I am a physician who is currently in the first year of Instructorship in the Division of Nephrology, having completed 3 years as a postdoctoral fellow in the NIH T32 training program in Vascular Biology and Hypertension following a clinical fellowship in Nephrology. My goal in seeking a Mentored Research Career Development Award is to acquire the additional necessary training, practical experience, and knowledge to develop into an independent clinical investigator committed to a career in patient-oriented research. My undergraduate and clinical training, as well as my postdoctoral training, has been steadfastly devoted to the development of a career in academic medicine. I have excelled as a trainee and have taken full advantage of the opportunities afforded to me. My unique fellowship training has led me to pursue a research career devoted to understanding the physiologic role of the vasculature in the progression and development of chronic kidney disease (CKD), with a long-term career goal to identify and/or develop CKD-specific therapies. The work I propose is timely and cutting-edge with the potential to significantly improve clinical outcomes for the large patient population with CKD. Receipt of a K-Award is a crucial next step in my trajectory toward becoming an independent investigator. My outstanding mentoring team, the well-established record of research career development activities at UAB, and the "protected time" afforded by this K23, will allow me to progress toward achieving my long-term career goal of becoming an independent clinical investigator in the area of CKD-specific therapies. My mentors and I have developed an intensive career development plan as outlined in Table I and within this proposal. I will commit 80% effort to this research project and its associated career development program.

**Table 1. Outline of Proposal**

**1. Candidate's Background**
- A. Undergraduate & Clinical Training
- B. Postdoctoral Training
- C. Graduate Studies

**2. Career Goals and Objectives**

**3. Career Development Plan**
- A. Vascular Function Testing
- B. Physiologic Measures
- C. Didactic Coursework
- D. Professional Development
- E. Mentorship Team
- F. Mentorship Plan
- G. Comp. Activities & Timeline

**4. Research Strategy**
- A. Significance
- B. Innovation
- C. Approach
- D. Future Directions

## 1. CANDIDATE'S BACKGROUND

A. Undergraduate and Clinical Training: I graduated *summa cum laude* from Trinity University where I studied engineering science. It was at Trinity University where I discovered my intense scientific curiosity and began developing skills of critical thinking through engineering design and problem solving. Applying these skills in medical school allowed me to excel, particularly in physiology, and I took on leadership positions in education and teaching. While I was attracted to the science of research throughout my internal medicine residency, it was not until the first year of my nephrology fellowship that I actively participated in research. For my first study, I designed and successfully performed a cross-sectional study in close collaboration with two radiologists. Work from this study, "Pneumonia in hemodialysis patients: a challenging diagnosis," was presented at the National Nephrology Young Investigators Forum and published in 2013.

B. Postdoctoral Training: My second year of nephrology fellowship combined work as Chief Fellow with the start of my postdoctoral clinical research fellowship on a T32 training grant (PI: Suzanne Oparil, MD) under the primary mentorship of David A. Calhoun, MD. During my first year as a postdoctoral fellow, I was immersed in clinical trial research in Dr. Calhoun's lab with roles as sub investigator and lead investigator. My work as a lead investigator on one of his pivotal trials investigating aldosterone excess in resistant hypertension [R01 HL075614 (Calhoun)], allowed me to gain first-hand experience working on an R01-level clinical trial and culminated in an oral presentation at the National American Heart Association Scientific Sessions in my second year of postdoctoral training. In this study, I was introduced to performing vascular function testing on study participants. These tests included measuring blood vessel response to endogenous vasodilators, which is an important measure in my proposed research. Ultimately, I plan to expand upon the vascular function testing techniques developed for our resistant hypertension population in order to answer questions of vascular function in kidney disease. In this way, I am building on my experiences as a postdoctoral clinical researcher, which also include patient recruitment techniques, clinical trial troubleshooting, ensuring study participant rights and safety, randomization and masking techniques, data analysis, and manuscript preparation. At the end of my second year of postdoctoral training, I was awarded the Walter B. Frommeyer, Jr., Fellowship in Investigative Medicine, a competitive fellowship awarded to "outstanding physician-scientists." Receipt of this award provided research funding for a feasibility study to define the vascular phenotype of Liddle's Syndrome. In total the work from my postdoctoral training has produced 8 first author publications in peer-review journals, 2 book chapters, a departmental pilot grant, and 2 oral presentations at national conferences.

C. Graduate Studies: In order to build a strong foundation in clinical trial design, I enrolled into the Master's Program at UAB at the start of my 2nd year of postdoctoral training. My coursework has been personalized to include formal training in clinical trial design, biostatistics, and public health, with expected completion in May 2016. Receipt of this K-Award will allow me the protected time to continue my studies.

**3. CAREER DEVELOPMENT PLAN:** My mentoring team and I have developed a systematic plan that will address needs in training and research experiences utilizing research and educational resources within UAB. The implementation of this plan will address the following needs:

1) Vascular function testing including i) performing noninvasive assessments of endothelial function and arterial stiffness; ii) biochemical measurement of endothelial function, (e.g., oxidative stress).
2) Physiologic measurements including i) reading and interpreting 48 hour ambulatory blood pressure monitoring (ABPM); ii) measuring GFR by iothalamate urine clearance
3) Didactic coursework to gain knowledge of advanced study design and biostatistical methodology.
4) Develop expertise in recruitment and performance of clinical trials.
5) Development of professional skills such as successful grant writing, networking, and communication skills that will contribute to success as an independent investigator.

A. Vascular function testing.

***(i)Noninvasive assessment of endothelial function and arterial stiffness.*** My Primary Mentor, David Calhoun, MD, Professor of Medicine and Director of Vascular Biology and Hypertension Clinic, has conducted many studies measuring ultrasound-guided flow-mediated dilation (FMD) of the brachial artery and pulse wave velocity between the carotid and femoral arteries. His laboratory techniques have been externally validated through multi-center clinical trials. This provides me an excellent opportunity to be trained in the skill of measuring brachial artery flow-mediated dilation and pulse wave velocity. While I have been introduced to these measurement techniques in a small number of study participants, dedicated training with Dr. Calhoun will allow me to acquire these measurements unsupervised. Becoming an expert in vascular function testing will equip me with a deep understanding of the testing limitations, which is critical to successful trial design and data analysis. In the first 3 months of year one, I will spend approximately 100 hours in Dr. Calhoun's laboratory becoming proficient in these techniques.

***(ii)Biochemical measurement of endothelial function*** **(e.g., oxidative stress)**. Oxidative stress has emerged as a leading biomarker of endothelial function. Developing a comprehensive understanding of free radical physiology and measurement limitations in humans will complement my training in noninvasive vascular testing and strengthen the results from my clinical trials of vascular function testing. Dr. Rakesh Patel's, PhD (Consultant), research focuses on nitric oxide and redox cell signaling in the vasculature. As part of his guided training in oxidative stress, I will join the Society for Free Radical Biology and Medicine and attend their yearly meetings. I will attend selected Free Radical Biology Seminars at UAB, offered through the Center for Free Radical Biology. Dr. Patel and I will meet monthly in the first year with the goal of gaining a level of expertise in

measuring oxidative stress in clinical trials. Regular meetings with Dr. Patel will foster future collaborations with basic scientists and promote the sharing of research methods across basic and clinical research fields.

B. Physiologic Measures: Ambulatory blood pressure monitoring (ABPM) for 24-48 hours characterizes the circadian rhythm of blood pressure. Beginning in year 2, I will devote 1 hour a week to reading and interpreting ABPM, which will be overseen by Dr. Calhoun. In addition to training in BP physiology, I will learn a gold standard method for measuring GFR in clinical trials. Beginning in year 3, my monthly meetings with Dr. Allon will be expanded to include training in urine clearance of iothalamate. Both iothalamate measured GFR (mGFR) and 48 hr ABPM will be employed in my proposed research project and potential future R01s.

C. Didactic Coursework: Didactic aspects of the plan have been designed to provide the knowledge and research skills that are needed to meet my overall career goal of becoming and independent clinical investigator. I will build on my ongoing Master's Program coursework with the study of advanced study design and biostatistical methodology. Some of the key courses I will take include:

- **Fundamentals of Clinical Research (EPI 607)**: specific tools for designing and conducting ethically sound clinical trials, integrating epidemiological and biostatistical approaches.
- **Survival Analysis (BST 665)**: Training in Kaplan-Meier estimation, parametric survival models, Cox proportional hazards regression models, competing risks models, and multiple events models.

Dr. Gary Cutter, an expert in clinical trials design and analysis, will serve as Content Expert in these areas.
I will also attend didactic conferences as part of the **Nephrology Research and Training Center's Seminar Series** and the **Vascular Biology and Hypertension Seminars**. These weekly conferences attract basic science and clinical researchers from within and outside UAB to present research focused on projects/experimental strategies that integrate basic concepts and clinical insights into translational research.

D. Professional Development

*(i) Intramural Activities*. The **Center for Clinical and Translational Science (CCTS) at UAB** provides access to numerous resources through the Research Commons, a physical and virtual hub designed to facilitate the use of programs and services by investigators and trainees. Specific resources that I will utilize during the training period are:

1) **Nascent Project Panels** are multidisciplinary, collaborative groups of expert faculty who engage in targeted discussions to positively impact in-process research plans, applications, and manuscripts.
2) **The Professional Skills Training Program** is an ongoing series that includes practical assistance in the areas of scientific writing, scientific presentations, career development and leadership.
3) **Research Methods and Analysis Seminar Series**, a monthly series which provides secondary datasets available for analysis, fosters networking and collaborations campus- and nation-wide.
4) **Training Interdisciplinary Emerging Research Scholars**, a monthly gathering of postdocs, K Scholars and junior faculty with an interest in an academic translational research career. Its design is to encourage networking and promote collaborative learning, and is guided by senior faculty.

*(ii) Presentation of Research and Networking Activities*. I will present at a minimum of one to two national conferences per year with abstract submissions to the **American Society of Nephrology (ASN) Kidney Week**, **American Heart Association (AHA) Scientific Sessions**, or **National Kidney Foundation (NKF) Spring Clinical Meeting**. Attending these meetings will allow for oral or poster presentations of my research findings and networking opportunities with leaders in nephrology and vascular research.

E. Mentorship Team: I have organized an experienced, interdisciplinary mentoring team with excellent research credentials that will work together to ensure success with the proposed research project and my development as an independent clinical investigator (Table 2). My Primary and Co-mentors will share the mentoring

**Table 2. Expertise and meeting schedule for mentors and consultants**

| | Areas of Expertise | Meeting Frequency |
|---|---|---|
| **Primary Mentors** | | |
| David Calhoun, MD | Aldosterone physiology, vascular function testing in resistant hypertension, clinical trial design and implementation | Weekly |
| Michael Allon, MD | Vascular access and function in CKD, K23 & K24 mentoring experience | Monthly |
| **Consultants and Research Facilitators** | | |
| Rakesh Patel, PhD | Measures of oxidative stress | Monthly in the 1st year then Quarterly |
| Anupam Agarwal, MD | Leadership, career and professional development | Quarterly |
| Gary Cutter, PhD | Statistical methods in clinical trials | Quarterly |

responsibilities as outlined below.

*(i) Mentors:* **Primary Mentor: David Calhoun, MD,** serves as the Medical Director of the Vascular Biology and Hypertension Program and as my primary mentor during my postdoctoral training. He is a worldwide expert in aldosterone excess in resistant hypertension, and his research has been well funded with current NIH R01 funding through 2019. His ongoing studies are investigating the pathophysiology of hypertension refractory to medical therapy, obstructive sleep apnea, and aldosterone excess. These clinical trials have regularly included vascular function testing with published data on over 200 participants. Overall, Dr. Calhoun has a greater than 20 year experience as an independent clinical investigator and provides an ideal role model for a young, developing clinical investigator. He and I will continue to meet weekly to discuss my research projects. He will oversee training in both FMD and ABPM reading and interpretation. Importantly, he will provide ongoing guidance in the management of clinical trials.

**Table 3. Benchmarks for candidate evaluation by mentors**

| Time | Career | Research |
|---|---|---|
| 6 months | Training in responsible conduct of research | Completion of vascular function training (oxidative stress & FMD) |
| 12 months | Completion of Master's Degree in Biostatistics | Recruitment of 12 participants for Aims 1 & 2 |
| 18 months | Participation in TIERS<br>Attend Southern Salt and Water Kidney Club and Society for Free Radical Biology and Medicine* | External evaluation of source documentation and adverse event reporting. |
| 24 months | Submission of 2 research manuscripts in peer reviewed journals<br>Attend Southern Society of Clinical Investigators* | Recruitment of 36 participants for Aims 1 & 2 and 12 for Aim 3 |
| 36 months | Oral presentation at ASN Kidney Week and/or AHA Scientific Sessions | Completion of training in ABPM interpretation.<br>Recruitment of 50 participants for Aims 1 & 2 and 20 for Aim 3 |
| 48 months | Manuscript submission for Aims 1 & 2<br>Preparation of R01 | Completion of training in iothalamate measured GFR. Recruitment complete for Aims 1 & 2, 30 for Aim 3 |
| 60 months | Manuscript submission for Aim 3<br>Submission/resubmission of R01 | Recruitment complete for Aim 3 |

*In addition to yearly ASN and AHA conferences
ABPM = ambulatory blood pressure monitoring

**Co-Mentor: Michael Allon, MD,** is a Professor of Medicine in the Division of Nephrology at UAB. He is an expert in clinical trials involving vascular access in the late stages of CKD, with active NIDDK funding investigating fistula non-maturation. He has experience as a patient-oriented research mentor, having held an NIDDK K24 mentoring grant and serving as a K23 Primary Mentor (Ivan Maya). With his mentoring experience, Dr. Allon will ensure that I meet my benchmarks for evaluation (Table 3) and provide the needed support to get back on schedule should unanticipated problems arise. In addition, he will oversee training in clinical nephrology-related research methods including measurement of GFR using iothalamate clearance.

*(ii) Consultants and Content Experts:* In order to enhance the mentorship of Drs. Calhoun and Allon, I have assembled an advisory team of consultants, content experts, and research facilitators to advise in the research plan implementation, monitor my progress through the career development program, and identify opportunities for the next steps in my research. My advisory team includes:

**Anupam Agarwal, MD**, UAB Nephrology Division Director. Dr. Agarwal serves as the PI for the NIDDK funded O'Brien Center and will assist with the resources available in the Center including the Biostatistical Core and the Bioanalytical Core for measures of oxidative stress. In addition, he is a leader in nephrology and will provide insights into my career development.

**Rakesh Patel, PhD**, Professor of Pathology and Chair for the Gordon Research Conferences of Oxygen Radicals, 2016. Dr. Patel researches redox cell signaling pathways involved in the regression of inflammation and their role in chronic inflammatory diseases. He is an expert in measuring nitric oxide metabolites and assessing their role in biological processes, and their therapeutic potential. He will provide expert training in reactive oxygen species and ensure appropriate measurement of oxidative stress in the proposed research project. In addition, he will be instrumental in formulating hypotheses for future RO1 grant proposals.

**Gary Cutter, PhD**, Professor of Biostatistics and Head of the Section on Research Methods and Clinical Trials. Dr. Cutter is the Biostatistical Resource Director for the O'Brien Center. He is an expert in the design, analysis, conduct, and interpretation of clinical trials and will guide the statistical methods of this research proposal as well as assist in the design of future clinical trials to be proposed as part of an R01 submission.

F. Mentorship Plan: Each of my mentors is committed to my development into an independent clinical investigator. In addition to the individual meetings outlined in Table 2, my entire team will convene every 6 months in the first 2 years and then yearly to evaluate my progress. These group meetings will ensure that I am meeting my career and research benchmarks (Table 3). If I fall behind, meeting frequency will increase, as needed. The goal of these add-on meetings will be to identify the problem(s) impeding my progress and define a plan to get back on schedule.

G. Complementary Activities & Timeline: To complement my research activities, I will devote 20% effort to clinical duties and teaching in the Nephrology Division. This time will be dedicated to a one half day a week outpatient clinic, 4 weeks/year of attending on the renal consult service at UAB, and attending the Nephrology Division journal clubs, and Renal and Medical Grand Rounds.

**Table 4. Integrated timeline for career development plan and research activities**

| Year | Career development activities | Research activities |
|---|---|---|
| 1st | - Coursework in epidemiology, fundamentals of clinical research, advanced statistical analysis, and responsible conduct of research<br>- Attend weekly didactic research conferences: Nephrology Research and Training Center Seminars, Free Radical Biology Seminars, and Vascular Biology and Hypertension Seminars<br>- Weekly meeting with Dr. Calhoun, monthly meetings with Drs. Allon and Patel, quarterly meetings with Drs. Agarwal and Cutter | - Participant recruitment and data collection, including performing vascular function testing, for Aims 1 and 2.<br>- Frommeyer Fellowship Manuscript submission |
| 2nd | - Attend weekly didactic research conferences: Nephrology Research and Training Center Seminars, Free Radical Biology Seminars, and Vascular Biology and Hypertension Seminars<br>- Continue weekly meeting with primary mentor, monthly meetings with co-mentor, and quarterly meetings with collaborators. | - Participant recruitment and data collection, including performing vascular function testing for all 3 Aims. |
| 3rd | - Attend weekly didactic research conferences: Nephrology Research and Training Center Seminars, Free Radical Biology Seminars, and Vascular Biology and Hypertension Seminars<br>- Continue weekly meeting with primary mentor, monthly meetings with co-mentor, and quarterly meetings with collaborators<br>- Presentation at UAB's Vascular Biology and Hypertension Seminars | - Continued participant recruitment for all 3 Aims.<br>- Preliminary data analysis<br>- Abstracts submitted to national conferences |
| 4th | - Continue weekly meeting with primary mentor, monthly meetings with co-mentor, and quarterly meetings with collaborators<br>- Attendance and presentation at ASN Kidney Week<br>- Attendance and presentation at AHA Scientific Sessions<br>- Attend weekly didactic research conferences: Nephrology Research and Training Center Seminars, Free Radical Biology Seminars, and Vascular Biology and Hypertension Seminars | - Study completion of Aims 1 & 2.<br>- Manuscript(s) for Aims 1 & 2 prepared<br>- grant submission to the ASN – Carl Gottschalk award<br>- Preparation and submission of an R01 Award |
| 5th | - Attendance and presentation at ASN Kidney Week<br>- Attendance and presentation at AHA Scientific Sessions<br>- Continue weekly meeting with primary mentor, monthly meetings with co-mentor, and quarterly meetings with collaborators | - Study completion of Aim 3.<br>- Manuscript for Aim 3 prepared<br>-Revision and resubmission of R01 Award |

**2. CAREER GOALS AND OBJECTIVES:** My long-term goal is to become an independent clinical investigator and a leader in the field of vascular function as it relates to CKD and hypertension. My experiences as a postdoctoral clinical researcher have provided me a foundational level of competence in performing clinical trials that are designed to answer specific physiologic questions. My ongoing coursework in biostatistics and clinical trial design has advanced my skills of data analysis and understanding of bias and power as it relates to trial design. In order to continue progress toward achieving my career goals, I need to develop expertise in four additional content areas: (1) vascular function testing, (2) advanced study design and biostatistical methodology, (3) recruitment and performance of clinical trials and (4) focused mentorship and career development through the UAB Center for Clinical and Translational Science's Training Interdisciplinary Emerging Research Scholars (TIERS). My mentors and I have developed a detailed career development plan to fill these gaps with additional training and mentored research experiences that will allow me to successfully transition into an independent clinical investigator. A K23 award is a vital component to my continued, upward career trajectory and offers the ideal mechanism to transition towards independence. Under the mentorship of Drs. Calhoun (Primary Mentor) and Allon (Co-Mentor), experts in vascular function testing and conducting clinical trials, I will perform original research in the vascular physiology of CKD. Results from my research proposal will offer insights into therapeutic targets for patients with CKD and, importantly, create the background for an R01 proposal.

## RESUBMISSION MODIFICATIONS

Introduction: We thank the reviewers for their careful review and excellent suggestions. We are encouraged by the impact score of 30 and their comments … "The research proposal is clinically relevant … The research plan has good training potential … Research is logical, relevant, and achievable … Outstanding mentor, co-mentor, and consultants." **Based on recommendations by the reviewers we have revised the application with changes to the Career Development Plan and Research Strategy denoted by vertical line along the left margin and referenced in our responses below.**

1) Mentorship Team *"The mentorship plan should be strengthened and more clearly outlined…Except for weekly meetings with primary mentor, the frequency of individual meetings … is not defined. Also, annual mentorship team meetings are insufficient…No description of evaluation plan…Dr. Allon's complementary role in providing expertise … is unclear…Overlap with his primary mentor R01."*

***Response***: A separate mentorship plan section has been added, which includes (i) a timetable of benchmarks for candidate evaluation during each of the team meetings (ii) a contingency strategy of add-on meetings to address unmet benchmarks, and (iii) better defined mentor roles with meeting times **(Section 3.F. & Tables 2 & 3).** Individual meeting times have been outlined in Table 2. Mentorship team meetings have been increased to every 6 months in the first 2 years with benchmarks for candidate evaluation at these time points outlined in Table 3. Meeting frequency will increase if the candidate is falling behind **(Section 3.F & Tables 2 & 3).** Benchmarks for mentors to evaluate the candidate's progress have been detailed in a table of career and research timelines **(Section 3.F, Table 3).** Dr. Allon will oversee training in clinical nephrology-related research methods including GFR measurements. His mentoring experience will be especially valuable during add-on meetings when a trajectory change in the candidate's career or research is needed **(Section 3.F).** Dr. Calhoun's current R01 focuses on refractory hypertension and has no overlap with this proposal **(Calhoun Biosketch).**

2) Training Plan *"…research activities timeline is without details regarding completion of specific aims within the timeline…It is unclear how much time will be dedicated to learn how to measure the FMD with ultrasound…The laboratory training plan in biomarkers is not well described."*

***Response***: Thank you. Recruitment goals for each aim have been added in Table 3 and completion further clarified in Table 4 **(Sections 3.E & 3.F).** The 1st 3 months of year 1 are dedicated to training in the measurement of FMD with Doppler flow **(Section 3.Ai).** The biomarker section has been narrowed to focus on markers of oxidative stress and the training plan outlined in **Section 3.A.ii.** as well as in **Dr. Rakesh Patel's letter**. Training in ambulatory blood pressure monitoring and GFR has been added **(Section 3.B).**

3) Research Strategy: *"The project has some critical design flaws…Patients with diabetes per se usually have endothelial dysfunction ...The inclusion of well-controlled diabetic patients, despite the paired analysis, may lead to variability…The cut-off values for each of the biomarkers selected were not reported…Lack preliminary ROC analysis, in at least a few of the selected biomarkers… Methods and assays for measuring biomarkers are not mentioned and can be critically-important. A table with the various methods/techniques should be included…Small changes in FMD (2%) will be considered biological significant, and it is unclear how the presence/absence of diabetes, other anti-HT medications, caffeine, tobacco, etc., will affect these measurements…Aim 3, many confounding variables can affect the outcome of proteinuria independently of the spironolactone treatment…Inclusion of time control studies for FMD are not mentioned or planned… The research plan is too limited to be turned into an R01 and the small sample size may not take the entire funded period to complete."*

***Response***: Thank you for these concerns. Diabetic patients have been excluded (Table 5). Biomarker measurements have been focused to markers of oxidative stress, and plasma and urine $F_2$-isoprostanes will be measured by liquid chromatography tandem-mass spectrometry with accuracy and precision included in **Section 4.C.1.2.2.** Participants will be contacted 1-2 days prior to FMD measurement to limit potential confounders on the morning of FMD measurement **(Section 4.C.2.1.5).** In CKD, FMD measurements range from 0 to 8%; therefore a change in FMD by 2% is considered significant **(Section 4.C.2.1.3.1).** Aim 3, the statistical analysis has been changed to include treatment group as a covariate in the general linear model and the number of potential adjusted covariates increased to 6 in the sample size calculation **(Sections C.4.1.3 & C.4.1.4).** The crossover study design will minimize unmeasured confounders. Time control studies have been previously performed, and 5 minute occlusion has been adopted as the standard time [18]. ABPM and GFR have been added to Aims 3 and sample sizes expanded to 58 and 42 participants **(Section 4.D).**

Additional revisions include clarification of the choice of amiloride as the active control **(Section 4.B.2.1)** and a diagram describing the proposed methods for assessing endothelial function **(Figure 1).**