Particles Conjugated with a Ligand to VCAM-1 Exhibit Selective, Avid and Focal Adhesion to Sites of Atherosclerosis. *Biotechnology and Bioengineering*. 101(2):400-407. 2008.
9. Mark A Christman II, Douglas J Goetz, Eric Dickerson, Kelly D McCall, Christopher J Lewis, Fabian Benencia, Mitchell J Silver, Leonard D Kohn, and **Ramiro Malgor**. Wnt5a is Expressed in Murine and Human Atherosclerotic Lesions *Am J Physiol Heart Circ Physiol* 294: H2864-2870. 2008.
10. Kim, W.B., Lewis, C.J., McCall, K., **Malgor, R.**, Kohn, A.D., Moon, R.T., Kohn, L.D. Overexpression of Wnt-1 in Thyrocytes Enhances Cellular Growth But Suppresses Transcription of the Thyroperoxidase Gene via Different Signaling Mechanisms. *Journal of Endocrinology* 193(1):93-106, 2007.
11. McCall, K.D., Harii, N., Lewis, C.J., **Malgor, R.**, Kim, W.B., Saji, M., Kohn, A.D., Moon, R.T., Kohn, L.D. High Basal Levels of Functional Toll-like Receptor 3 (TLR3) and Non-Cannonical Wnt5a Are Expressed in Papillary Thyroid Cancer (PTC) and Are Coordinately Decreased by Phenylmethimazole Together with Cell Proliferation and Migration. *Endocrinology* 148 (9):4226-4237, 2007.

Papers Presented (last 5 years out of 49 total)

1. Oxidized LDL increases Wnt5a expression in human monocyte-derived macrophages. P.M. Bhatt, C.J. Lewis, D.L. House, D.J. Goetz and R. Malgor Wnt 2011 meeting Los Angeles CA June30-July 3, 2011.
2. Human Atherosclerotic Lesions with Advanced Histopathological Features Showed Higher Expression of Wnt5a. Beth Gregory , Denise House , Mitchell J. Silver and Ramiro Malgor. Atherosclerosis, Thrombosis and Vascular Biology 2011 Scientific Sessions. Chicago April 28 -30 2011. Poster 226 pp 90 Abstract book
3. *In vitro* expression of Wnt5a in urothelial carcinoma and its effect on cellular migration. Seth Crouser, Denise House, Christopher Lewis, **Ramiro Malgor** Experimental Biology 2011, Washington DC April 9-13 Poster C142 pp 251 Abstract book.
4. Effects of GH and IGF-1 treatments on body composition, glucose metabolism and hepatic gene expression in obese/diabetic mice with nonalcoholic fatty liver disease. List EO, Blischak JD, Berryman DE, Lubbers E, **Malgor R**, Kopchick JJ The 5th International Congress of the GRS and the IGF Society. New York, NY, Oct 3-7, 2010. GH and IGF Research vol.20(suppl#1)Oct, 2010.
5. Upregulation of Wnt5a transcripts in human monocytes treated with ox-LDL. P. M. Bhatt, C.J. Lewis, D.L. House, D.J. Goetz and **R. Malgor**. BMES 2010 Annual Fall Meeting, Austin, Texas, October 6 - 9, 2010
6. The Effects of GH, IGF-1 or Combined GH/IGF-1 Therapies on Type 2 Diabetes and Nonalcoholic Fatty Liver Disease (NAFLD) in Mice. List EO, Berryman DE, Bower B, Blischak JD, Wright-Piekarski J, Lubbers E, **Malgor R**, and Kopchick JJ. The Endocrine Society 92st Annual Meeting, San Diego, CA, June 19-22, 2010.

7. Wnt5a expression in human atherosclerotic plaques is elevated in macrophage-rich and complicated plaques. **R. Malgor**, C.J. Lewis, C. Keller, L.Kohn, M.J. Silver, D.J. Goetz. 78th European Atherosclerosis Society Congress, Hamburg Germany, 2010. abstract 1128
8. Expression of Wnt5a correlates with histopathological features of bladder urothelial carcinoma. **R Malgor**, C. Brockett, D. Grecco, D. House and K.McCall. Experimental Biology 2010, Anaheim, CA Abstract LB451.
9. The expression of Wnt5a in Human Atherosclerotic Lesions is Higher in Areas with Advanced Disease **Ramiro Malgor**, Christopher J. Lewis, Chad M. Keller, Denise House, Mitchell J. Silver, Leonard D. Kohn, Douglas J. Goetz. 2009. Wnt Meeting Georgetown University, Washington DC June 11-14.
10. Wnt5a and Toll Like Receptor Cross-signaling in Pathogenesis of Atherosclerosis **Ramiro Malgor**, Chad M. Keller, Christopher J. Lewis, Douglas J. Goetz, Mitchell J. Silver, Leonard D. Kohn. BMES 2008 Annual Fall Meeting, St Louis October, 2008.
11. Impact of diet-induced obesity and type 2 diabetes on coronary endothelial adhesion molecule expression in mice. Shawn B. Bender, **Ramiro Malgor**, Richard E. Klabunde. American Society for Investigative Pathology 2007 Annual meeting at Experimental Biology 2007 April 28-May 2, 2007 Washington DC
12. Wnt5a is Expressed in Murine and Human Atherosclerotic Lesions. **R. Malgor**, M.A.Christman, E. Dickerson, K. McCall, M.J. Silver, D.J. Goetz and L.D. Kohn. Wnt Meeting La Jolla San Diego, June 21-23, 2007.
13. Coordinate Expression of Toll-like Receptor 3 (TLR3) and Wnt5a in Papillary Thyroid Cancer (PTC); Phenylmethimazole Decreases Both in addition to PTC Cell Proliferation and Migration. K. McCall, N. Harii, C. Lewis, **R. Malgor**, W. Kim, M. Saji, A.D. Kohn, R.T. Moon, and L.D. Kohn. The 88th Annual Endocrine Society Meeting, Boston, Massachusetts, June 24-27, 2006. Abstract Book, p. 791

OTHER SUPPORT FOR Dr. Karen Coschigano

A. Previous University Funding (last three years)

RSAC small award entitled “Establishment of a Pathogen-Free Mouse Colony”; K Coschigano, PI; 11/15/11 – 11/15/13; \$8000 (funded)
Ohio University’s Program to Aid Career Exploration (PACE) 2011-2012; funded for 300 hours at minimum wage
Ohio University Heritage College of Osteopathic Medicine Laboratory Technician/Research Assistant (LT-RA) Program award; position funded for two years (April 2012-March 2014); Fabian Benencia, Karen Coschigano, Ramiro Malgor, Kelly McCall, co-PIs
Ohio University’s Program to Aid Career Exploration (PACE) 2010-2011; funded for 300 hours at minimum wage
RSAC small award entitled “Investigation of the correlation of degree of inflammatory infiltrate with the degree of diabetic nephropathy in mice”; K Coschigano, PI; 07/10/09 – 06/30/11; \$6500 (funded)
Ohio University’s Program to Aid Career Exploration (PACE) 2009-2010; funded for 300 hours at minimum wage
BMS Department support of an undergraduate student - \$1,800 (June – October 2012)
Research Incentive: \$1,700 remaining
HTC Tutorial Funds: \$1,500 remaining

B. External Funding (last three years)

NIH NIDDK AREA 1 R15 DK081192-01
Title: Cross-talk between growth hormone and inflammation pathways in kidney damage
Duration: 03/10/2008 – 02/28/2012
Investigators: K Coschigano, PI; R Malgor, co-I; R Hikida, co-I
Total costs: \$221,250 (funded)
1 publication; numerous presentations

NIH Availability of Recovery Act Funds for Administrative Supplements
NIH NIDDK AREA 1R15DK081192-01S2
Title: Cross-talk between growth hormone and inflammation pathways in kidney damage
Duration: 12/20/09 – 11/30/11
Investigators: K Coschigano, PI; R Malgor, co-I; R Hikida, co-I
Total costs: \$34,185 (funded)

NIH Summer Research Experiences for Students and Science Educators supplement
NIH NIDDK AREA 1 R15 DK081192-01S1
Title: Cross-talk between growth hormone and inflammation pathways in kidney damage
Duration: 7/15/2009 – 6/30/2011
Investigators: K Coschigano, PI; R Malgor, co-I
Total costs: \$16,103 (funded)

C. Sustainability

Potential sponsors for continuation support: NIH/NIDDK R15 mechanism

Other Support – Dr. Ramiro Malgor

Ongoing Research Support

Title: Understanding Wnt5a and TLR-4 cross-signaling during atherosclerosis

Agency: NIH **Type:** 1 R15 HL092545

Period: 04/01/2009-03/31/2012

The goal of this project is to study the role of Wnt5a signaling in the pathogenesis of atherosclerosis and dissect the possible connection to Toll-like receptors signaling.

Role: PI

Title: TLR Signal Inhibition: A Novel Therapeutic Paradigm

Agency: NIH via a subcontract from the Interthyr Corporation **Type:** R42: AI066618 (Phase II)

Period: 9/22/09-08/31/11

The goal of this study is to develop a novel agent with a new therapeutic paradigm: Inhibition of Toll-like receptor (TLR) expression and signaling in non-immune cells.

Role: Co-Investigator

PI: Kohn

Title: The Role of Toll-Like Receptor-3 in ITD & Efficacy of a Novel Therapeutic

Agency: NIH **Type:** 1R15

Period: 09/30/2010-08/31/2012

The goal of this project is to study the role of Toll-like Receptor 3 pathway in the pathogenesis of diabetes type 1 and the effect of a novel drug in this signaling.

Role: Co-Investigator

PI: McCall

Title: Cross-talk between growth hormone and inflammation pathways in kidney damage

Agency: NIH **Type:** 1R15DK081192

Period: 03/10/2008-02/28/2010

Extension: 08/01/2009-07/31/2011

The goal of this project is to study the link between growth hormone signaling and inflammation for kidney alterations related with diabetes.

Role: Co-Investigator

PI: Coschigano

Completed Research Support

Title: Acquisition of a Laser Microdissection System

Agency: Ohio University 1804 Fund Program

Period: 07/22/08-06/30/10

The goal of this project was the acquisition of a Laser Microdissection System.

Role: PI

BUDGET AND JUSTIFICATION

| | |
|--|-----------------|
| Consumable Supplies..... | \$10,000 |
| Real-time RT/PCR reagents for RNA analysis, western blot and immunohistochemical reagents and antibodies for protein analysis, Milliplex kits for protein quantitation | |
| Student Wages..... | \$3,600 |
| Hourly wages for undergraduate student worker [(\$8/hour x 10 hours/week x 15 weeks) + (\$8/hour x 30 hours/week x 10 weeks)] | |
| Other..... | \$800 |
| Publication costs (including color pages) | |
| Total budget | \$14,400 |
| Matching funds from PI KCoschigano's Accounts..... | \$2,400 |
| Total Baker Fund request..... | \$12,000 |

RECOMMENDED REVIEWERS

Holly Brown-Borg, PhD
Department of Pharmacology, Physiology & Therapeutics
School of Medicine & Health Sciences
University of North Dakota
501 North Columbia Road
Grand Forks, ND 58202-9037
Email: holly.brown.borg@med.und.edu
Phone: (701) 777-3949
Fax: (701) 777-4490

Holly Brown-Borg is a prominent researcher in the growth hormone and aging field. We met at a meeting and have a common collaborator, but we have never collaborated.

Dominic Cosgrove, Ph.D.
Director of Basic Research, Center for the Study and Treatment of Usher Syndrome
Director, Gene Expression Laboratory
Boys Town National Research Hospital
Center for Hereditary Communication Disorders
555 N. 30th St., Omaha, NE 68131
Email: cosgrove@boystown.org
Phone: (402) 498-6334
Fax: (402) 498-6331

Dominic Cosgrove is a prominent researcher in the fields of renal fibrosis and Alports glomerular disease. He served as a consultant on a previous NIH grant of mine, advising me in a glomerular isolation technique. We met twice at meetings but have had no other interactions.

Sonia Q. Doi, MD, PhD
Research Associate Professor
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Uniformed Services University
4301 Jones Bridge Road
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E-mail: sonia.doi@usush.mil
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Fax: (301) 295-3557

Sonia Doi is a prominent researcher in the fields of growth hormone and nephropathy. I have sent her mice for her studies (I have been included on meeting abstracts for this reason) and we have met occasionally at meetings, but we have not co-authored any publications.

Michal M. Masternak, PhD
Associate Professor
Burnett School of Biomedical Sciences
College of Medicine
BBS 333

University of Central Florida
Orlando, FL
Email: Michal.Masternak@ucf.edu
Phone: 407.266.7113
Fax: 407.266.7002

Michal Masternak is a prominent researcher in the field of growth hormone and aging. We have a common collaborator but we have never met or collaborated.

Wyatt McMahon, PhD
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FAX: (540) 231-2606

Wyatt McMahon is a bioinformatician who studied gene expression in mouse kidney. He helped to analyze a microarray experiment of mine several years ago. We met once at a meeting but have not interacted in over a year. We have not co-authored anything.

APPENDIX

Letter of Collaboration from Dr. Ramiro Malgor (following)



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UNIVERSITY

College of Osteopathic Medicine

October 10th 2012

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Dr. Karen Coschigano PhD
Heritage College of Osteopathic Medicine
Ohio University

Dear Karen,

I am writing this letter to confirm my willingness to continue our collaborative work focused on Diabetic Nephropathy. I would like to express my enthusiasm and support to assist with the morphological and immunohistochemical assessments as needed, as well as with the preparation of the manuscript.

I truly look forward to working with you on this project, which builds upon our earlier collaborations. I strongly believe these studies will help us understand the relationship between STAT5 and diabetic nephropathy.

I look forward to our continued work together on this important study.

Sincerely,

Ramiro Malgor MD
Associate Professor of Pathology
Heritage College of Osteopathic Medicine
Ohio University