PI: **Petriello, Michael Curtis**	Title: TMAO is a biomarker of dioxin-like pollutant exposure and cardiometabolic disease	
Received: 11/13/2017	FOA: PA16-193	Council: 05/2018
Competition ID: FORMS-D	FOA Title: NIH PATHWAY TO INDEPENDENCE AWARD (PARENT K99/R00)	
1 K99 ES028734-01A1	Dual: HL	Accession Number: 4111618
IPF: 2793601	Organization: UNIVERSITY OF KENTUCKY	
Former Number:	Department: Internal Medicine	
IRG/SRG: ZES1 LAT-D (K1)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 88,700 Year 2: 90,375 Year 3: 249,000 Year 4: 249,000 Year 5: 249,000	Animals: Y Humans: Y Clinical Trial: N Current HS Code: E4 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Michael Petriello	University of Kentucky Research Foundation	PD/PI
Andrew Morris	University of Kentucky Research Foundation	Other (Specify)-Mentor
Richard Charnigo	University of Kentucky Research Foundation	Other (Specify)-Co-Mentor
Susan Smyth	University of Kentucky Research Foundation	Other (Specify)-Co-Mentor

Reference Letters

Xabier Arzuaga	U.S. Environmental Protection Agency	05/19/2021
Jonathan Brown	Cleveland Clinic	05/19/2021
Sudha Biddinger	Boston Children's Hospital	05/19/2021
Bernhard Hennig	University of Kentucky	05/19/2021

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
ES028734

b. Agency Routing Number

2. DATE SUBMITTED	**Application Identifier**	**c. Previous Grants.gov Tracking Number**
2017-11-13		

5. APPLICANT INFORMATION **Organizational DUNS*:** 939017877

Legal Name*: University of Kentucky Research Foundation
Department:
Division:
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001

Person to be contacted on matters involving this application
Prefix: First Name*: Lauren Middle Name: Parrish Last Name*: McMahan Suffix:
Position/Title: Research Project Administrator
Street1*: 500 S Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001
Phone Number*: 8592574652 Fax Number: 8593231060 Email: ospa@uky.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-616033693-C4

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*	**10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER**
National Institutes of Health	TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
TMAO is a biomarker of dioxin-like pollutant exposure and cardiometabolic disease

12. PROPOSED PROJECT		**13. CONGRESSIONAL DISTRICTS OF APPLICANT**
Start Date*	Ending Date*	KY-006
07/01/2018	06/30/2023	

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Michael Middle Name: C Last Name*: Petriello Suffix:
Position/Title: Post-Doctoral Trainee
Organization Name*: University of Kentucky Research Foundation
Department: Internal Medicine
Division: Medicine
Street1*: 1095 V.A. Drive
Street2: 306 Health Science Research Bldg
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40536-0305
Phone Number*: (859) 257-3760 Fax Number: Email*: michaelcpetriello@uky.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$940,401.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$940,401.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Kim Middle Name: C Last Name*: Carter Suffix:
Position/Title*: Associate Director
Organization Name*: University of Kentucky Research Foundation
Department: Sponsored Projects Administrat
Division: Research
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001
Phone Number*: 8592579420 Fax Number: 8593231060 Email*: ospa@uky.edu

Signature of Authorized Representative*
Kim C Carter

Date Signed*
11/13/2017

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter1002093645.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Kentucky Research Foundation
Duns Number:	939017877
Street1*:	500 South Limestone
Street2:	
City*:	Lexington
County:	Fayette
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40526-0001
Project/Performance Site Congressional District*:	KY-006

Additional Location(s) File Name:

Funding Opportunity Number: PA-16-193. Received Date: 2017-11-13T09:45:56.000-05:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ● Yes ❍ No

If YES, check appropriate exemption number: ☐ 1 ☐ 2 ☐ 3 ☑ 4 ☐ 5 ☐ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number 00005295

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ● No

IACUC Approval Date: 06-12-2017

Animal Welfare Assurance Number A3336-01

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Summary1002093472.pdf
8. Project Narrative*	Narrative1002093474.pdf
9. Bibliography & References Cited	BIBLIOGRAPHY_K99_revision1002093616.pdf
10.Facilities & Other Resources	Facilities_and_Resources_K991002093648.pdf
11.Equipment	Equipment1002093484.pdf

Funding Opportunity Number: PA-16-193. Received Date: 2017-11-13T09:45:56.000-05:00

Dioxin-like organic pollutants persist in the environment and, because of their bioaccumulation in adipose tissue can be detected in the blood of most individuals. Exposure to these pollutants causes diabetes and its complications of obesity and cardiovascular disease in animal models. These observations can likely be translated to humans because several large longitudinal epidemiological studies have associated serum levels of these pollutants, for example polychlorinated biphenyls (PCBs) with an increased risk of cardiovascular disease and type 2 diabetes. The variability in inter-individual responses to increased body burdens of these pollutants observed in these epidemiological studies can likely be explained by the additional contributions of genetic and other environmental risk factors, the most powerful of which is clearly the diet. The goal of this proposal is to provide the applicant with mentored training and early career research support to become an independent investigator studying interactions between diet, nutrition and environmental exposures as determinants of human disease. To accomplish this the candidate will be mentored by an interactive group of established investigators with complementary expertise in analytical chemistry, multivariate statistics, and preclinical models of cardiovascular and metabolic disease. This training will be accomplished through participation in an original research project studying a mechanism that could link diet and exposure to dioxin-like persistent organic pollutants to cardiovascular disease risk. Increased circulating levels of a diet derived metabolite, trimethylamine N-oxide (TMAO) are associated with coronary artery disease and diabetes risk in humans. The precursor of TMAO, trimethylamine (TMA) is generated from dietary substrates (choline containing lipids and carnitines) by the gut microbiota. TMA is oxidized to TMAO by hepatic Flavin-containing monooxygenases, predominantly the FMO3 isoform. We have found that exposure to dioxin-like PCBs strongly increases FMO3 expression in the liver to amplify formation of TMAO from dietary sources in animal models and that exposure to dioxin like pollutants positively associates with circulating TMAO levels in a highly exposed human population. These observations lead us to propose our overarching hypothesis that induction of FMO3 expression is a mechanism linking coplanar PCB exposure to the development of cardiovascular and related metabolic diseases and that circulating TMAO levels are a biomarker of systemic dioxin-like pollutant exposure in humans. We will test this hypothesis in the following aims. 1: To test the hypothesis that a diet high in TMAO precursors can exacerbate dioxin-induced cardiometabolic disease *in vivo*. 2: To test the hypothesis that FMO3 and/or gut microbiota are required for dioxin-induced cardiometabolic disease *in vivo*. 3 To test the hypothesis that elevated TMAO levels in dioxin-like pollutant-exposed individuals result from increased FMO3 activity/expression.

PROJECT NARRATIVE

We hypothesize that dietary factors can interact with pollutant exposures to modulate associated chronic diseases. Results from our mechanistic studies will identify biomarkers, such as TMAO, that link diet, toxicant exposure, and cardiometabolic diseases which are important within the contexts of human cumulative risk assessment and environmental public health.

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FACILITIES AND OTHER RESOURCES

All necessary facilities to complete the proposed studies are available to Dr. Petriello through interactions with his mentoring team or through other well-defined collaborations. As a postdoctoral fellow in the Center for Cardiovascular Research, Dr. Petriello has been designated space at the University of Kentucky (UK) College of Medicine, as detailed below. The University of Kentucky is unique among land grant universities in that all five health-related colleges (Dentistry, Health Sciences, Medicine, Nursing, Pharmacy, and Public Health) and the other UK colleges (Agriculture, Arts and Sciences, Design, Business and Economics, Communications and Information Studies, Education, Engineering, Fine Arts, Law, and Social Work) are located on the same campus in Lexington, Kentucky. This constellation of programs has enabled UK to develop extraordinarily productive collaborations across diverse disciplines.

Laboratory:

Dr. Morris occupies 2000 sq ft of space on the second floor of the newly constructed Biomedical Biosciences Research Building, including 250 sq ft tissue culture room, a 250 sq ft dedicated microscope room. The UK-SRC research support core laboratory which Dr Morris directs is in 1000 sq ft of laboratory space on the lower floor of the Biomedical Biosciences Research Building. General laboratory equipment available for this proposed research includes Refrigerated table-top and micro- centrifuges and ultracentrifuges, acrylamide/agarose gel electrophoresis and electroblotting equipment, power supplies, gel-dryer, Odyssey Infrared Imaging System, UV transilluminator, digital camera for gel documentation, analytic balances, Pharmacia dual pump FPLC system with controller and UV monitor, single and multi-channel peristaltic pumps, in-line UV monitor, chart recorders, three fraction collectors, chromatography columns, three thermocyclers, electroporator, rotary evaporator, vacuum apparatus, pH meter, orbital shakers, refrigerator and freezers (-70ºC and -20ºC), fume hoods, centrifugal evaporator/concentrator, rotary evaporator, thin layer chromatography tanks, bacterial incubators, probe and bath sonicators. BioTek Powerwave 200 absorbance and Flx800 fluorescence plate readers with computer control and software for data acquisition and analysis, LiCor Odyssey Infrared imager, Packard Tri-Carb Liquid Scintillation Counter. Mass spectrometry related instrumentation is detailed within the equipment page and includes multiple LC/MS and GC/MS instruments.

As stated within his accompanying support letter, Dr. Petriello will also have access to all resources of the Superfund Research Center, directed by Dr. Bernhard Hennig. Facilities include modern laboratory space totaling approximately 1,500 square feet of laboratory space on the 5th floor in the Wethington Health Sciences Building. The laboratory is equipped with standard infrastructure (benches, fume hoods, sinks, distilled water and glassware) and is well equipped for conducting molecular, biochemical, physiological and tissue culture studies and can accommodate up to 10 researchers. Shared dark and cold rooms, equipment rooms containing autoclaves, Millipore Milli-Q water systems, ultracentrifuges, scintillation counters, a gamma counter, X-ray film developing unit, two different calcium imaging systems (Zeiss Attofluor and Quantex), confocal laser scanning microscope (Olympus FluoView 300), metabolic phenotyping equipment for measurement of whole body metabolism in mice, etc., also are available. Drs. Morris's and Petriello's offices and research labs, are in close proximity in an adjacent research building (Biomedical Biological Sciences Research Building)

Other resources that are available include: Printing, Photography, Electronics and Machine Departments, Biostatistics and Computer Science Divisions as well as DNA Sequence, Cell Analysis & Sorting and Cellular Structure Facilities, and DNA Microarray Core Facility. The Cell Analysis & Sorting Facility currently houses a Bacton-Dickinson dual (dye) laser FACStar Plus Cell Sorter and FAScan analyzer, a Consort 32 computer system with software programs for immunofluorescence analysis, cell analysis, and kinetics programs. The Cellular Structure Facility provides high resolution transmission (TEM), scanning transmission (STEM), and scanning (SEM) microscopes, as well as high-brightness guns and X-ray analysis equipment.

Animal: The experiments outlined in Aims 1,2 of the K99/R00 require wild-type and genetically modified mice. Mice will be housed in controlled-access, pathogen-free microbarrier facilities equipped with laminar flow rooms and sterile changing stations and managed by the UK Division of Laboratory Animal Resources (DLAR). Our animal facilities at UK are fully accredited by AAALAC and operated under the statement of assurance of compliance with the PHS Policy on the Humane Care and Use of Laboratory Animals. Dedicated rooms for sensitive strains of animals allow for highly individualized animal care processes, mandated by specific investigations, including those requiring breeding. Surgical procedures are performed in the Experimental Surgery Suite, a fully staffed sterile animal-operating complex with appropriate facilities and equipment

available for postsurgical care. DLAR staff includes four full-time veterinarians who are responsible for animal health and disease control. Research support services include complete surgical resources, pathology, breeding colony management, and species-specific training. DLAR clinical laboratory services cover preventive medicine, diagnostic, and animal definition programs. Available laboratory procedures include blood chemistries, hematology, rodent serologies, bacterial culture and sensitivity testing, and parasite identification. Veterinary staff, licensed veterinary technicians, surgical technicians, and animal research analysts are available to the investigators for consultation or assistance.

Computer: In total, Dr.Petriello has access to 12 Pentium III or better work stations and attached printers in offices and laboratories. Computers are connected to the HP Superdome Cluster (1.792 TFLOPS) at the University of Kentucky, which allows Medline literature searches, electronic-mail communication, statistical data analysis, etc. All computers are configured with standard application software for Windows systems, including Microsoft Office Suite 2007, Adobe Photoshop, EndNote and SigmaPot/Stat. Computers are integrated into the UK local area network, which provides high-speed Internet and network connections, an enterprise storage and backup system for the management of critical research data, and secure remote access, as needed, via a Cisco VPN solution for all University computer users. In addition, email and virus/spam protection are provided to all employees. Access to Information technology support personnel and resources are provided through the College of Medicine. The laboratory uses an Access database and a server maintained by the Department of Internal Medicine.

Office: Dr. Petriello has approximately 100 sq. ft. of fully furnished office space located in the Biomedical Biological Sciences Research Building adjacent to the laboratories and additional office space in the Wethington Building. The office provides phone, fax, copy, and printing support and convenient access to full-time administrative assistants within the College of for grants management and general staff assistance. Information technology support personnel and resources are provided by the College of Medicine.

Clinical: The University of Kentucky (UK) has a long tradition of support for clinical and translational research resources through dedicated facilities and resources as well as key initiatives to foster collaboration. The Clinical Services core is a unit housed within the UK Center for Clinical and Translational Science (CCTS) that provides critically important infrastructure to aggregate, integrate, and enhance services, and thereby establish an optimal setting for controlled clinical investigations, including NIH and industry-sponsored clinical trials. 2 members of Dr. Petriello's mentoring team, Dr. Smyth and Charnigo are active members of the CCTS. Physical facilities includes state-of-the-art inpatient and outpatient units that are equipped to support phase I through IV drug and medical device trials and to provide support for investigator-initiated research involving human subjects. The Inpatient Unit, designated as the Special Care Unit (5SCU), occupies 5,954 sq. ft. on the fifth floor north wing of University Hospital. Nursing care is provided by the hospital for inpatient protocols. The unit includes a reception area for patients and their families, a registration area, a nurse's station, eight rooms housing up to 15 patient beds, an exam room, a specimen-handling and processing facility, a metabolic kitchen, equipment storage, and space for program and administrative offices. The unit is supplied with standard hospital emergency equipment (oxygen, suction, emergency cart with defibrillator, etc).The specimen-processing lab contains two refrigerated and low-speed centrifuges for separation of serum and plasma, and -20°C and -80°C freezers for specimen storage. The Outpatient Unit, located on the third floor of University Hospital, totals 3,787 sq. ft. and includes a reception area for patients and their families, patient registration, six dedicated exam rooms and a procedure/phlebotomy room, specimen-handling and processing facilities, and offices for clinical research nurses and staff. Exam rooms are equipped with an exam table and cardiac chair for assessment of vital signs, lab sampling, and/or brief interviews. A small private room is also available for conferencing, consultation, neuropsychological testing, consenting, or questionnaire administration. The specimen-processing room contains a refrigerated centrifuge for separation of serum and plasma and access to dry ice. The Biochemical Analysis Laboratory, located near the Inpatient Unit, provides investigators access to assays that are not routinely done within the clinical laboratory setting, as well as, the capability to run assays on the Luminex, Affymetrix gene array, Immulite, chemiluminescence plate reader and to perform endotoxin analysis. This laboratory is networked with other laboratories to further enhance assay capabilities.

Other: The University provides an array of advanced resources through a number of highly specialized facilities that support this project, as described below.

Markey Cancer Center Free Radical Biology Core.

LiCor Oddyssey Infra Red Blot Imaging System. Controlled by a computer running image studio software for data acquisition and analysis.
Two Agilent HPLC systems with fluorescence and electrochemical detection capabilities. Include computers for instrument control, data aqusition and analysis.
Seahorse XF24 Extracellular Flux Analyzer. Includes a computer running the manufacturer's XFWave software for instrument control, data acquisition and analysis.
Thermo LTQ Velos Orbitrap Mass Spectrometer with Electron Transfer Dissociation and Eksigent Nano LC system. This system includes gas generators and a workstation computer for instrument control and data acquisition as well as access to computers for offline data analysis and database searching.
Bruker EMX ESR spectrometer. Equipped with temperature control and sample handling systems for studies in live cells and tissues.

The Free Radical Biology Core may be utilized in future studies depending on the outcome of Aims 1,2 of the K99 proposal (e.g., use of Seahorse Analyzer to investigate FMO3's role in oxidative stress).

Resource Center for Stable Isotope-Resolved Metabolomics Facility (RC-SIRM), directed by Drs. Teresa Fan, Richard Higashi, Andrew Lane, and Hunter Moseley provides state of the art access to metabolomics instrumentation and bioinformatics. As stated within the accompanying support letter, Dr. Petriello will also have access to all resources of the RC-SIRM

The MicroArray Facility, directed by Kuey-Chu Chen, PhD, Department of Molecular and Biomedical Pharmacology, provides comprehensive state-of-the-art microarray services and resources for the analysis of gene expression, including Affymetrix GeneChip Technology, preparation of RNA samples, and bioinformatics support for data reduction and analysis. Fee-based services include assessment of RNA samples with an Agilent 2100 Bioanalyzer as well as RNA labeling, chip hybridization, chip scanning, and data collection. Available instrumentation by Affymetrix includes a GCS 3000 7G scanner, GeneChip Fluidics Station 450, GeneChip Hybridization Oven 640, and a statistical and bioinformatics software computer workstation. The facility's newest addition is a second-generation nCounter Analysis System from NanoString Technologies for direct measurement of copies of RNA and microRNA with high sensitivity and accuracy, which can be used to genotype DNA samples for copy number variations. In addition, the facility houses a high-throughput Roche LightCycler 480 Real-Time PCR System with automated MagNA Lyser for tissue preparation and two MagNA Pure Compacts for isolation of nucleic acids. The PCR system offers 96- and 384-well plate capacity on a fee-based service basis.

The MicroArray core may be utilized depending on the outcomes related to experiments within Aim 1,2.

Proposal Development Office.
The UK Proposal Development Office (PDO) is a major resource within the suite of services provided by the Office of the Vice President for Research to support strategic research development across campus through targeted resource and service delivery. The office provides an array of pre-award proposal development and consultation services to faculty and staff to assist them in securing extramural funding for their scholarly activities. Primary PDO services include assistance in individual proposal planning and development, proposal review and critique. If Dr. Petriello remains at UK to continue his R00 phase, The PDO will help Dr. Petriello to prepare his subsequent first R01. PDO also provides workshop training in grant-seeking and proposal development skills that will be available to Dr. Petriello during the K99 phase. Grant-related workshop offerings focus on agency or sponsor requirements, specific funding mechanisms, and diverse topics relevant to effective grantsmanship. Workshop services are also available to investigators whose funded projects incorporate career development or training activities and can be developed in conjunction with specific training needs.

EQUIPMENT

Cell Culture: Two laminar flow hoods, four CO_2 incubators, one phase (Nikon TS100) and one fluorescence (Zeiss) inverted microscope.

General Laboratory Equipment: Refrigerated table-top and micro- centrifuges and ultracentrifuges, acrylamide/agarose gel electrophoresis and electroblotting equipment, power supplies, gel-dryer, Odyssey Infrared Imaging System, UV transilluminator, BioRad Chemi Doc Touch gel/blot imaging system (multiplex fluorescence, chemiluminesence), analytic balances, GE/Pharmacia dual pump FPLC system with controller and UV monitor, Agilent Quaternary HPLC system with absorbance and fluorescence detectors and microplate compatible fraction collector, rotary evaporator, vacuum apparatus, pH meter, orbital shakers, refrigerator and freezers (-70ºC and -20ºC), thin layer chromatography tanks, probe and bath sonicators. BioTek Powerwave 200 absorbance and Flx800 fluorescence plate readers with computer control and software for data acquisition and analysis, Biotek Synergy multimode plate reader (includes injector system for luciferase assays), LiCor Odyssey Infrared imager.

Confocal and Wide Field Microscopes: Nikon TE 2000 fully motorized inverted motorized microscope with 20, 40, 60 X objectives, DIC capabilities. Nikon A1R resonance scanning four laser confocal system. This microscope also has a wide field fluorescence illuminator with fluorescence filter sets and a Cascade CCD camera. Computer running Nikon Elements software for data acquisition and analysis. At the Lexington VAMC we have a more advanced Nikon A1R system with a highly sensitive GaAsP detector and spectral imaging capabilities. This instrument is configured for use with live cells as well as fixed specimens.

Multiplex analyzers: Biorad Bioplex 200 suspension array reader and Luminex MAGPIX analyzers with dedicated computers running manufacturer's software for instrument control and data analysis.

Molecular biology resources. Three BioRad 1000 series muti block thermocyclers, Thermo Quant Studio 7 real time PCR system with 384 well microfluidic array capability, BioRad electroporation system. Incubators and shakers for bacterial culture, nanodrop 8000 high throughput DNA spectrophotometer.

Small Animal Surgical Resources: A dual-headed surgical dissecting microscope (Leica M691) with digital recording system, 2 dissecting microscopes (Nikon SMZ 800 with camera port), Doppler Processing Work Station (Indus) with 10 and 20 M-Hx pulsed Doppler, perivascular flow meter with 0.5PSB, 0.7PSB, and 1.5 PSL probes (Transonics T400), THM 100 temperature and EKG board (Indus Instruments), mouse tail cuff blood pressure systems CODA (Kent Scientific), pressure-volume conductance system (Millar Instruments), mouse pulse oximeter (Starr), I-STAT system for blood gas and blood chemistry analysis, ABC vet Analyzer for complete blood counts, surgical equipment, instrument sterilizer, bipolar hand coagulator with footswitch (Kirwan Surgical Products), thermocouple thermometer for monitoring rectal temperature (WPI); inhaled isoflurane anesthetic mixing chamber for rodents, small animal respirator. The lab has a small animal imaging system (Vevo 770 biomicroscopy system with 30 and 40 MHz probes from Visual Sonics) and equipment for hyperinsulinemic-euglycemic clamps, including tether system (INSTECH), multi-infusion pump (Harvard Apparatus), and dual infusion pump (Harvard Apparatus).

CV Physiologic Core. The Physiologic core is located within the Division of Animal Laboratory Resources (DLAR) space within the Biopharm Complex and BBSRB and dedicated laboratory space in the BBSRB. The core is under the direction of Dr. Lisa Cassis and is staffed by Dr. Wendy Katz (PhD) who developed SOPs and facilitates standardized training for CVRC faculty and trainees. The core is equipped with: DSI radiotelemetry platforms (20) and data analyzer and 20 radiotelemetry implants; TSE LabMaster indirect calorimetry system that includes 48 acclimation chambers, and 24 calorimetry chambers instrumented to record chamber emperature, oxygen and carbon dioxide concentrations, food and water consumption and activity in the X-axis; TSE PhenoMaster metabolic cage system, comprising 8 chambers for separation and collection of urine and feces with concurrent recording of food and water intake; EchoMRI Whole Body Composition Analyzer for quantification of fat and lean mass in conscious mice; Visitech tail cuff (16 platforms)

and Coda tail cuff platforms (20) for systolic blood pressure measurements in mice; Vevo 2100 ultrasound with several scanheads; Infusion pumps and glucose analyzers for hyperinsulinemic euglycemic clamps in mice; FPLC systems (2) for analyzing plasma lipoproteins; and dissecting microscopes (5) with camera and image analysis software for quantifying atherosclerosis

Mass Spectrometry Equipment: This instrumentation is contained in an institution wide facility core directed by Dr Morris and can be used for research conducted by members of the laboratory in accordance with the business operations of the facility core .

Agilent 7890 GC/ 5975 mass selective detector. This instrument is configured with an electron impact ion source and dual inlets with the ability to run samples simultaneously in parallel on separate columns connected to the mass selective detector or an electron capture detector and controlled by a workstation computer running Agilent Chemstation software.

Agilent 7890B Gas Chromatograph/ 7000C triple quadrupole mass spectrometer. This instrument is configured with an autosampler and multimode inlet capable of compressed air or liquid nitrogen assisted cooling and integrated microfluidics to enable column and inlet switching between electron capture, flame ionization and a triple quadrupole mass spectrometer detector and integration of gas selection and column backflushing into automated workflows. The mass spectrometer can be configured with electron impact or chemical ionization sources and is controlled by workstation computer running Agilent Mass Hunter Software.

Two AB Sciex 4000 Q-Trap triple quadrupole linear ion trap mass spectrometer systems. Two completely separate ABSciex 4000 Q-Trap hybrid linear ion trap triple quadrupole mass spectrometers with ABSciex “Turbo V” electrospray and chemical ionization sources. One of these systems can also be operated with a vaccum MALDI ion source (ABSciex Flashquant). Each system can be operated with a Shimadzu HPLC system comprising an autosampler, column oven multiple pumps, switching valves and a controller and is connected to a workstation computer running AB Sciex Analyst and Multiquant Software for instrument control and data analysis.

ABSciex 6500 plus Q-Trap triple quadrupole linear ion trap mass spectrometer system. This instrument is located at the Lexington VA Medical Center and available for use by university of Kentucky investigators with approval from that facility. The instrument is configured with a Shimadzu Nexera UPLC system including a rack changing autosampler and column oven and connected to a workstation computer running AB Sciex Analyst and Multiquant Software for instrument control and data analysis.

ABSciex 5600 Quadrupole time of flight mass spectrometer. This instrument incorporates an AB Sciex electrospray and chemical ionization source and is configured for operation with either a complete automated Shimadzu HPLC system (of identical configuration to those used with the 4000 Q-Trap instruments), a or with a Eksigent microflow HPLC system that is configured for use with either capillary HPLC columns or perform automated direct infusion. This microflow system operates with a low dispersion electrode insert that provides some of the sensitivity benefits of nano flow electrospray ionization with increased robustness in comparison to glass emitter electrodes. This instrument can also be configured with an Advion Nanomate robotic chip based nano electrospray ionization source for sample analysis by direct infusion.

Thermo Q-Exactive quadrupole orbitrap mass spectrometer. This is a quadrupole orbitrap mass spectrometer system that provides high sensitivity and high mass resolution for quantitative and qualitative analysis. The system is configured for use with a Thermo/Dionex U3000 UPLC chromatography system and is interfaced with a workstation computer running software for instrument control, data acquisition and analysis.

Sample preparation. The laboratory contains two Gilson Gradient HPLC systems with absorbance, fluorescence and evaporative light scattering detectors. Other equipment in the laboratory for sample

preparation for GC or LC MS includes two N-Evap N2 evaporator systems, three Biotage Turbo Vap evaporators (one configured for 4 ml vials, the other for 96 well plates), two LabConco centrifugal evaporator/concentrators, a Genevac Rocket Solvent Evaporator System, a Thermo/Dionex Accelerated Solvent Extraction System, two Supelco Solid Phase extraction systems, a Matrix Scientific Well Mate multiwell plate dispenser, a Gilson 215 Robotic Liquid Handling System configured for multiwell plate and cartridge based Solid Phase Extraction with workstation computer running Gilson Trilution Software, two multi sample vortexers, heating blocks for lipid phosphate analysis and chemical derivatization and a Biotek Multimode absorbance/fluorescence reader.

Data analysis. In addition to the instrument control computers, the facility contains two workstation computers running instrument control software and software packages for data analysis that are available for off-line processing of data collected using the primary instrument control computers. All of the computer systems in the laboratory have redundant hard drives and are networked for data transfer and backup.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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Department: Internal Medicine
Division: Medicine
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Phone Number*: (859) 257-3760 Fax Number:

E-Mail*: michaelcpetriello@uky.edu

Credential, e.g., agency login: MICHAEL.PETRIELLO

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2015

Attach Biographical Sketch*: File Name: Petriello_K99_biosketch1002093584.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12519448 Funding Opportunity Number: PA-16-193 . Received Date: 2017-11-13T09:45:56.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Andrew Middle Name Last Name*: Morris Suffix:

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Division: Medicine
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County: Fayette
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Zip / Postal Code*: 40536-0509

Phone Number*: 8593233749 Fax Number:

E-Mail*: a.j.morris@uky.edu

Credential, e.g., agency login: AMORRIS

Project Role*: Other (Specify) Other Project Role Category: Mentor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Morris_Biosketch_Petriello_Final1002158161.pdf

Attach Current & Pending Support: File Name: Morris_Other_Support1002158162.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: Susan Middle Name Last Name*: Smyth Suffix:

Position/Title*: Professor
Organization Name*: University of Kentucky Research Foundation
Department: Internal Medicine
Division: Medicine
Street1*: 741 South Limestone Street
Street2: BBSRB B347
City*: Lexington
County: Fayette
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Province:
Country*: USA: UNITED STATES
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Phone Number*: 859-323-2274 Fax Number:

E-Mail*: susan.smyth@uky.edu

Credential, e.g., agency login: SUSAN_SMYTH

Project Role*: Other (Specify) Other Project Role Category: Co-Mentor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Smyth_biosketch_Petriello11002093651.pdf

Attach Current & Pending Support: File Name: Smyth1002093613.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: Richard Middle Name J Last Name*: Charnigo Suffix:

Position/Title*: Professor
Organization Name*: University of Kentucky Research Foundation
Department: Dept Of Biostatistics
Division: Public Health
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Street2: Multidisciplinary Science Building 203
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 40526-0001

Phone Number*: 859-218-2072 Fax Number:

E-Mail*: richard.charnigo@uky.edu

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Co-Mentor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Charnigo_Biosketch_Petriello_K99_Nov_171002093575.pdf

Attach Current & Pending Support: File Name: Charnigo_othersupport1002093614.pdf

Tracking Number: GRANT12519448 Funding Opportunity Number: PA-16-193 . Received Date: 2017-11-13T09:45:56.000-05:00

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Michael C. Petriello, PhD**

eRA COMMONS USER NAME (credential, e.g., agency login): Michael.Petriello

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Muhlenberg College, Allentown, PA	BS	05/2010	Biology / Envi. Sci
University of Kentucky, Lexington, KY	PhD	05/2015	Toxicology
University of Kentucky, Lexington, KY	Postdoctoral	09/2016	Superfund Research Ctr
Harvard Medical (Boston Children's Hospital), Boston, MA	Vis. Scientist	Current	Endocrinology
University of Kentucky, Lexington, KY	Fellow	Current	Cardiovas. Res. Center

A. Personal Statement

I am a broadly trained research scientist with a strong background in molecular toxicology, the environmental health sciences, analytical instrumentation, nutrition, and metabolic diseases. As a member of the University of Kentucky Superfund Research Center (UK-SRC), my doctoral work focused on identifying novel cross-talk mechanisms linking exposure to persistent organic pollutants to increased risk of chronic inflammation. Additionally, I examined if lifestyle modifications, such as increasing the consumption of diets high in anti-inflammatory bioactive nutrients could decrease the toxicity of environmental pollutants. As a UK-SRC postdoctoral scholar, I received interdisciplinary training that provided me with a unique skillset to gauge the impact of hazardous waste exposure on communities and to determine effective means to communicate my research findings to stakeholders. In addition to my graduate and postdoctoral studies, I have had the unique opportunity to advance my scientific career by working in the lab of Dr. Sudha Biddinger at Boston Children's Hospital (affiliated with Harvard Medical School). As a visiting scientist, I examined the role of the xenobiotic detoxification enzyme Flavin-containing monooxygenase 3 (FMO3) in glucose and insulin signaling. Returning to Kentucky I received a T32 training grant within the Cardiovascular Research Center and began working with Dr. Andrew Morris on identifying clinically relevant biomarkers in humans that link dietary choices and metabolic disease. This collaboratively designed postdoctoral experience has substantially increased my knowledge of mass spectrometry as well as cardiovascular disease and associated metabolic disorders. Thanks to the Superfund Research Program and the emphasis of my scientific mentors, I have built multiple strong relationships with environmental health scientists, which will continue to ground and focus my research even as I advance my training in additional areas. During my second and third years as a post-doc I have begun to collaborate closely with representatives from the CDC and NIH to examine the importance of my preclinical observations in a human population highly exposed to environmental pollutants. My diverse background has created a strong personal interest in identifying links between nutrition, pollutant exposures, and cardiovascular disease, but I currently lack knowledge in key areas which limits my research's significance and clinical translatability. In my K99 application, I aim to investigate biomarkers that link environmental exposures, diet, and metabolic diseases in human populations and to test mechanisms of toxicity using mouse models of cardiometabolic disease. My career development plan is focused on making me a competitive environmental health scientist in the world of "big data" and "omics" technologies. I will receive a strong foundation in targeted mass spectroscopy and metabolomics, statistical analysis of large data sets, as well as in the biology of cardiometabolic disease. This new training in combination with my background as a mechanistic toxicologist, will help to position me to contribute to the field of environmental health as a

successful independent scientist. Below are a list of publications that have been submitted or accepted since the original K99/R00 submission of last year:

1. **Petriello MC**, Brandon JA, Hoffman JB, Wang C, Tripathi H, Latif-Abdel A, Ye X, Li, X, Lee E, Soman S, Barney J, Wahlang B, Hennig B, and Morris AJ,: Dioxin-like PCB 126 increases systemic inflammation and accelerates atherosclerosis in lean LDL receptor deficient mice. *Toxicol Sci*. 2017 Oct. *Under review*.

2. **Petriello MC,** Charnigo R, Sunkara M, Soman S, Pavuk M, Birnbaum L, Morris AJ, Hennig B: Relationship between serum Trimethylamine N-oxide and exposure to dioxin-like pollutants. *Environ Res.* 2017 Oct. *Under review*

3. **Petriello MC**, Hoffman J, Morris AJ, Hennig B: Emerging roles of xenobiotic detoxification enzymes in metabolic diseases. *Reviews on Environmental Health* Mar 1;32(1-2):105-110. doi: 10.1515/reveh-2016-0050

4. **Petriello MC**, Hoffman J, Charnigo R, Vsevolozhskaya O, Morris AJ, Hennig B: Dioxin-like PCB 126 increases intestinal inflammation and disrupts gut microbiota and metabolic homeostasis. *In prep.*

B. Positions and Honors

Positions and Employment

2008-2010 Research Scholar, Department of Environmental Science, Muhlenberg College, Allentown, PA – Laboratory of Dr. Jason Kelsey
2012 Lecturer, Graduate Center for Toxicology, University of Kentucky, Lexington, KY.
2010-2015 Graduate Research Assistant, Graduate Center for Toxicology, University of Kentucky, Lexington, KY – Laboratory of Dr. Bernhard Hennig.
2015-2016 Postdoctoral scholar, University of Kentucky Superfund Research Center, University of Kentucky, Lexington, KY – Project 1 Post-doc – Laboratory of Dr. Bernhard Hennig.
2015-Present Visiting Scientist, Boston Children's Hospital, Harvard Medical School, Boston, MA – Laboratory of Dr. Sudha Biddinger
2016-Present Postdoctoral fellow, University of Kentucky Cardiovascular Research Center, University of Kentucky, Lexington, KY Laboratories of Dr. Andrew Morris and Susan Smyth

Other Experience and Professional Memberships

2012-present Member – Ohio Valley Chapter of Society of Toxicology
2012-present Member – Society of Toxicology
2013-present Member – Society of Environmental Toxicology
2015- Fighting with Food student representative
2015 EPA Task Force for new PCB assessment
2016 Organizing committee for Central and Eastern European Conference on Health and Environment (CEECHE 2016)
2016-present Member – European Atherosclerosis Society
2016-present Editorial Board, Journal of Nutritional Biochemistry

Honors/Awards

2006 Eagle Scout Recipient
2008 Merck Scholar Summer Research Award
2009 Dean's Grant Summer Research Award
2010 Presidential Scholarship
2013 Superfund Annual Meeting Plenary Speaker Award
2013 SETAC Travel Award
2013 SOT Annual Meeting Travel Award
2014 CEECHE 2014 Meeting Travel Award
2016 September 2016 NIEHS Paper of the Month Award
2016 European Atherosclerosis Society (EAS) 2015 Annual Meeting Best Poster Award

2016 EAS 2015 Meeting Travel Support Award
2017 Society of Toxicology Cardiovascular Toxicology Specialty Section Postdoctoral Award
2018 Keystone Symposia Future of Science Fund scholarship

C. Contribution to Science

1. Exposure to polychlorinated biphenyls, and especially dioxin-like congeners has been linked to multiple pro-inflammatory diseases. Understanding associated mechanisms may help to elucidate possible effective therapeutic strategies to counteract the deleterious effects of environmental pollutants. My earliest publications examined novel signaling pathways encompassing cross-talk between pro-inflammatory and antioxidant mediators critical to PCB-induced vascular inflammation. These publications document that certain types of PCBs can cause inflammation in cells that line blood vessels which may eventually lead to atherosclerosis and heart disease. With a better understanding of how these toxicants elicit an inflammatory response, I was then able to show that certain bioactive food components could work through some of these same pathways to decrease inflammation and protect against the toxicity of coplanar PCBs. These publications added to the growing body of evidence that lifestyle modifications could be a sensible means of preventing toxicant-induced disease. Other groups have now began investigating how healthful nutrition can modulate the toxicity of other environmental pollutants including heavy metals and airborne contaminants.

a. **Petriello MC**, Newsome B, Hennig B: Influence of nutrition in PCB-induced vascular inflammation. Environmental Science Pollution Research. 2014 May;21(10):6410-8. PMID: 23417440
b. **Petriello MC**, Newsome B, Han SG, Murphy M, Eske K, Sunkara M, Morris A, Hennig B: Green tea diet decreases PCB 126-induced oxidative stress in mice by upregulating antioxidant enzymes. Journal of Nutritional Biochemistry. 2014 Feb;25(2):126-35. PMID: 24378064
c. **Petriello MC**, Han SG, Newsome B, Hennig B: PCB 126 toxicity is modulated by cross-talk between caveolae and Nrf2 signaling. 2014 Jun 1;277(2):192-9. PMID: 24709675.
d. **Petriello MC**, Newsome BJ, Dziubla TD, Hilt JZ, Bhattacharyya D, Hennig B. Modulation of persistent organic pollutant toxicity through nutritional intervention: emerging opportunities in biomedicine and environmental remediation. Sci Total Environ. 2014 Sep 1;491-492:11-6. PMID: 25586614

2. PCBs have long been known for their possible cancer causing and detrimental neurological effects, but knowledge on the impacts of PCBs on the cardiovascular system have been lacking. I have advanced my initial *in vitro* and *in vivo* studies investigating inflammation to now focus more specifically on models of cardiometabolic disease (e.g., atherosclerosis). These publications document that dioxin-like PCBs can accelerate atherosclerosis and increase circulating mediators of cardiometabolic disease. Specifically I have developed and characterized a novel mouse model to study toxicant-accelerated atherosclerosis. In this model, Ldl receptor deficient mice are fed a lowfat atherogenic diet that prevents adipose expansion and subsequent pollutant sequestration while still promoting atherosclerosis. These publications added to the growing body of evidence that dioxin-like pollutants increase systemic inflammation and may be an independent risk factor for the development of cardiovascular diseases.

a. Murphy MO, **Petriello MC**, Han SG, Sunkara M, Morris AJ, Esser K, Hennig B. Exercise protects against PCB-induced inflammation and associated cardiovascular risk factors. Environ Sci Pollut Res Int. 2016 Feb;23(3):2201-11. PMID: 25586614
b. Wahlang B, Perkins JT, **Petriello MC**, Hoffman JB, Stromberg AJ, Hennig B. A compromised liver alters polychlorinated biphenyl-mediated toxicity. Toxicology. 2017 Apr 1;380:11-22. doi: 10.1016/j.tox.2017.02.001. Epub 2017 Feb 2. PubMed PMID: 28163111
c. Wahlang B, Barney J, Thompson B, Wang C, Hamad OM, Hoffman JB, **Petriello MC**, Morris AJ, Hennig B. PCB126 exposure increases risk for peripheral vascular diseases in a liver injury mouse model. Toxicol Sci. 2017 Aug 31. PMID: 28973532.
d. **Petriello MC**, Brandon JA, Hoffman JB, Wang C, Tripathi H, Latif-Abdel A, Ye X, Li, X, Lee E, Barney J, Wahlang B, Hennig B, and Morris AJ,: Dioxin-like PCB 126 increases systemic inflammation and accelerates atherosclerosis in lean LDL receptor deficient mice. Toxicol Sci. 2017 Oct. *Submitted.*

3. Identifying new biomarkers of pollutant exposure in preclinical and clinical settings is a critically important emerging paradigm that may lead to better and more personalized human intervention strategies. Working as a lead scientist for the UK-SRC we found that exposures to chlorinated pollutants elicit epigenetic changes

including histone modifications and microRNA alterations in key proteins related to inflammation and cardiovascular disease. Most recently in mice, I discovered that exposure to dioxin-like PCBs can increase a circulating biomarker of cardiovascular disease called trimethylamine-N-oxide (TMAO). This increase may be due, at least in part, by PCB-induced upregulation of a critical enzyme for TMAO formation known as Flavin-containing monooxygenase 3 (FMO3). To examine if these interesting observations can be recapitulated in a human population, I have begun collaborating with the CDC to make associations with TMAO and dioxin-like pollutant exposures in the highly exposed Anniston, Alabama cohort.

a. Liu D, Perkins JT, **Petriello MC**, Hennig B: Exposure to coplanar PCBs induces endothelial cell inflammation through epigenetic regulation of NF-κB subunit p65. 2015 Oct 28; S0041-008X(15)30118-6. doi: 10.1016/j.taap.2015.10.015. PMID: 26519613.
b. **Petriello MC,** Hoffman J, Sunkara, Wahlang B, Perkins JT, Morris AJ, Hennig B: Dioxin-like pollutants increase hepatic flavin containing monooxygenase (FMO3) expression to promote synthesis of the pro-atherogenic nutrient biomarker Trimethylamine N-oxide from dietary precursors. Journal of Nutritional Biochemistry. 2016 April 1; 33:145-153. PMID: 27155921
c. Wahlang B, **Petriello MC,** Perkins JT, Shen S, Hennig B: Polychlorinated biphenyl exposure alters the expression profile of microRNAs associated with vascular diseases. 2016 Sep; 35:180-187. PMID: 27288564
d. **Petriello MC,** Charnigo R, Sunkara M, Soman S, Pavuk M, Birnbaum L, Morris AJ, Hennig B: Relationship between serum Trimethylamine N-oxide and exposure to dioxin-like pollutants. *Environ Res. Revisions submitted*

Complete list of Published Work (18 manuscripts):
https://www.ncbi.nlm.nih.gov/sites/myncbi/1TyLshqP-YdAY/bibliography/40910356/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

5T32HL091812-08 Smyth, S (PI) 09/2016-07/2018
Clinical Scholars in Cardiovascular Science
The goal of this program is to prepare clinical scholars to assume leadership positions directing research in the field of cardiovascular medicine.
Role: Postdoctoral Training Fellow

Completed Research Support

P42ES007380-17A1S1 Hennig, B (PI) 05/2016-03/2017
Nutrition and Superfund Chemical Toxicity
The purpose of this supplement award was to support collaboration between Dr. Sudha Biddinger and Dr. Michael Petriello and transfer technologies between Boston Children's Hospital and University of Kentucky
Role: Fellow/Visiting Scientist

13PRE15860000 Hennig, B, Morris A, Petriello M (MPI) 07/2013-06/2015
Novel Methodologies to Study Anti-Inflammatory Nitro-Fatty Acids
The goal of this American Heart Association Great Rivers Affiliate Predoctoral Fellowship project was to….
Role: Graduate Student Fellow

5T32ES007266-22 Vore, M (PI) 06/2011-06/2013
Training Grant in Molecular Mechanisms of Toxicity
The goal of this program is to educate and train scientists for research and education in academia, government and industry emphasizing cardiovascular disease, neurodegenerative disease, and cancer.
Role: Doctoral Training Fellow

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Andrew J. Morris, PhD**

eRA COMMONS USER NAME (credential, e.g., agency login): AMORRIS

POSITION TITLE: Professor of Cardiovascular Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Bristol, Bristol, United Kingdom	BSc	1984	Biochemistry
University of Birmingham, Birmingham, United Kingdom	PhD	1988	Biochemistry
University of North Carolina, Chapel Hill, NC	Postdoctoral	1991	Pharmacology

A. Personal Statement

I will serve as the primary mentor for Dr. Petriello's K99/R00 training. My personal research program broadly concerns the role of lipid metabolism and signaling in cardiovascular and metabolic disease and employs multidisciplinary approaches spanning clinical research, preclinical models biochemistry as well as cell and molecular biology. I have been continuously supported by federal funds for more than 20 years. My interest in analytical chemistry led me to develop and direct a facility core at the University of Kentucky that provides institution-wide small molecule mass spectrometry services and participate in a number of personal and programmatic research efforts in the broad areas of diet and environmental disease that benefit from my capabilities in analytical chemistry. This includes direction of the analytical core components of our NIEHS supported P42 (Superfund) and P30 (Environmental Disease) centers. My relationship with Dr. Petriello stems from our shared interest in understanding how diet and nutrition interact with environmental exposures to impact on human disease risk. Dr. Petriello's research training and independent program will therefore benefit substantially from instrumentation and professional staff that I have assembled to conduct research mouse models and mass spectrometry based approaches. I am committed to the mentoring and career development of early stage investigators. For the past seven years, I have been an active member of a COBRE in Cardiovascular Disease and Obesity where I direct the analytical core and mentor junior faculty including several individuals who have competed successfully for NIH R-level research support. I am currently the mentor of an NIH/NCI K01 recipient and a co mentor for a K99/R00 awardee (K99 HL122505 -PI: Nagareddy) who recently obtained a tenure track appointment at the University of Alabama at Birmingham. Dr. Petriello's accomplishments and promise are comparable to the very best individuals I have interacted with at his career stage. Support of Dr. Petriello with a K99/R00 award would be an appropriate way to recognize his potential an appropriate next step in his career and a worthwhile investment of NIEHS funds. Recent publications demonstrating my expertise in analytical chemistry approaches for environmental disease research include:

a. Baker NA, Karounos M, English V, Fang J, Wei Y, Stromberg A, Sunkara M, Morris AJ, Swanson HI, Cassis LA. Coplanar polychlorinated biphenyls impair glucose homeostasis in lean C57BL/6 mice and mitigate beneficial effects of weight loss on glucose homeostasis in obese mice. *Environ Health Perspect.* 2013 121:105-10. doi: 10.1289/ehp.1205421. PMID: 23099484
b. Baker NA, Shoemaker R, English V, Larian N, Sunkara M, Morris AJ, Walker M, Yiannikouris F, Cassis LA. Effects of Adipocyte Aryl Hydrocarbon Receptor Deficiency on PCB-Induced Disruption of Glucose Homeostasis in Lean and Obese Mice. *Environ Health Perspect.* 2015 PMID: 25734695
c. Sui Y, Park SH, Helsley RN, Sunkara M, Gonzalez FJ, Morris AJ, Zhou C. 2014. Bisphenol A increases atherosclerosis in pregnane X receptor-humanized ApoE deficient mice. *J Am Heart Assoc* 3:e000492. PMCID: 4187496
d. Petriello, M.C., Hoffman, J.B., Sunkara, M., Wahlang, B, Perkins, J.T., Morris, A.J. and Hennig, B. Dioxin-Like pollutants increase hepatic flavin dependence monooxygenase expression to promote

synthesis of the pro-atherogenic biomarker trimethylamine N-oxide from dietary precursors. *J. Nutr. Biochem,* in press 2016, 33:145-53. PMCID:27155921

B. Positions and Honors

Positions and Employment

1991-1993 Research Assistant Professor, Department of Pharmacology, University of North Carolina (UNC)-Chapel Hill, Chapel Hill, NC
1993-2001 Assistant, Associate Professor, Department of Pharmacology, State University of New York (SUNY)-Stony Brook, Stony Brook, NY.
2001-2005 Associate Professor, Department of Cell Biology, UNC-Chapel Hill, Chapel Hill, NC
2005-Present Endowed Professor, Cardiovascular Medicine & Pharmacology University of Kentucky
2005-Present Investigator, Lexington Veterans Affairs Medical Center, Lexington KY

Other Experience and Professional Memberships

1999, 2001 Co-Chair, FASEB Conference on Phospholipase D
2000-2005 Editorial Board, Journal of Biological Chemistry
2001-2010 Associate Editor, the Biochemical Journal
2004 Chair, FASEB Conference on Phospholipases
2005, 2007 Co-Chair, FASEB Conference on Lysophospholipids and Related Mediators
2010-present Editorial Board, Journal of Biological Chemistry
2016-present Associate Editor, Molecular Pharmacology

Honors

1983-87 SERC CASE Predoctoral Fellowship Award
1990 American Heart Association Young Investigator Award
1990-92 Patrick J. Mitchell Fellowship, American Heart Association North Carolina Affiliate
1994 NIH Director's Shannon Award.

C. Contributions to Science

1. **Identification and characterization of inositol lipid and phosphate metabolizing enzymes and studies of G-protein regulated phospholipase C.** My interests in lipid metabolism and signaling stem from graduate studies conducted with Bob Michell and Peter Downes at the University of Birmingham in the UK. Bob and Pete made seminal discoveries about the link between agonist-stimulated inositol lipid turnover and "Ca^{2+} signaling" that eventually led to the realization that inositol 1,4,5 tris phosphate was an intracellular second messenger. I worked on the metabolism of inositol 1,4,5 trisphosphate. These studies included the identification and characterization of enzyme activities responsible for complete dephosphorylation of this molecule as well as kinase activities that could convert it to so-called "higher" inositol phosphates that had until then only been observed in plants. Analytical approaches developed for these studies were used to identify the lipid products of phosphoinositide 3-kinase. As a post-doctoral fellow and research faculty member with Ken Harden at the University of North Carolina I investigated the regulation of the inositol 1,4,5 tris phosphate generating enzyme phospholipase C. We identified the beta class of these enzymes as regulated by heterotrimeric G-proteins providing molecular identification of the machinery responsible for agonist stimulated inositol lipid signaling including one of the first descriptions of a G-protein beta gamma subunit regulated effector.
   a. **Morris AJ**, Storey DJ, Downes CP, Michell RH. Dephosphorylation of 1D-myo-inositol 1,4-bisphosphate in rat liver. Biochem J 254:655-660, 1988. PMCID: PMC1135135
   b. **Morris AJ**, Murray KJ, England PJ, Downes CP, Michell RH. Partial purification and some properties of rat brain inositol 1,4,5-trisphosphate 3-kinase. Biochem J 251:157-163, 1988. PMCID: PMC1148977
   c. **Morris AJ**, Waldo GL, Downes CP, Harden TK. A receptor and G-protein-regulated polyphosphoinositide-specific phospholipase C from turkey erythrocytes. I. Purification and properties. J Biol Chem 265:13501-13507, 1990.
   d. Waldo GL, Boyer JL, **Morris AJ**, Harden TK. Purification of an AlF4- and G-protein beta gamma-subunit-regulated phospholipase C-activating protein. J Biol Chem 266:14217-14225, 1991
2. **Structure, regulation and function of eukaryotic phospholipases D.** As an independent investigator I continued my interest in phospholipases and was fortunate to collaborate with Joanne Engebrecht who had serendipitously identified the first eukaryotic phospholipase D gene. This gene defined a large family of related enzymes. With another collaborator, Mike Frohman, I contributed to a body of highly cited work that reported the sequences and biochemical activities of these enzymes and then used a range of genetic and cell biological approaches to investigate their functions. These studies revealed roles in cell signaling pathways that control cell survival, motility and membrane trafficking and organelle dynamics.

a. Hammond SM, Altshuller YM, Sung TC, Rudge SA, Rose K, Engebrecht J, **Morris AJ**, Frohman MA. Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family. J Biol Chem 270:29640-29643, 1995.
b. Rose K, Rudge SA, Frohman MA, **Morris AJ**, Engebrecht J. Phospholipase D signaling is essential for meiosis. Proc Natl Acad Sci U S A 92:12151-12155, 1995. PMCID: PMC40314
c. Sciorra VA, Rudge SA, Prestwich GD, Frohman MA, Engebrecht J, **Morris AJ**. Identification of a phosphoinositide binding motif that mediates activation of mammalian and yeast phospholipase D isoenzymes. EMBO J 18:5911-5921, 1999. PMCID: PMC1171657
d. Huang H, Gao Q, Peng X, Choi SY, Sarma K, Ren H, **Morris AJ**, Frohman MA. PiRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. Dev Cell 20:376-387, 2011. PMCID: PMC3061402

3. **Synthesis and inactivation of bioactive lysophospholipids.** My studies of phospholipase D led me to identify integral membrane lipid phosphatase genes that we were surprised to find encoded proteins that could localize to the plasma membrane with active sites facing the extracellular space. This led us to explore the concept that these enzymes could function as regulators of extracellular signaling by bioactive lysophospholipids such as lysophosphatidic acid and sphingosine 1 phosphate. We have made important contributions to our understanding of the enzymology of the synthesis, transport and inactivation of these lipids that include studies of these integral membrane lipid phosphate phosphatase and the lysophosphatidic acid-generating enzyme autotaxin. To enable our research in this area I became invested in mass spectrometry-based methods to detect and quantitate these lipids at low levels in complex biological samples. I developed an institutional core facility for LC and GC coupled multistage mass spectrometry. Beyond our interests in lipid metabolism, these technologies have led me to contribute to research in metabolism, environmental toxicology, and natural products research and drug discovery.
   a. Roberts R, Sciorra VA, **Morris AJ**. Human type 2 phosphatidic acid phosphohydrolases. Substrate specificity of the type 2a, 2b, and 2c enzymes and cell surface activity of the 2a isoform. J Biol Chem 273:22059-22067, 1998.
   b. Albers HM, Dong A, van Meeteren LA, Egan DA, Sunkara M, van Tilburg EW, Schuurman K, van Tellingen O, **Morris AJ**, Smyth SS, Moolenaar WH, Ovaa H. Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. Proc Natl Acad Sci U S A 107:7257-7262, 2010. PMCID: PMC2867685
   c. Hausmann J, Kamtekar S, Christodoulou E, Day JE, Wu T, Fulkerson Z, Albers HM, van Meeteren LA, Houben AJ, van Zeijl L, Jansen S, Andries M, Hall T, Pegg LE, Benson TE, Kasiem M, Harlos K, Kooi CW, Smyth SS, Ovaa H, Bollen M, **Morris AJ**, Moolenaar WH, Perrakis A. Structural basis of substrate discrimination and integrin binding by autotaxin. Nat Struct Mol Biol 18:198-204, 2011. PMCID: PMC3064516
   d. Wu T, Kooi CV, Shah P, Charnigo R, Huang C, Smyth SS, **Morris AJ**. Integrin-mediated cell surface recruitment of autotaxin promotes persistent directional cell migration. FASEB J 28:861-870, 2014. PMCID: PMC3898650

4. **Role of bioactive lysophospholipids in cardiovascular disease.** My research into bioactive lysophospholipid metabolism and signaling has been highly collaborative driven in part by sharing of unique reagents and experimental approaches. This includes important work identifying roles for bioactive lysophospholipids lipids and the enzymes that make and degrade them in trafficking, mobilization and homing of lymphocytes and hematopoietic cells. My most significant recent contributions stem from a long term collaboration with Susan Smyth. One of the integral membrane lipid phosphatase genes we originally identified (PPAP2B) is dramatically upregulated in blood and vascular cells in settings of vascular inflammation and atherosclerosis. A common heritable variant of this gene is strongly associated with coronary artery disease risk in humans. The risk-associated allele inactivates an intronic enhancer that normally increases PPAP2B expression in response to inflammatory and atherogenic stimuli. In mice, PPAP2B deficiency is associated with accelerated atherosclerosis. These findings identify a normally protective role for PPAP2B in human atherothrombotic disease. A major focus of my personal research is now on understanding how changes in PPAP2B expression impact lipid metabolism and signaling to determine cardiovascular disease risk.
   a. Salous AK, Panchatcharam M, Sunkara M, Mueller P, Dong A, Wang Y, Graf GA, Smyth SS, **Morris AJ**. Mechanism of rapid elimination of lysophosphatidic acid and related lipids from the circulation of mice. J Lipid Res 54:2775-2784, 2013. PMCID: PMC3770090

b. Herzog BH, Fu J, Wilson SJ, Hess PR, Sen A, McDaniel JM, Pan Y, Sheng M, Yago T, Silasi-Mansat R, McGee S, May F, Nieswandt B, **Morris AJ**, Lupu F, Coughlin SR, McEver RP, Chen H, Kahn ML, Xia L. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. Nature 502:105-109, 2013. PMCID: PMC3791160
c. Panchatcharam M, Salous AK, Brandon J, Miriyala S, Wheeler J, Patil P, Sunkara M, **Morris AJ**, Escalante-Alcalde D, Smyth SS. Mice with targeted inactivation of ppap2b in endothelial and hematopoietic cells display enhanced vascular inflammation and permeability. Arterioscler Thromb Vasc Biol 34:837-845, 2014. PMCID: PMC4001868
d. Reschen ME, Gaulton KJ, Lin D, Soilleux EJ, **Morris AJ**, Smyth SS, O'Callaghan CA. Lipid-induced epigenomic changes in human macrophages identify a coronary artery disease-associated variant that regulates PPAP2B Expression through Altered C/EBP-beta binding. PLoS Genet 11:e1005061, 2015. PMCID: PMC4383549

5. **Science Education and Mentoring.** I am actively engaged in training and mentoring students, fellows, and early career investigators. Over the past 20 years, students and fellows receiving training in my laboratory have competed successfully for individual support from the American Heart Association (8 pre- or postdoctoral fellowship awards, three Beginning Grants-in-Aid, and two Scientist Development Grants) and the NIH (T and F32 awards, as well as currently active K01 and K99/R00 awardees) and gone onto successful careers as independent investigators in academia and industry. At UK, I have mentored two MD/PhD students and am a member of the MD/PhD program steering committee. I direct the Research Support Core of our NIEHS-funded Superfund Research Center and, together with the Training and Mentoring Core, have offered an annual workshop to affiliated trainees providing instruction in mass spectrometry for environmental disease research. As detailed in my personal statement, I have mentored several investigators who are now NIH-funded, independent researchers.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/andrew.morris.1/bibliography/40712481/public/?sort=date&direction=ascending

D. Research Support

Current

R01 HL120507 (MPI: Morris, A, Smyth, S) 01/04/15-03/30/19
NIH/NHLBI
Goals: To identify the cell types and mechanisms involved in protective effects of lipid phosphate phosphatase 3 against cardiovascular disease.
Role: MPI

I01CX001550 (PI: Morris, A.) 1/1/17-12/30/21
VA CSR&D Merit Review
Lysophosphatidic acid and cardiovascular disease risk.
Goals: To identify sources of atherogenic lipoprotein associated pools of lysophosphatidic acid and determine the mechanisms that their intravascular accumulation and signaling during atherogenesis.
Role: PI

1S10OD021753-01A1 (PI:Morris, A.) 3/1/17-2/28/18
Triple quadrupole mass spectrometer system
Goals: To obtain an ABSciex triple quadrupole HPLC coupled mass spectrometer system to replace a ~9 year old workhorse instrument in the mass spectrometry facilty core at the University of Kentucky.
Role: PI

BC150305P1 (PI: Morris, A) 10/01/15-09/30/18
DOD/USAMRAA
“Twist-ATX-LPAR1 signaling axis in obesity-associated triple negative breast cancer”
Goals: To delineate the function and regulation of Twist, and to explore the therapeutic potential of targeting Twist-ATX-LPAR1 axis in triple negative breast cancer and obesity.
Role: PI

1I01BX002769-01 (PI: Smyth, S) 10/01/10-09/30/18
VA BLR&D Merit Review
“Adipose autotaxin: a novel link between obesity and cardiovascular disease”
Goals: To test the hypothesis that adipose derived autotaxin and its product lysophosphatidic acid contribute to obesity associated cardiovascular disease.

Role: Co-PI

P20 GM103527 (PI: Cassis, L) 09/08/08-06/30/19
NIH/NIGMS
“Center of Research in Obesity and Cardiovascular Disease: Analytical Core”
Goals: I direct the analytical core of this center grant and serve as a mentor to junior faculty investigators
Role: Core director, mentor.

P42 ES007380 (PI: Hennig) 04/07/97-03/31/19
NIEHS
“Superfund Basic Research Program: Research Support Core”
Goals: Bioanalytical support for the University of Kentucky Superfund basic research program.
Role: Core director.

P42 ES007380 (PI: Hennig) 04/07/97-03/31/19
NIEHS
“Superfund Chemicals, Nutrition, and Endothelial Cell Dysfunction”
Goals: To identify mechanisms by which environmental pollutants impair vascular endothelial cell function to promote cardiovascular disease.
Role: Co-PI.

P30 ES026529 (Shi, X) 04/01/17-03/31/22
NIH/NIEHS
Center for Appalachian Research in Environmental Sciences
The overall goal of this application is to support an integrated core center to increase the efficiency and impact of environmental disease research at the University of Kentucky. My role is to direct an Analytical Core that provides bioanalytical and computational services to center-affiliated investigators.
Role: Core Lead (Analytical Core)

R01 ES023470(PI: Zhou) 09/26/13-06/30/18
NIH/NIEHS
“Endocrine disruptor mediated activation of PXR causes dyslipidemia”
Goals: To define the role of endocrine disrupting environmental pollutants in cardiovascular disease.
Role: Co-PI.

K01 CA197073 (PI: Onono, F) 07/01/15-06/31/20
NIH/NCI
“Intestinal phosphatidylcholine exposure and breast cancer risk”
Goals: This is a mentored career development award that will enable the recipient to become an independent investigator working on mechanisms that link obesity and cancer risk
Role: Mentor.

R01 HL123358 (PI: Zhou) 08/01/15-05/31/19
NIH/NHLBI
“A novel mechanism for ART-associated dyslipidemia and atherosclerosis”
Goals: To define the role of PXR in cardiovascular complications of HIV drug therapies.
Role: Co-PI

Completed Relevant Support

BX001984-01 (PI: Morris, A) 11/05/12-11/04/16
VA BLR&D Merit Review
“Association of a common variant of PPAP2B gene with cardiovascular disease”
Goals: To establish a mechanistic basis for the strong association of a common polymorphism in the PPAP2B gene with cardiovascular disease. (a competing renewal has been approved for funding starting 1/1/2017)
Role: PI

IS1BX003153 (PI: Morris, A) 10/01/2015-9/30/16
VA Shared Equipment Evaluation Program
“Acquisition of an HPLC Electrospray/chemical ionization triple quadrupole linear ion trap mass spectrometer system”
Goals: Purchase of an AB Sciex 6500 hybrid triple quadrupole linear ion trap mass spectrometer system
Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Smyth, Susan

eRA COMMONS USER NAME (credential, e.g., agency login): susan_smyth

POSITION TITLE: Jeff Gill Professor of Cardiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Mount Holyoke College, S Hadley, MA	AB	1987	Biological Sciences
University of North Carolina, Chapel Hill, NC	PHD	1992	Pharmacology
University of North Carolina, Chapel Hill, NC	MD	1994	Medicine
University Medical Center, Stony Brook, NY	Resident	1998	Internal Medicine
Mount Sinai Medical Center, New York, , NY	Fellow	2001	Cardiology

A. Personal Statement

I will serve as a Co-mentor for Dr Petriello's K99/R00 supported training providing particular support for clinical studies and investigations using mouse models of atherosclerosis. As a physician scientist, I combine clinical practice in cardiology with funded research focused on the molecular and cellular interplay of inflammation and thrombosis in the development and progression of cardiovascular disease, with a particular emphasis on ischemic vascular disease. We translate observations from experimental cellular systems and preclinical animal models to clinical settings, including acute coronary syndromes, venous thrombosis, and more recently sepsis. Central to much of our research is the use of mouse models in which genetic and pharmacologic strategies are employed to define roles for specific cell surface molecules and inflammatory mediators. Current work examines communication between blood and vascular cells, identification of novel thromboinflammatory pathways, and studies of lysophospholipid signaling pathways. Our work has resulted in the generation of unique reagents, animal models, technology and new therapeutic strategies for vascular diseases.

Of particular relevance to Dr Petrillo's application, I have been involved in education and training at many levels, including mentoring of medical and graduate students (>20) and postdoctoral fellows (10), junior faculty (8), and didactic teaching. While on the faculty at UK, I have had six graduate students perform doctoral dissertation research in my laboratory and all were supported by fellowship awards either from the NIH (F-series) or the American Heart Association. One of my current post-doctoral trainees recently received a K99/R00. Twelve of my former trainees have faculty appointments at academic institutions leading research efforts supported by extramural funding. I devote approximately 6 hours a week to programmatic direction of training and education in my role as Director of the MD/PhD Program. My mentoring efforts were recognized by the 2012 Mentor Award from the Center for Clinical and Translational Science. Based on my track record of performance, I am well prepared to provide mentoring and career development advice to Dr Petriello.

1. Smyth SS, Reis ED, Zhang W, Fallon JT, Gordon RE, Coller BS. Beta(3)-integrin-deficient mice but not P-selectin-deficient mice develop intimal hyperplasia after vascular injury: correlation with leukocyte recruitment to adherent platelets 1 hour after injury. Circulation. 2001 May 22;103(20):2501-7. PubMed PMID: 11369692.

2. Smyth SS, Reis ED, Väänänen H, Zhang W, Coller BS. Variable protection of beta 3-integrin--deficient mice from thrombosis initiated by different mechanisms. Blood. 2001 Aug 15;98(4):1055-62. PubMed PMID: 11493451.
3. Panchatcharam M, Miriyala S, Yang F, Rojas M, End C, Vallant C, Dong A, Lynch K, Chun J, Morris AJ, Smyth SS. Lysophosphatidic acid receptors 1 and 2 play roles in regulation of vascular injury responses but not blood pressure. Circ Res. 2008 Sep 12;103(6):662-70. PubMed PMID: 18703779; PubMed Central PMCID: PMC2637300.
4. Panchatcharam M, Salous AK, Brandon J, Miriyala S, Wheeler J, Patil P, Sunkara M, Morris AJ, Escalante-Alcalde D, Smyth SS. Mice with targeted inactivation of ppap2b in endothelial and hematopoietic cells display enhanced vascular inflammation and permeability. Arterioscler Thromb Vasc Biol. 2014 Apr;34(4):837-45. PubMed PMID: 24504738; PubMed Central PMCID: PMC4001868.

B. Positions and Honors

Positions and Employment

2001 - 2005	Assistant Professor, University of North Carolina, Chapel Hill, NC
2006 -	Adjunct Associate Professor, Cell & Molecular Physiology, University of North Carolina, Chapel Hill, NC
2006 - 2010	Associate Professor, Department of Internal Medicine, joint appointments in Physiology and Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY
2010 -	Professor, Department of Internal Medicine, Physiology and Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY

Other Experience and Professional Memberships

2005 -	Editorial Panel, Clinical Science
2005 - 2009	NHLBI, Parent Program Project Review Committee
2006 -	NHLBI, Strategic Planning Group on Inflammation in Ischemic Vascular Disorders
2007 -	Editorial Board, Arteriosclerosis, Thrombosis and Vascular Biology
2008 - 2009	Chair, Parent Program Project Review Committee, NIH/NHLBI
2008 - 2010	Chair, Vascular Biology Study Section, Region 1, American Heart Association
2009 - 2013	Member, NIH Thrombosis and Hemostasis Review Panel
2010 -	Associate Editor, Journal Thrombosis and Thrombolysis (section editor Fellow's Forum)
2012 -	Consulting Editor, Journal of Clinical Investigation
2013 – 2015	Chair, HEMA study section, Department Veterans Affairs
2016 –	Chair, NHLBI Outstanding Investigator review panel
2016 - 2020	Member, NHLBI Institutional Training Mechanism (NITM) Review Committee

Honors

2000	Faculty Scholar Award, American Society of Hematology
2003	Young Investigator Prize in Thrombosis, finalist , American Heart Association
2006	Special Recognition Award in Thrombosis, ATVB Council, American Heart Association
2009	Member, American Society of Clinical Investigation
2009	Stewart-Niewiarowski Award , Women in Vascular Biology
2013	Member, Association of University Cardiologists.
2013	Jeffrey M. Hoeg Award, American Heart Association
2015	Board of Governors, American College of Cardiology
2015	Chair elect, FASEB conference on Lysophospholipid Mediators in Health and Disease

C. Contribution to Science

1. Drawing on a long-standing interest in platelet biology, our group has established animal models for studying the role of platelet membrane proteins in thrombosis and the response to arterial injury, has delineated specific signaling systems responsible for mediating platelet-leukocyte interactions, and defined the contribution of inflammation to the arterial injury response and in the setting of cardiac hypertrophy.

a. Evangelista V, Pamuklar Z, Piccoli A, Manarini S, Dell'elba G, Pecce R, Martelli N, Federico L, Rojas M, Berton G, Lowell CA, Totani L, Smyth SS. Src family kinases mediate neutrophil adhesion to adherent platelets. Blood. 2007 Mar 15;109(6):2461-9. PubMed PMID: 17095622; PubMed Central PMCID: PMC1852189.

b. Totani L, Piccoli A, Dell'Elba G, Concetta A, Di Santo A, Martelli N, Federico L, Pamuklar Z, Smyth SS, Evangelista V. Phosphodiesterase type 4 blockade prevents platelet-mediated neutrophil recruitment at the site of vascular injury. Arterioscler Thromb Vasc Biol. 2014 Aug;34(8):1689-96. PubMed PMID: 24925970; PubMed Central PMCID: PMC4117992.

c. Ye S, Huang Y, Joshi S, Zhang J, Yang F, Zhang G, Smyth SS, Li Z, Takai Y, Whiteheart SW. Platelet secretion and hemostasis require syntaxin-binding protein STXBP5. J Clin Invest. 2014 Oct;124(10):4517-28. PubMed PMID: 25244094; PubMed Central PMCID: PMC4191053.

d. Yang F, Dong A, Ahamed J, Sunkara M, Smyth SS. Granule cargo release from bone marrow-derived cells sustains cardiac hypertrophy. Am J Physiol Heart Circ Physiol. 2014 Nov 15;307(10):H1529-38. PubMed PMID: 25239803; PubMed Central PMCID: PMC4233303.

2. My group's interest in understanding the functional interplay between thrombosis and inflammation has identified beneficial effects of antiplatelet drugs in infectious and inflammatory states, such as pneumonia and sepsis. This work has also spawned clinical trials of the effects of antithrombotic therapy on inflammation and the identification of biomarkers of ischemic heart disease. Ongoing research seeks to identify novel signaling pathways in platelets, as targets for future translational studies.

a. Pamuklar Z, Lee JS, Cheng HY, Panchatcharam M, Steinhubl S, Morris AJ, Charnigo R, Smyth SS. Individual heterogeneity in platelet response to lysophosphatidic acid: evidence for a novel inhibitory pathway. Arterioscler Thromb Vasc Biol. 2008 Mar;28(3):555-61. PubMed PMID: 18202325.

b. Selim S, Sunkara M, Salous AK, Leung SW, Berdyshev EV, Bailey A, Campbell CL, Charnigo R, Morris AJ, Smyth SS. Plasma levels of sphingosine 1-phosphate are strongly correlated with haematocrit, but variably restored by red blood cell transfusions. Clin Sci (Lond). 2011 Dec;121(12):565-72. PubMed PMID: 21749329; PubMed Central PMCID: PMC3174054.

c. Xiang B, Zhang G, Guo L, Li XA, Morris AJ, Daugherty A, Whiteheart SW, Smyth SS, Li Z. Platelets protect from septic shock by inhibiting macrophage-dependent inflammation via the cyclooxygenase 1 signalling pathway. Nat Commun. 2013;4:2657. PubMed PMID: 24150174; PubMed Central PMCID: PMC4217311.

d. Sexton TR, Wallace EL, Macaulay TE, Charnigo RJ, Evangelista V, Campbell CL, Bailey AL, Smyth SS. The effect of rosuvastatin on platelet-leukocyte interactions in the setting of acute coronary syndrome. J Am Coll Cardiol. 2015 Jan 27;65(3):306-7. PubMed PMID: 25614429.

3. Our work to identify novel inflammatory mediators of vascular disease led us to studying the role of bioactive lysophospholipids, including lysophosphatidic acid (LPA), in cardiovascular disease. LPA is a proteotypic member of a family of bioactive lipid phosphoric acids that function as receptor-active mediators with roles in cell growth, differentiation, apoptosis and development. Using our preclinical, animal models, we provided the first evidence of a pathologic role for LPA receptors in the response to arterial injury and established important roles for LPA signaling in thrombosis/hemostasis, vascular remodeling, and adipocyte function.

a. Panchatcharam M, Miriyala S, Yang F, Rojas M, End C, Vallant C, Dong A, Lynch K, Chun J, Morris AJ, Smyth SS. Lysophosphatidic acid receptors 1 and 2 play roles in regulation of vascular injury responses but not blood pressure. Circ Res. 2008 Sep 12;103(6):662-70. PubMed PMID: 18703779; PubMed Central PMCID: PMC2637300.

b. Pamuklar Z, Federico L, Liu S, Umezu-Goto M, Dong A, Panchatcharam M, Fulkerson Z, Berdyshev E, Natarajan V, Fang X, van Meeteren LA, Moolenaar WH, Mills GB, Morris AJ, Smyth SS. Autotaxin/lysopholipase D and lysophosphatidic acid regulate murine hemostasis and thrombosis. J Biol Chem. 2009 Mar 13;284(11):7385-94. PubMed PMID: 19139100; PubMed Central PMCID: PMC2652269.

c. Cheng HY, Dong A, Panchatcharam M, Mueller P, Yang F, Li Z, Mills G, Chun J, Morris AJ, Smyth SS. Lysophosphatidic acid signaling protects pulmonary vasculature from hypoxia-induced remodeling.

Arterioscler Thromb Vasc Biol. 2012 Jan;32(1):24-32. PubMed PMID: 22015657; PubMed Central PMCID: PMC3241874.

d. Federico L, Ren H, Mueller PA, Wu T, Liu S, Popovic J, Blalock EM, Sunkara M, Ovaa H, Albers HM, Mills GB, Morris AJ, Smyth SS. Autotaxin and its product lysophosphatidic acid suppress brown adipose differentiation and promote diet-induced obesity in mice. Mol Endocrinol. 2012 May;26(5):786-97. PubMed PMID: 22474126; PubMed Central PMCID: PMC3355557.

4. The lysophospholipase D autotaxin (ATX) catalyzes the hydrolysis of circulating or cell-associated lysophosphatidylcholine (LPC) to generate LPA. We have identified pathways for the production of LPA in the circulation and discovered key structural and functional elements of ATX that regulate the generation of LPA along cell surfaces. This work elucidated an integrin-dependent pathway for localization of ATX along the surface of platelets and other vascular cells that concentrates the production of LPA. Current investigations are focused on identifying mechanistic links between dietary intake of choline-containing lipids, adipose-derived ATX, and atherosclerotic cardiovascular disease risk.

a. Albers HM, Dong A, van Meeteren LA, Egan DA, Sunkara M, van Tilburg EW, Schuurman K, van Tellingen O, Morris AJ, Smyth SS, Moolenaar WH, Ovaa H. Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. Proc Natl Acad Sci U S A. 2010 Apr 20;107(16):7257-62. PubMed PMID: 20360563; PubMed Central PMCID: PMC2867685.

b. Hausmann J, Kamtekar S, Christodoulou E, Day JE, Wu T, Fulkerson Z, Albers HM, van Meeteren LA, Houben AJ, van Zeijl L, Jansen S, Andries M, Hall T, Pegg LE, Benson TE, Kasiem M, Harlos K, Kooi CW, Smyth SS, Ovaa H, Bollen M, Morris AJ, Moolenaar WH, Perrakis A. Structural basis of substrate discrimination and integrin binding by autotaxin. Nat Struct Mol Biol. 2011 Feb;18(2):198-204. PubMed PMID: 21240271; PubMed Central PMCID: PMC3064516.

c. Fulkerson Z, Wu T, Sunkara M, Kooi CV, Morris AJ, Smyth SS. Binding of autotaxin to integrins localizes lysophosphatidic acid production to platelets and mammalian cells. J Biol Chem. 2011 Oct 7;286(40):34654-63. PubMed PMID: 21832043; PubMed Central PMCID: PMC3186383.

d. Wu T, Kooi CV, Shah P, Charnigo R, Huang C, Smyth SS, Morris AJ. Integrin-mediated cell surface recruitment of autotaxin promotes persistent directional cell migration. FASEB J. 2014 Feb;28(2):861-70. PubMed PMID: 24277575; PubMed Central PMCID: PMC3898650.

5. LPA can be hydrolyzed and inactivated by lipid phosphate phosphatase (LPP) enzymes present on cell membranes. A genome-wide association studies (GWAS) identified the PPAP2B gene encoding LPP3 as a novel loci associated with coronary artery disease susceptibility. We were among the first to demonstrate that LPPs regulate lysophopholipid signaling and have established that LPP3 expression in mice is critical to attenuate inflammation, reduce smooth muscle cell proliferation, and maintain endothelial barrier function following vascular injury in an LPA-dependent manner. Current studies focus on how heritable human variation in PPAP2B predisposes an individual to the development and/or complications of atherosclerosis, which should ultimately translate into the validation of novel pathways for disease prevention.

a. Panchatcharam M, Miriyala S, Salous A, Wheeler J, Dong A, Mueller P, Sunkara M, Escalante-Alcalde D, Morris AJ, Smyth SS. Lipid phosphate phosphatase 3 negatively regulates smooth muscle cell phenotypic modulation to limit intimal hyperplasia. Arterioscler Thromb Vasc Biol. 2013 Jan;33(1):52-9. PubMed PMID: 23104851; PubMed Central PMCID: PMC3524385.

b. Salous AK, Panchatcharam M, Sunkara M, Mueller P, Dong A, Wang Y, Graf GA, Smyth SS, Morris AJ. Mechanism of rapid elimination of lysophosphatidic acid and related lipids from the circulation of mice. J Lipid Res. 2013 Oct;54(10):2775-84. PubMed PMID: 23948545; PubMed Central PMCID: PMC3770090.

c. Panchatcharam M, Salous AK, Brandon J, Miriyala S, Wheeler J, Patil P, Sunkara M, Morris AJ, Escalante-Alcalde D, Smyth SS. Mice with targeted inactivation of ppap2b in endothelial and hematopoietic cells display enhanced vascular inflammation and permeability. Arterioscler Thromb Vasc Biol. 2014 Apr;34(4):837-45. PubMed PMID: 24504738; PubMed Central PMCID: PMC4001868.

d. Reschen ME, Gaulton KJ, Lin D, Soilleux EJ, Morris AJ, Smyth SS, O'Callaghan CA. Lipid-induced epigenomic changes in human macrophages identify a coronary artery disease-associated variant that

regulates PPAP2B Expression through Altered C/EBP-beta binding. PLoS Genet. 2015 Apr;11(4):e1005061. PubMed PMID: 25835000; PubMed Central PMCID: PMC4383549.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/myncbi/susan.smyth.1/bibliography/40331475/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

R01 HL120507-01A1 SMYTH (PI) 2015/04/01-2019/03/31
National Heart, Lung and Blood Institute (NHLBI)
Lipid phosphate phosphatase 3 as a novel atherosclerosis suppressor
This study will functional validate PPAP2B as a modifier of risk for development of coronary artery disease.
Role: PI

TL1TR001997-01 SMYTH (PI) 2016/08/15-2020/05/31
NRSA Training Core - Kentucky Center for Clinical and Translational Science
Role: PI

I01 BX002769-01 SMYTH (PI) 2015/01/01-2018/12/31
Biomedical Laboratory Research & Development (BLRD)
Adipose autotaxin: a novel link between obesity and cardiovascular disease
This study defines the contribution of adipose secreted autotaxin to inflammation and atherosclerosis.
Role: PI

T32 HL091812-07 SMYTH (PI) 2008/06/01-2019/05/31
National Heart, Lung and Blood Institute (NHLBI)
Clinical Scholars In Cardiovascular Science
Role: PI

NIH/NIGMS 5P20GM103527-07 (Cassis) 2008/9/08-2018/07/31
Center of Research in Obesity and Cardiovascular Disease
This grant establishes a Center of Biomedical Research Excellence in Obesity and Cardiovascular Disease and provides mentoring to junior faculty to facilitate their development as independent investigators.
Role: Mentor

NIH/NHLBI 1R01HL123927-01A1 (Li, Zhenyu) 2014/09/25-2018/06/30
Crosstalk between membrane traffic proteins and integrin activation
The objective of this project is to identify a novel binding partner that plays an important role in αIIbβ3 outside-in signaling and link membrane traffic proteins and integrin activation in platelets.
Role: Co-investigator

5UL1TR000117-04 KERN (PI) 2011/06/01-2020/02/29
NIH/NCATS Kentucky Center for Clinical and Translational Science
The University of Kentucky Center for Clinical and Translational Science (CCTS) is the academic home for the discipline of clinical and translational science, dedicated to growing the clinical and translational science research teams of the future
Role: TL1/T32 Director and Education co-Director

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Richard Charnigo

eRA COMMONS USER NAME (credential, e.g., agency login): RICH.CHARNIGO

POSITION TITLE: Professor of Biostatistics, and Professor of Statistics, at the University of Kentucky

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Case Western Reserve University, Cleveland OH	B.S.	05/1997	Mathematics
Case Western Reserve University, Cleveland OH	M.S.	08/1999	Mathematics
Case Western Reserve University, Cleveland OH	Ph.D.	08/2003	Statistics

A. Personal Statement

Dr. Charnigo will serve as a Co-Mentor for Dr. Petriello's K99/R00 supported training, providing expertise in the application of multivariate statistics to Dr. Petriello's proposed research. Dr. Charnigo is a statistician in the administrative core of the NIH-funded (P20) Center of Research in Obesity and Cardiovascular Disease at the University of Kentucky. Dr. Charnigo has been the principal investigator on grants from the National Science Foundation (2007-2011) and Army Research Office (2012-2013; 2016-2017). He has been the dissertation advisor to twelve Ph.D. students in Statistics (eight completed) and two Ph.D. students in Epidemiology and Biostatistics (both completed), and the supervisor to thirteen graduate and two undergraduate research assistants. Dr. Charnigo has worked with Dr. Petriello to analyze data from biomarker measurements conducted using samples from the Anniston Community Health Survey, generating data that are presented in the accompanying proposal. He also has collaborated with Dr. Petriello's mentors, Drs. Morris and Smyth, as exemplified by the publications below. Accordingly Dr. Charnigo has an ongoing record of interactions with Dr. Petriello and his mentors and is clearly very well qualified for his role in Dr. Petriello's training and career development.

1. Pamuklar, Zehra; Lee, Jin; Cheng, Hsin-Yuan; Pantcharam, Manikandan; Steinhubl, Steven; Morris, Andrew; Charnigo, Richard; Smyth, Susan (2008). "Individual Heterogeneity in Platelet Response to Lysophosphatidic Acid: Evidence for a Novel Inhibitory Pathway." *Arteriosclerosis, Thrombosis, and Vascular Biology*: Volume 28, pp. 555-561.

2. Selim, Samy; Sunkara, Manjula; Salous, Abdel; Berdyshev, Evgeny; Bailey, Alison; Campbell, Charles; Charnigo, Richard; Morris, Andrew; Smyth, Susan (2011). "Plasma levels of sphinosine 1 phosphate are strongly correlated with hematocrit but variably restored by red blood cell transfusions." Clinical Science: Volume 121, pp. 565-572.

3. Wu, Tao; Vander Kooi, Craig; Huang, Cai; Shah, Pritom; Charnigo, Richard; Smyth, Susan; Morris, Andrew (2014). "Integrin-Mediated Cell Surface Recruitment of Autotaxin Promotes Directional Cell Migration." The FASEB Journal: Volume 28, pp. 861-870.

4. Sexton, T.; Wallace, E.; Chen, A.; Charnigo, Richard; Reda, H.; Ziada, K.; Gurley, J.; Smyth, S. (2016). "Thromboinflammatory Response and Predictors of Outcomes in Patients Undergoing Transcatheter Aortic Valve Replacement." *Journal of Thrombosis and Thrombolysis*: Volume 41, pp. 384-393.

B. Positions and Honors

Positions and Employment

2003-2009	Assistant Professor, University of Kentucky, Lexington KY
2009-2013	Associate Professor, University of Kentucky, Lexington KY
2013-	Professor, University of Kentucky, Lexington KY
2017	Chair of Biostatistics Department, University of Kentucky, Lexington KY

Other Experience and Professional Memberships

2011-	Member, Delta Omega Honorary Society in Public Health
2012-2016	President, Kentucky Chapter of American Statistical Association
2013-2014	Reviewer, Natural Sciences and Engineering Research Council of Canada
2016	Temporary Member, NIH CICS Study Section

Honors

2010	Golden Apple Teaching Award, University of Kentucky
2014-2015	University Research Professor, University of Kentucky

C. Contributions to Science

1. With his collaborators, and supported by grants from the National Science Foundation and Army Research Office, Dr. Charnigo has developed theory and methodology for self-consistent nonparametric regression. More specifically, Dr. Charnigo has addressed problems of how to simultaneously estimate a mean response function along with its derivatives at near optimal rates and how to choose tuning parameters when derivative estimation is prioritized by the underlying scientific application. Indeed, he has applied his methodology to data sets on human development, liver function, Raman spectroscopy (to infer chemical composition), and simulated radiation scattering (to characterize nanoparticles).

a. Charnigo, Richard; Srinivasan, Cidambi (2011). "Self-Consistent Estimation of Mean Response Functions and their Derivatives." *Canadian Journal of Statistics*: Volume 39, pp. 280-299.

b. Charnigo, Richard; Francoeur, Mathieu; Kenkel, Patrick; Mengüç, M. Pinar; Hall, Benjamin; Srinivasan, Cidambi (2011). "Estimating Quantitative Features of Nanoparticles Using Multiple Derivatives of Scattering Profiles." *Journal of Quantitative Spectroscopy and Radiative Transfer:* Volume 112, pp. 1369-1382.

c. Charnigo, Richard; Srinivasan, Cidambi (2015). "A Multivariate Generalized C_p and Surface Estimation." *Biostatistics*: Volume 16, pp. 311-325.

d. Charnigo, Richard; Feng, Limin; Srinivasan, Cidambi (2015). "Nonparametric and Semiparametric Compound Estimation in Multiple Covariates." *Journal of Multivariate Analysis*: Volume 141, pp. 179-196.

2. With his collaborators, Dr. Charnigo has also developed theory and methodology for inferring the number of components in various kinds of mixture models. More specifically, Dr. Charnigo has established procedures for testing homogeneity in mixture models featuring vector within-component parameters when one component parameter is known and scalar within-component parameters when no component parameter is known. He has also created a test for two versus three components in a normal location mixture model when one component mean is known and developed an information-theoretic criterion for selecting the number of components in a mixture model. Practical applications have included the analysis of birthweight and microarray data.

a. Charnigo, Richard; Sun, Jiayang (2004). "Testing for Homogeneity in Mixture Distributions via the L_2 Distance Between Competing Models." *Journal of the American Statistical Association*: Vol. 99, pp. 488-498.

b. Charnigo, Richard; Sun, Jiayang (2010). "Asymptotic Relationships between the D-Test and Likelihood Ratio-Type Tests for Homogeneity." *Statistica Sinica*: Volume 20, pp. 497-512.

c. Charnigo, Richard; Chesnut, Lorie W.; LoBianco, Tony; Kirby, Russell S. (2010). "Thinking Outside the Curve, Part I: Modeling Birthweight Distribution." *Biomedcentral Pregnancy and Childbirth*: Vol. 10, Article 37.

d. Charnigo, Richard; Fan, Qian; Bittel, Douglas; Dai, Hongying (2013). “Testing Unilateral versus Bilateral Normal Contamination.” *Statistics and Probability Letters*: Volume 83, pp. 163-167.

3. Dr. Charnigo has contributed expertise to numerous projects in cardiovascular research, including both animal and human studies. The four items below are illustrative. The first was the culmination of a working group convened by the Duke Clinical Research Institute, at which Dr. Charnigo was an invited presenter. The other three were deemed sufficiently important to merit editorial comment when they were published. In fact, Dr. Charnigo’s University Research Professorship for 2014-2015 was awarded in part for him to address an outstanding controversy about digoxin and mortality in the AFFIRM study.

a. Rao, Sunil; Eikelboom, John; Steg, Gabriel; Lincoff, A. Michael; Weintraub, William; Bassand, Jean-Pierre; Rao, A. Koneti; Gibson, C. Michael; Petersen, John; Mehran, Roxana; Manoukian, Steven; Charnigo, Richard; Lee, Kerry; Moscucci, Mario; Harrington, Robert (2009). “Standardized Reporting of Bleeding Complications for Clinical Investigations in Acute Coronary Syndrome: A Proposal from the Academic Bleeding Consensus (ABC) Multidisciplinary Working Group.” *American Heart Journal*: Volume 158, pp. 881-886.

b. Yiannikouris, Frederique; Karounos, Michael; Charnigo, Richard; English, Victoria; Rateri, Debra; Daugherty, Alan; Cassis, Lisa (2012). “Adipocyte-specific deficiency of angiotensinogen decreases plasma angiotensinogen concentration and systolic blood pressure in male mice.” *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*: Volume 302, pp. 244-251.

c. Yiannikouris, Frederique; Gupte, Manisha; Putnam, Kelly; Thatcher, Sean; Charnigo, Richard; Rateri, Debra; Daugherty, Alan; Cassis, Lisa (2012). “Adipocyte Deficiency of Angiotensinogen Prevents Obesity-Induced Hypertension in Male Mice.” *Hypertension*: Volume 60, pp. 1524-1530.

d. Whitbeck, M.; Charnigo, Richard; Khairy, P.; Ziada, K.; Bailey, A.; Zegarra, M.; Shah, J.; Morales, G.; Macaulay, T.; Sorrell, V.; Campbell, C.; Gurley, J.; Anaya, P.; Nasr, H.; Bai, R.; DiBiase, L.; Booth, D.; Jondeau, G.; Natale, A.; Roy, D.; Smyth, S.; Moliterno, D.; Elayi, C. (2013). “Increased Mortality among Patients Taking Digoxin - Analysis from the AFFIRM Study.” *European Heart Journal*: Vol. 34, pp. 1481-1488.

MyBibliography link:
http://www.ncbi.nlm.nih.gov/sites/myncbi/richard.charnigo.1/bibliography/43873163/public/?sort=date

D. Selected Research Support

Below are grants on which I am currently supported. My participation on #8 and #9 is via an intergovernmental personnel act between the Lexington VA and me. My participation on #11 is via cost share.

1. W911NF-17-1-0040 Charnigo (PI) 12/14/16 – 12/13/17
Army Research Office
ALMOST-SMOOTH NONPARAMETRIC REGRESSION AND PATTERN RECOGNITION
The proposed project entails creating new statistical methodology and providing corresponding mathematical justification, for nonparametric estimation of “almost smooth” mean response functions and related pattern recognition. Role: Principal Investigator

2. R01DA043519 Bada and Leggas (MPI) 5/1/17 – 2/28/22
NIH
NON-OPIATE TREATMENT AFTER PRENATAL OPIATE EXPOSURE TO PREVENT POSTNATAL INJURY TO THE YOUNG BRAIN (NO-POPPY)
The objective of this application is to evaluate the effectiveness of clonidine, an α2 adrenergic receptor agonist, as a treatment for neonates with neonatal abstinence syndrome, in a randomized clinical trial. Role: Co-Investigator.

3. R01DK112136 Schoenberg (PI) 8/1/17 – 7/31/22
NIH
COMMUNITY TO CLINIC NAVIGATION TO IMPROVE DIABETES OUTCOMES
Since diabetes has reached epidemic proportions in the US, especially among low income and rural residents, we aim to test a refine and promising program called “Community to Clinic Navigation” (CCN) that combines

diabetes self-management education with tailored patient navigation to improve clinical care. Role: Co-Investigator.

4. R01HL134731 Webb and de Beer (MPI) 1/1/17 – 12/31/20
NIH
SERUM AMYLOID A, INFLAMMASOME ACTIVATION, AND ABDOMINAL AORTIC ANEURYSMS
This project investigates the role of serum amyloid A (SAA) in the initiation and progression of abdominal aortic aneurysms (AAA) and tests the hypothesis that systemic SAA promotes AAA by activating the NRLP3 inflammasome in macrophages that have been recruited to an established AAA. Role: Co-Investigator.

5. R01HL131925 Zhou (PI) 4/1/16 – 2/29/20
NIH
ROLE OF IKKβ IN OBESITY AND ATHEROSCLEROSIS
This is an R01 application intended to test the hypothesis that IKKβ is a critical regulator of adipogenesis and atherogenesis, and that overnutrition-induced activation of IKKβ promotes adipocyte differentiation, adipocyte inflammation and atherosclerosis. Role: Co-Investigator

6. R01HL123358 Zhou (PI) 8/01/15 – 5/31/19
NIH
A NOVEL MECHANISM FOR ART-ASSOCIATED DYSLIPIDEMIA AND ATHEROSCLEROSIS
The goal of this project is to investigate a novel mechanism linking antiretroviral (ARV) drugs with dyslipidemia and cardiovascular disease (CVD). Role: Co-Investigator

7. R01HL120507 Smyth and Morris (MPI) 4/01/15 – 3/31/19
NIH
LIPID PHOSPHATE PHOSPHATASE 3 AS A NOVEL ATHEROSCLEROSIS SUPPRESSOR
Completion of these studies promises to provide valuable insight into the mechanism(s) by which extracellular bioactive lipid mediators influence the development of ischemic heart disease and provide novel and innovative targets to predict, prevent and treat coronary artery disease. Role: Statistician

8. I01 CX000975 Tannock (PI) 4/1/14 – 3/31/18
VA-ORD
THE ASSOCIATION OF SAA WITH APOB LIPOPROTEINS AFFECTS CARDIOVASCULAR RISK
The central hypothesis of this grant is that the shift of SAA from HDL to apoB-containing lipoproteins in insulin resistant conditions such as Metabolic syndrome and diabetes contributes to the increased atherosclerosis and cardiovascular disease observed in these populations. Role: Statistician.

9. I01 CX000773 Webb (PI) 1/1/14–12/31/17
VA-ORD
HDL REMODELING IN THE METABOLIC SYNDROME
The central hypothesis of this proposal is that TG enrichment of HDL in MetS predisposes the particle to remodeling by intravascular factors and impedes selective lipid uptake by SR-BI. Consequently, TG-enriched HDL in MetS is more susceptible to rapid clearance and less capable of supporting reverse cholesterol transport. Role: Statistician.

10. R01ES023470 Zhou (PI) 9/26/13 - 6/30/18
NIH/NIEHS
ENDOCRINE DISRUPTOR MEDIATED ACTIVATION OF PXR CAUSES DYSLIPIDEMIA
The goal of this project is to investigate a novel mechanism linking endocrine disrupting chemical (EDC) exposure and hyperlipidemia. Role: Co-Investigator

11. Tannock and Gong (MPI) 2/23/16 – 11/30/17
Washington University
EATING AT THE RIGHT TIME – A NOVEL APPROACH TO CORRECT NON-DIPPING BLOOD PRESSURE

This project will test whether nighttime eating increases the prevalence of non-dipping BP, and daytime restricted food intake will restore normal BP dipping at night. Role: Co-Investigator

12. P20GM103527 Cassis (PI) 9/08/08 – 7/31/18
NIH/NCRR
CENTER OF RESEARCH IN OBESITY AND CARDIOVASCULAR DISEASE
The objective of this Center is to identify mechanisms linking the epidemic of obesity to the high incidence of cardiovascular diseases in the obese population and to develop promising junior project investigators.
Role: Administrative core

13. R01HL073085 Cassis (PI) 6/03/03 – 4/30/18
NIH/NHLBI
ANGIOTENSIN – A LINK BETWEEN OBESITY AND HYPERTENSION
These studies focus on the renin-angiotensin system of adipocytes as a mediator of obesity-hypertension in males versus females. Since obesity is the primary cause of hypertension in the US, and the prevalence of hypertension is increasing more rapidly in females than males, our studies will define mechanisms for these differences and uncover novel targets for drug therapy. Role: Statistician

Morris, Andrew J.

ACTIVE

5R01HL120507-03 Smyth, Morris (MPIs) 04/01/15 – 03/31/20 1.0 Cal Mnths
NIH/NHLBI $360,204
Lipid Phosphate Phosphatase 3 as a Novel Atherosclerosis Suppressor
The goal of this study is to use pre-clinical models to identify the mechanisms involved in protective effects of lipid phosphate phosphatase 3 against cardiovascular disease with a focus on foam cell formation in atherosclerosis.
OVERLAP: None

W81XWH-16-1-0067 Morris (PI) 03/15/16 – 03/14/19 .7 Cal Mnths
DOD/USAMRAA $70,000
Define the Twist-ATX-LPAR1 signaling axis in promoting obesity-associated triple negative breast cancer
The goal of this project is to test the hypothesis that transcriptional regulation of autotaxin and lysophosphatidic acid receptors by twist is important for obesity associated risk and progression of triple negative breast cancer.
OVERLAP: None

1S10OD021753-01A1 Morris (PI) 03/15/17 – 04/14/18 0.0 Cal Mnth
NIH/ Office of the Director $352,381
Triple Quadrupole Mass Spectrometer System
This application replaces heavily used mass spectrometry instrumentation in the University of Kentucky small molecule mass spectrometry facility core.
OVERLAP: None

CX00155-05 No. Morris (MPI) 01/01/17 – 12/31/20 5.0 Cal Mnths
VA BLR&D Merit Review $150,000
Lysophosphatidic acid and cardiovascular disease risk
The goal of this project is to test the hypothesis that association of the bioactive lipid lysophosphatidic acid with atherogenic lipoproteins is a determinant of cardiovascular disease risk.
OVERLAP: None

1P30ES026529-01A1 (Shi) 04/01/16 – 03/31/21 1.2 Cal Mnth
NIH/NIEHS $140,000 (direct cost for the core)
Center for Appalachian Research in Environmental Sciences – Analytical Services Core
The overall goal of this application is to support an integrated core center to increase the efficiency and impact of environmental disease research at the University of Kentucky.
OVERLAP: None

5P42ES007380-20 Hennig (PI) 04/07/97 – 03/31/19 .7 Cal Mnth
NIH/NIEHS $218,921 (direct cost for the core)
Nutrition and Superfund Chemical Toxicity – Research Support Core
The goal of this center is to explore the paradigm that healthy nutrition and exercise can reduce Superfund chemical toxicity through research projects and core service support.
OVERLAP: None

5P42ES0073380-20 Hennig (PI) 04/07/97 – 03/31/19 .35 Cal Mnths
NIH/NIEHS $250,000
Superfund Chemicals, Nutrition, and Endothelial Cell Dysfunction
The goal of this study is to identify mechanisms by which environmental pollutants impair vascular endothelial cell function to promote cardiovascular disease.
OVERLAP: None

5P20GM103527-10 Cassis (PI) 09/08/08 – 07/31/18 .35 Cal Mnths
NIH/NIGMS $100,000 (direct cost for the core)
Center of Research in Obesity and Cardiovascular Disease – Analytical Core
The Center aims to identify mechanisms linking the epidemic of obesity to the high incidence of cardiovascular disease in the obese population and to develop promising junior project investigators.
OVERLAP: None

1P20GM121327-01 03/01/17 – 12/31/21 .6 Cal Mnths
NIH/NIGMS
University of Kentucky Center for Cancer and Metabolism
This award supports a program of training and infrastructure to enable the career development of early stage independent investigators working in the area of cancer and metabolism. I am a mentor for two of these investigators.
OVERLAP: None

5R01HL123358-03 Zhou (PI) 08/01/15 – 05/31/19 0.35 Cal Mnths
NIH/NHLBI $250,000
A Novel Mechanism for ART-Associated Dyslipidemia and Atherosclerosis
The goal is to investigate a novel mechanism linking antiretroviral (ARV) drugs with dyslipidemia and cardiovascular disease.
OVERLAP: None

5R01ES023470-05 Zhou (PI) 09/26/13 – 06/30/18 0.48 Cal Mnths
NIH/NIEHS $225,000
Endocrine Disruptor Mediated Activation of PXR Causes Dyslipidemia
The goal is to investigate a novel mechanism linking endocrine disrupting chemical exposure and hyperlipidemia.
OVERLAP: None

5R01DK107646-03 Kern (PI) 09/21/15 – 07/31/18 0.6 Cal Mnths
NIH/NIDDK $338,141
Cold Induced Changes in Human Subcutaneous White Adipose
The goal of this study is to define the mechanisms and physiological consequences of cold-induced "browning" of while adipose tissue. I will make measurements of lipids and related metabolites for this project. I will make measurements using stable isotope tracers to monitor glucose and fatty acid metabolism for this project. This award provides no support for my personal research program.
OVERLAP: None

5K01CA197073-02 Onono (PI) 07/01/15-06/31/20 0.0 Cal Mnths
NIH/NCI $140,000 total direct costs current year
Intestinal phosphatidylcholine exposure and breast cancer risk
This is a mentored career development award that will enable the recipient to become an independent investigator working on mechanisms that link obesity and cancer risk. I am the primary mentor.
OVERLAP: None

PENDING

1P30GM127211-01 04/01/18 – 03/31/23 1.2 Cal Mnths
NIH/NIGMS $125,000 (direct cost for core)
Center of Research in Obesity and Cardiovascular Disease (COBRE Phase III)
The goal of this phase III program is to further develop and expand upon obesity as an underlying risk factor for the development of cardiovascular diseases, type 2 diabetes, cancer and neurodegenerative diseases by transitioning the research core facilities developed in Phase I and II to independence.
OVERLAP: None

1R01CA231293-01 Spassieva (PI) 04/01/18 – 03/31/23 1.2 Cal Mnths
NIH/NCI $250,000
(PQ12) Deoxysphingolipids and Taxane-induced Peripheral Neuropathy
The goal is to test the hypothesis that the neurotoxic effect of taxanes is mediated by deoxysphingolipids due to serine palmitoyltransferase up regulation.
OVERLAP: None

1R01- 07/01/18 – 06/30/23 1.0 Cal Mnths
NIH $250,000
Biogenesis and Function of Lancefield Group A Carbohydrate Expressed by Streptococcus pyogenes
The goal of this study is to re-examine group A streptococcus structure and characterize its biosynthesis and function.
OVERLAP: None

Smyth, Susan

ACTIVE

5R01HL120507-03 Smyth, Morris (MPIs) 04/01/15 – 03/31/20 2.4 Cal Mnths
NIH/NHLBI $360,204
Lipid Phosphate Phosphatase 3 as a Novel Atherosclerosis Suppressor
The goal of this study is to use pre-clinical models to identify the mechanisms involved in protective effects of lipid phosphate phosphatase 3 against cardiovascular disease with a focus on foam cell formation in atherosclerosis.
OVERLAP: None

BX002769 Smyth (PI) 10/01/11 – 09/30/18 1.5 Cal Mnths
VA Merit Award $150,000
Adipose autotaxin: A Novel Link between Obesity and Cardiovascular Disease
The goal of this study is characterize the role of the lysophospholipase D autotaxin and its product LPA in regulation of adipogenesis and obesity and CV complications.
OVERLAP: None

5UL1TR001998-02 Kern (PI) 08/15/16 – 05/31/20 2.4 Cal Mnths
NIH/NCATS $2,471,209
Kentucky Center for Clinical and Translational Science (CCTS)
The goal of this CTSA is to continue to champion innovation in the full spectrum of clinical and translational science while building the workforce of the future, engaging communities, and advancing the network of multi-site clinical trials.
OVERLAP: None

5P20GM103527-10 Cassis (PI) 09/08/08 – 07/31/18 1.2 Cal Mnths
NIH/NIGMS $356,046 (direct cost for core)
Center of Research in Obesity and Cardiovascular Disease – Administrative Core
This grant establishes a Center of Biomedical Research Excellence in Obesity and Cardiovascular Disease and provides mentoring to junior faculty to facilitate their development as independent investigators.
OVERLAP: None

1P30ES026529-01A1 (Shi) 04/01/16 – 03/31/21 0.6 Cal Mnth
NIH/NIEHS $295,481 (direct cost for the core)
Center for Appalachian Research in Environmental Sciences – Administrative Core
The overall goal of this application is to support an integrated core center to increase the efficiency and impact of environmental disease research at the University of Kentucky.
OVERLAP: None

1R01DA043938-01A1 Stoops (PI) 09/01/2017 – 05/31/22 0.3 Cal Mnths
NIH/NIDA $446,470
Cardiovascular, Immune and Psychosocial Benefits of Reduced Cocaine Use
This study aims to demonstrate that reduced COC use improves physiological and biochemical indicators of cardiovascular and immune fitness, as well as psychosocial function.
OVERLAP: None

5R01HL123927-04 Li (PI) 09/25/14 – 06/30/18 0.6 Cal Mnths
NIH/NHLBI $285,207
Crosstalk between Membrane Traffic Proteins and Integrin Activation
The objective of this project is to identify a novel binding partner that plays an important role in αIIbβ3 outside-in signaling, but also for the first time establish a link between membrane traffic proteins and integrin activation in platelets.
OVERLAP: None

PENDING

1R01HL142640-01 Li (PI) 07/01/18 – 06/30/23 0.6 Cal Mnths
NIH/NHLBI $297,000
Inflammasome activation triggers systemic coagulation in sepsis
The goal of this study is to test the hypothesis that inflammasome is an important trigger of systemic coagulation in sepsis.
OVERLAP: None

1U01HL143508-01 Smyth (MPI) 07/01/18 – 06/30/20 0.7 Cal Mnths
NIH/NHLBI $347,794
PROJECT MISSION: Developing a Multilevel Implementation Strategy for Syncope OptImalCare through engagement
This study will identify barriers and facilitators for implementation of an evidence-based high-value approach to diagnosis and management of patients presenting with syncope.
OVERLAP: None

Charnigo, Richard

ACTIVE

W911NF-17-1-0040 Charnigo (PI) 12/14/16 – 12/13/17 1.24 Acad Mnths
Army Research Office $79,535 0.94 Sum Mnths
Almost-Smooth Nonparametric Regression and Pattern Recognition
The project creates new statistical methodology and provides mathematical justirication for nonparametric estimation of 'almost smooth' mean response functions and related pattern recognition.
OVERLAP: None

596-D77016 Charnigo (PI) 11/01/16 – 03/31/18 .9 Acad Mnths
Veterans Affairs Medical Center $27,335 .1 Sum Mnths
Intergovernmental Personnel Act with Lexington VA for Biostatistical Analysis FY17 - FY18
The goal is to provide biostatistical expertise to the VAMC during the planning and design of experiments and to assist with data interpretation and publication.
OVERLAP: None

1R01DK112136-01A1 Schoenberg (PI) 08/01/17 – 07/31/22 0.54 Acad Mnths
NIH/NIDDK $412,917 0.18 Sum Mnths
Community to Clinic Navigation to Improve Diabetes Outcomes
The project combines an evidence-based intervention to improve diabetes self-management with individually tailored patient navigation to improve appropriate clinical care.
OVERLAP: None

1R01DA043519-01 Bada (PI) 05/01/17 – 02/28/22 0.45 Acad Mnths
NIH/NIDA $277,803 0.15 Sum Mnths
Non-opiate Treatment after Prenatal Opiate Exposure to Prevent Postnatal Injury to the Young Brain (No-POPPY)
The goal is to establish the best pharmacological treatment for Neonatal Abstinence Syndrome (NAS) and determine how treatment of NAS affects long-term developmental outcomes.
OVERLAP: None

1R01HL134731-01 Webb, deBeer (MPIs) 01/01/17 – 12/31/20 0.45 Acad Mnths
NIH/NHLBI $365,055 0.15 Sum Mnths
Serum Amyloid A, Inflammasome Activation, and Abdominal Aortic Aneurysms
The goal is to test the hypothesis that angiotensin II acts at the AT1aR receptor to stimulate adipocyte SAA production to promote AAA development in obese mice.
OVERLAP: None

5R01HL120507-03 Smyth, Morris (MPIs) 04/01/15 – 03/31/19 0.45 Acad Mnths
NIH/NHLBI $360,204 0.15 Sum Mnths
Lipid Phosphate Phosphatase 3 as a Novel Atherosclerosis Suppressor
The goal of this study is to use pre-clinical models to identify the mechanisms involved in protective effects of lipid phosphate phosphatase 3 against cardiovascular disease with a focus on foam cell formation in atherosclerosis.
OVERLAP: None

5R01HL131925-02 Zhou (PI) 04/01/16 – 02/29/20 0.3 Acad Mnths
NIH/NHLBI $250,000 0.2 Sum Mnths
Role of IKKß in Obesity and Atherosclerosis
The goal is to test the hypothesis that IKKß is a critical regulator of adipogenesis and atherogenesis.
OVERLAP: None

5R01HL123358-03 Zhou (PI) 08/01/15 – 05/31/19 0.18 Acad Mnths
NIH/NHLBI $250,000 0.06 Sum Mnths
A Novel Mechanism for ART-Associated Dyslipidemia and Atherosclerosis
The goal is to investigate a novel mechanism linking antiretroviral (ARV) drugs with dyslipidemia and cardiovascular disease.
OVERLAP: None

5R01ES023470-05 Zhou (PI) 09/26/13 – 06/30/18 0.27 Acad Mnths
NIH/NIEHS $225,000 0.09 Sum Mnths
Endocrine Disruptor Mediated Activation of PXR Causes Dyslipidemia
The goal is to investigate a novel mechanism linking endocrine disrupting chemical exposure and hyperlipidemia.
OVERLAP: None

4R01DK100892-04 Graf (PI) 09/20/13 – 08/31/18 0.27 Acad Mnths
NIH/NIDDK No-cost extension 0.09 Sum Mnths
The Role of Hepatic Insulin Resistance on SR-BI Dependent HDL Cholesterol Uptake and Metabolism
The goal is to examine the extent to which impaired insulin signaling alters HDL-mediated reverse cholesterol transport.
OVERLAP: None

5101 CX000975-03 Tannock (PI) 04/01/14 – 03/31/18 0.45 Acad Mnths
VA-ORD $19,954
The Association of SAA with APOB Lipoproteins Affects Cardiovascular Rish
The goal is to test the hypothesis that the shift of SAA from HDL to apoB-containing lipoproteins in insulin resistant conditions such as metabolic syndrome and diabetes contributes to the increased atherosclerosis and cardiovascular diseased observed in these populations.
OVERLAP: None

5101 CX000773-03 Webb (PI) 01/01/14 – 12/31/17 0.45 Acad Mnths
VA-ORD $19,954
HDL Remodeling in Metabolic Syndrome
The project tests the hypothesis that TG enrichment of HDL in MetS predisposes the particle to remodeling by intravascular factors and impedes selective lipid uptake by SR-BI.
OVERLAP: None

PO 6800415 Powell (subaward PI) 02/09/15 – 08/31/18 0.27 Acad Mnths
Geisinger Health System No-cost extension 0.09 Sum Mnths
Exploring the Role of Dyssynchrony in Pediatric Heart Disease with MRI
The goal of this project is to address the problem of pediatric heart failure by adapting a relatively new, highly successful therapy for adult heart failure called cardiac resynchronization therapy into a treatment option for children with heart failure.
OVERLAP: None

5P20GM103527-10 Cassis (PI) 09/08/08 – 07/31/18 0.9 Acad Mnths
NIH/NIGMS $356,046 (direct for core) 0.3 Sum Mnths
Center of Research in Obesity and Cardiovascular Disease COBRE – Administrative Core
The Center aims to identify mechanisms linking the epidemic of obesity to the high incidence of cardiovascular disease in the obese population and to develop promising junior project investigators.
OVERLAP: None

5R01HL073085-13 Cassis (PI)	06/03/03 – 04/30/18	0.27 Acad Mnths
NIH/NHLBI	$256,458	0.09 Sum Mnths

Angiotensin- A link Between Obesity and Hypertension

These studies focus on the renin-angiotensin system of adipocytes as a mediator of obesity-hypertension in males versus females.

OVERLAP: None

PENDING

No ID Charnigo (PI)	04/01/18 – 03/31/20	0.45 Acad Mnths
Childrens Mercy Hospital and Clinics	$22,233	0.15 Sum Mnths

Effects of the Affordable Care Act on Adult Smoking Cessation: A National Study

This study examines the effects of the Affordable Care Act (ACA) on rates of smoking cessation and utilization of tobacco cessation treatments among U.S. adults by analyzing two large nationally representative samples before and after implement of the ACA.

OVERLAP: None

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OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Tracking Number: GRANT12519448 Funding Opportunity Number: PA-16-193 . Received Date: 2017-11-13T09:45:56.000-05:00

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4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator / Change of Institution Section

☐ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

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OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (RESUBMISSION)	Introduction1002093615.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	Candidate_sections_11_10_171002093678.pdf
Research Plan Section	
3. Specific Aims	Specific_aims_revision1002093520.pdf
4. Research Strategy*	Proposal_unlinked1002093680.pdf
5. Progress Report Publication List (for RENEWAL applications only)	
6. Training in the Responsible Conduct of Research	Training_in_the_Responsible_Conduct_of_Research1002093501.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	Combined_Mentor_Statements1002093681.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	Support_letters1002158159.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	DESCRIPTION_OF_INSTITUTIONAL_ENVIRONMENT1002093506.pdf
11. Institutional Commitment to Candidate's Research Career Development	Institutional_Commitment1002093676.pdf
Human Subject Section	
12. Protection of Human Subjects	Non_Human_subject_review_final1002158157.pdf
13. Data Safety Monitoring Plan	
14. Inclusion of Women and Minorities	
15. Inclusion of Children	
Other Research Plan Section	
16. Vertebrate Animals	VERTEBRATE_ANIMALS1002093512.pdf
17. Select Agent Research	SELECT_AGENTS1002093511.pdf
19. Consortium/Contractual Arrangements	
19. Resource Sharing	
20. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Key1002093509.pdf
Appendix	
21. Appendix	

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Citizenship*:

U.S. Citizen or Non-Citizen National?* ☑ Yes ❑ No

If no, select most appropriate Non-U.S. Citizen option

- ❑ With a Permanent U.S. Resident Visa
- ❑ With a Temporary U.S. Visa
- ❑ Not Residing in the U.S.

If with a temporary U.S. visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, also check here: ❑

Tracking Number: GRANT12519448 Funding Opportunity Number: PA-16-193. Received Date: 2017-11-13T09:45:56.000-05:00

INTRODUCTION – Response to Reviewer's Comments (changes in text denoted by line in margin).
We appreciate the instructive comments provided by reviewers of the previous application; their insights have been most helpful in modifying this K99/R00 application. Responses to specific criticisms appear below.
Candidate. *First author publications are slow (Rev 3)* Multiple manuscripts that were in development at the first submission have now been submitted or accepted for publication *including* findings that TMAO is positively associated with dioxin-like pollutant exposure in the ACHS-II cohort after adjustment for covariates known to associate with TMAO and pollutants. I also published a manuscript using Ldlr -/- mice that showed PCB 126-accelerated atherosclerosis in a low fat high cholesterol model that will be used within this revised application. *Limited training… in statistics (Rev 1)* I completed an advanced biostatistics course as a graduate student and since the first submission have helped organize and took part in a day long statistics workshop for trainees of the UK Superfund Research Program. Also, I worked closely with my biostatistics mentor Dr. Charnigo during the data analysis portion of our collaborative manuscript, learning about multiple linear regression modeling. *K99 phase in all three major areas is ambitious and not well articulated (Rev 1)* We agree the submission was overly ambitious and thus have eliminated the population-based nutritional intervention study (Aim 3).
Career Development Plan/Career Goals & Objectives/Plan to Provide Mentoring. *Large number of mentors…(In)adequate frequency of standing meetings with mentor and co-mentors. (Rev 1) Mentoring plan for each of the mentors.. is not clearly defined (Rev 2)* With the revision of Aim 3 we have removed Drs. Moser and Zhang. We have proposed to increase official committee meetings from twice a year to 4 times a year, and clarified how often I will meet with each mentor individually. Also, the co-mentors have worked together to revise the mentoring plan to better show how they will interact as a group and with me. *Metabolomics local academic community seems limited to his mentor's laboratory (Rev 1)* In addition to Dr. Morris, who is an expert in targeted quantitation of small molecules, we have now added a support letter from the Metabolomics group (Resource Center for Stable Isotope-Resolved Metabolomics) at UK. Drs. Moseley, Lane, Fan, and Higashi will provide institutional support. Also, I will take part in their regular seminar series and annual Metabolomics Research Symposium. *Must develop a clear evaluation plan (Rev 2)* We have added a table within the career development plan outlining milestones and have made it clearer how mentors will oversee the progress toward the proposed milestones. We have made it clearer that one of Dr. Morris' primary responsibilities will be to help in manuscript preparation and submission and to assist in the transition from R00 to subsequent R01 submission. *Overly ambitious* (Rev2) The human intervention study was beyond the scope.
Research Plan. Concerns related to (1) overly ambitious aims, (2) lack of details related to what variables will be adjusted for in the multivariate models and what information is known about the diets of the Anniston cohort, (3) unclear how specific branched chain fatty acids are markers of dairy consumption and what other nutrient biomarkers of TMAO will be identified, (4) the use of untargeted metabolite profiling was seen as a positive by some reviewers, but specific details were lacking, (5) a lack of impact of the human benzydamine FMO3 activity study and lack of genotype information, (6) a lack of studies focusing on the gut microbiota, (7) a lack of details related to data analysis (8) focusing on an adenovirus model of atherosclerosis instead of purchasing and breeding Ldlr knockout animals. (1) We now have now reorganized the aims and made them more focused. We have removed the epidemiological intervention study focused on surveying environmental pollutants in a KY population (2) We have adjusted for covariates including race, sex, bmi, and age. We also have access to a diet questionnaire that investigated nutrition patterns related to dairy, red meat, eggs, and fish consumption (3) In our preliminary human study we showed that dairy consumption significantly associated with TMAO (from questionnaire responses) thus we wanted to follow up on this observation using more quantitative markers. We have also included information on other nutrient metabolites we will be investigating. (4) We have included more details related to the targeted and unbiased studies proposed. (5) As suggested by the reviewer we have removed this study from the revised application. (6) We now provide extensive new detail on experimental approaches, especially concerning studies of gut microbiota. We have also provided novel preliminary data showing PCB 126 exposure can decrease gut microbiota diversity in Ldlr -/- mice. (7) We have added new experimental details related to analytical data collection, *in vivo* studies, and statistics. (8) We agree that generating appropriate mouse strains would be valuable. Thus, we will breed Ldlr, FMO3 double knockouts. We have also included new preliminary data related to accelerated atherosclerosis.
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s *Letter from Dr. Biddinger (makes) no commitments and overly ambitious (Revs 1,2)*. A detailed letter is added and we propose one primary and two co-mentors.

CANDIDATE'S BACKGROUND

My interest in environmental health sciences stems from service trips to Appalachia where the impact of environmental stressors on susceptible populations became clear to me. We entered these communities with the major goal of patching roofs, but in talking with the families we learned of the daily struggles the people of Appalachia face. The high incidence of chronic inflammatory disease including CVD and diabetes and the proximity to sources of environmental hazards (e.g., coal burning and locally contaminated rivers) struck me. Beyond these exposures, extreme poverty and poor nutrition are a few of the stressors that I noticed that could increase the risk of developing chronic, non-communicable inflammatory diseases in these communities.

Potential to develop into a successful, independent investigator: My experiences as a highly motivated and independently funded molecular toxicologist, in combination with my current and proposed interdisciplinary training prepares me for a successful independent research career. My development is summarized below:

Pre-Doctoral Experience: My interest in environmental disease led me to major in Biology and Environmental Science at **Muhlenberg College in Allentown, Pennsylvania**. My advisor Dr. Jason Kelsey introduced me to toxicology and I examined the effects of chlorinated pollutants on plants and animals. My first published work showed that interactions between earthworms and plants modulated uptake of the pesticide DDE. I was awarded a summer research grant to utilize mass spectrometry-based metabolomics. This early research strengthened my interest in how the environment can impact living systems. My desire to help susceptible human populations led me to focus my subsequent doctoral work on studies using mammalian systems.

Doctoral Experience: I enrolled at the **University of Kentucky's Graduate Center for Toxicology**, and began working as a Superfund Research Center (UK-SRC) trainee in the laboratory of Dr. Bernhard Hennig, the Director of the UK-SRC. My thesis work focused on mechanisms of polychlorinated biphenyl-induced endothelial cell inflammation and how bioactive nutrients may protect against associated toxicities. I showed that cross-talk between xenobiotic-related proteins was critical for PCB-induced endothelial cell dysfunction and inflammation. During my doctoral training, I published six manuscripts (four first authored) and received multiple travel awards to present our research at national/international conferences. I was also awarded an American Heart Association Fellowship to study metabolites using mass spectrometry based approaches.

Postdoctoral Experience: UK offers a rich and collaborative atmosphere for training in environmental disease research where I could build on the strong relationships I already established while also benefiting from complementary training and career development opportunities. Therefore, I accepted a postdoctoral position with the UK-SRC focused on exploring novel mechanisms of pollutant-induced disease and fostering collaborations between the five SRC projects. As a UK-SRC scholar, I received interdisciplinary training (e.g., stakeholder engagement and scientific translation) by working with community groups and governmental agencies to promote the goals of the SRC. This gave me skills that are generally not available to most benchtop environmental health scientists. I assisted in the organization and implementation of communication workshops, community meetings, and webinars all related to the impacts of stressors like diet and environmental exposures. During my first year, I published one first author manuscript related to my new area of research and also co-authored two other manuscripts related to nutrition and toxicology. I showed that dioxin-like pollutants can upregulate an enzyme (flavin-containing monoxygenase 3 – FMO3) leading to increased production of a diet-related biomarker of CVD. To further advance and broaden my training, I took a visiting scientist position in the lab of Dr. Sudha Biddinger at **Harvard Medical School (BCH)** and helped to identify a novel link between FMO3/TMAO and diabetes and kidney disease (publication in preparation). Although my research experiences to date largely employed cell and animal models, recent advances in analytical technologies (e.g., mass spectrometers with high sensitivity and/or high resolution) are beginning to make studying environmental contributions to human disease risk more feasible. In particular, my studies of FMO3 and its product TMAO in mice exemplify the type of research I would like to pursue independently to illuminate the interplay between diet and environmental exposures as determinants of human disease risk. The K99 phase of this application describes additional training in the collection, analysis, and interpretation of biomarkers and health outcomes data from large population based studies. During my time at UK I met a leading scientist in the field of small molecule/biomarker quantitation, Andrew Morris, who invited me to join the **UK Cardiovascular Research Center** to continue my postdoctoral training. Dr. Morris will serve as the primary mentor for this K99/R00 training. Currently, I am in my second year of postdoctoral studies with Dr. Morris (NRSA funded), focusing on clinically relevant biomarkers in humans that link environmental exposures and metabolic disease. I have obtained and published exciting new data associating elevated circulating

TMAO levels with exposure to dioxin-like pollutants in humans as well as the characterization of a novel mouse model of toxicant-accelerated atherosclerosis. While I have developed mechanistic expertise in cell culture and animal models of inflammation as a toxicologist, with the proposed training, I am committed to adding the skills necessary to address 21st century environmental health problems.

CAREER GOALS AND OBJECTIVES

Current and long-term research and career goals: My long-term goal is to direct an independent research program that employs multidisciplinary approaches to identify and validate mechanisms linking diet and environmental disease. My 5 year research goal is to understand mechanisms that link nutrition and toxicology, which may lead to translatable prevention strategies that limit the impact of environmental determinants of metabolic disorders such as diabetes, obesity, and CVD. My training goal is to build on my experiences and build additional knowledge in analytical chemistry, biostatistics, and cardiovascular medicine so that I will be better positioned to research clinically relevant environmental health issues. While my American Heart Association-funded doctoral training, and current NRSA T32-funded postdoctoral training provided a strong background in mechanistic toxicology, additional training supported through the K99 mechanism would allow me to study how dietary stressors and environmental exposures can interact to modulate cardiometabolic diseases and better prepare me to collaborate with scientists working with human cohorts. This requires my current expertise in molecular toxicology, and proposed training objectives of analytical chemistry, multivariate statistics, and cardiovascular medicine. This is a rare combination of skills, but one that I believe is necessary to holistically approach seemingly intractable health problems. This training will help me to occupy a unique niche within the research community and will be a springboard to a successful independent career transition.

Progression of prior research to K99/R00 research phases: During my graduate studies I developed expertise in cell culture and animal models to study toxicant-induced inflammation. Using molecular and biochemical techniques I studied the cross-talk of multiple signaling pathways including the Aryl hydrocarbon receptor (AhR) and Nrf2. As I graduated and began collaborating with Dr. Sudha Biddinger (**BCH)** I hypothesized that there may be a link between dioxins and TMAO because there is some evidence that FMO3 can be regulated by AhR. I published that Dioxin-like pollutants could strongly increase FMO3 mRNA and protein expression to promote synthesis of TMAO from dietary precursors. Through this manuscript I was able to begin to increase my knowledge in analytical chemistry with Dr. Morris (e.g., targeted quantitation of TMAO via LC-MS). It was critically important to attempt to replicate our findings in a human population. Therefore we have now begun collaborating with the CDC to quantitate TMAO in the Anniston Community Health Survey-II, which is a study with available high quality dioxin measurements. In our first related manuscript I showed that exposure to dioxin-like pollutants was positively associated with TMAO among females (except at high BMIs) but not among males. **Justification of need for further mentored career development:** My strong foundation in xenobiotic-related signaling mechanisms, allows me the ability to make logical hypotheses related to the effects of dioxins on cardiometabolic diseases (e.g., the link between FMO3/TMAO and dioxins), but what has become clear through collaborative interactions during my postdoctoral studies is a need to develop expertise in the complementary approaches of analytical chemistry, multivariate statistics, and cardiovascular medicine if I wish to fulfil my overall career goals. The training component of this proposal is designed to provide a broad foundation in CVD research through a focus on targeted/untargeted metabolomics, statistical analyses of large data sets, and animal models of atherosclerosis. The availability of technologies to monitor multiple environmental exposures (i.e., exposomics) promises to make exposure assessment an important component of approaches to personalized medicine. Accordingly, additional didactic and hands-on experience with big-data and multivariate statistics will make me a more competitive next-generation environmental health scientist. While I have some experience with targeted analyses, I need to extend my capabilities to encompass untargeted approaches used to identify novel biomarkers. I will gain experience with non-biased approaches under the guidance of my primary mentor and collaborators located in The Resource Center for Stable Isotope-Resolved Metabolomics (RC-SIRM) at the University of Kentucky. Finally, a lack of classically trained, mechanistic toxicologists with practical experiences working with large population-based datasets (e.g., Anniston ACHS-II Study) represents a gap in current environmental health sciences. My mentoring team has access to datasets and model systems that will allow me to identify interactions between exposures and nutrition that will form the basis for my independent research.

Description of candidate's current NRSA-funded fellowship program: My current NIH/NHLBI T32 funded

position is designed to be “year 0” of my mentored K99 training. Accordingly, I am focused on using HPLC GC coupled multistage/high resolution mass spectrometry to build on the observations I have made linking diet and environmental exposure to circulating biomarker signatures.

Plans to separate scientifically from mentor and advance to research independence: My long-term goal is to direct an independent research program that employs multidisciplinary approaches to identify and validate mechanisms linking diet and environmental disease. This is unique from the focuses of all my mentors and collaborators. Dr. Morris is an expert in developing/applying mass spectrometry methods to quantify biological molecules. While Dr. Morris is interested in understanding how diet impacts human disease, my focus on chemical exposures is unique and provides a basis to develop full independence. Drs. Smyth and Charnigo are interested in studying genetic determinants of CVD, but have not focused on the impact of chemical exposures. During the K99 phase the research described below will allow me to work with Dr. Morris to refine the training plan for the R00 phase of the award. I expect that the R00 portion will provide me with the time and resources necessary to identify conceptually similar relationships between diet and chemical stressors that would then form the basis for future funding applications (e.g., the TMAO/dioxin association as framework). I will begin applying for tenure track faculty positions between the first and second years of K99 training. In the event that I remain at UK, this research would be conducted independently from my mentors, but I would take advantage of infrastructure/support provided by the programmatic awards and resources available.

CANDIDATE’S PLAN FOR CAREER DEVELOPMENT/TRAINING ACTIVITIES DURING AWARD PERIOD

Proposed career development activities: Through my two years of proposed training (K99 phase) I will take part in both didactic and hands on mentoring activities aimed at increasing my capabilities in three areas: 1) Analytical chemistry/mass spectrometry, 2) biostatistical analyses of large data sets, and 3) preclinical models of cardiovascular disease. The knowledge gained will complement my previous experiences to shape an independent research career in mechanistic experimental studies with human epidemiological investigations.

Objective 1: Develop expertise in analytical chemistry with a focus on quantitating biomarkers of pollutant exposure and diet using HPLC and GC coupled high resolution mass spectrometry. During my K99 training, I will work closely with Dr. Morris to gain experience in analytical chemistry that will provide opportunities for training in data analysis and statistics. Specifically, as explained within the proposal, this will include: 1) Quantitation of biomarkers of diet which are known to significantly associate with TMAO (e.g., dairy and eggs), and 2) Use of untargeted GC and HPLC coupled multistage/high resolution mass spectrometry to identify novel metabolite associations with TMAO and pollutant exposure. To accomplish this objective I will gain proficiency with instrumentation and data analysis software through multiple hands-on and didactic training mechanisms. I will receive training on instrumentation from ABSciex, Agilent and Thermo Fisher. I will become proficient in the upkeep and operation of these systems while also gaining experience in instrument control and data acquisition software. I have experience using triple quadrupole instruments for targeted quantitation of small molecules but I will extend my training to use high resolution (time of flight or orbitrap) instruments to generate untargeted information for unbiased identification of biomarkers. Analysis of the data from these instruments will require the use of proprietary and open source software/databases data dependent acquisition workflows. I will initially extract and analyze samples from the Anniston Community Health Survey-II. These studies will act as a vehicle for hands on training, but also will directly lead to manuscript publication. As recommended by Dr. Morris, I will complete formal **didactic training** at Agilent Instrumentation’s Agilent University, which involves in-depth workshops. These trainings focus on cutting edge instrumentation and software packages and also provide a means to cultivate important industry/academic relationships that will be pivotal during my independent career. To gain expertise in working with metabolomics I will first attend the “Mass Profiler Professional (MPP) Workshop” which focuses on a state of the art platform that enables differential sample analysis including integrated identification and annotation of compounds. My mentors will decide whether attending workshops provided by Agilent, the manufacturers of our other instrumentation (e.g.,Thermo- Compound Discoverer) or widely used xcms platform would be beneficial. I am also fortunate that UK houses one of six NIH Regional Comprehensive Metabolomics Resource Centers. Our center provides state of the art instrumentation and data analysis capabilities to enable systems biochemistry research. As detailed in the letter of support from the training director of this Center, Theresa Fan, I will participate in their annual 12 day workshop that focusses on global metabolomics and also take part/present in the Metabolomics monthly seminar series. Finally, our collaboration with the CDC (ACHS-II) presents a unique **hands-on**

training opportunity in quantitation of pollutants from large cohorts which is challenging to accomplish in an academic laboratory. I will visit our CDC collaborators to learn about analysis of pollutants in plasma (2018). **Objective 2: Obtain formal, in-depth training in multivariate biostatistics and informatics.** Dr. Richard Charnigo (Director of Biostatistics- UK). I will receive training in statistical packages (e.g., SAS, R, etc.) to determine biomarkers of pollutant exposure, diet, and disease risk. Gaining experience in informatics and biostatistics will prepare me to more effectively process large population-based data sets in the future, which will grow in importance as more expansive "exposomic" data sets that integrate healthcare records and pollutant exposures become available. I will complete the following UK **graduate level courses**, as recommended by the mentoring team during the first year of my training: 1) CPH535 (SAS programming), 2) CPH630 (Biostatistics II), 3) BMI 730 (Principles of Clinical Informatics). As I become more comfortable with multivariate statistics, I will utilize the large ACHS-II dataset to gain **hands on training** during year 2 by building multivariate models investigating associations between dioxins, TMAO, and covariates. **Objective 3: Receive training in clinical, translational, and experimental CVD research.** Working with Co-Mentor Dr. Susan Smyth (Physician Investigator) will help me to develop an independent research career based on combining clinical and preclinical CVD studies. During the second year of my training (K99 phase) I will complete the UK Center for Clinical and Translational Sciences' (CCTS) **Certificate in Clinical and Translational Science**. I will attend the CCTS' seminar series and annual research conference. Classes are offered during fall and spring: BSC 731(Methods and Technologies in CCTS); BSC 732 (Interdisciplinary Protocol Development); BSC 733 (Seminar in Clinical and Translational Science); BSC 534 (Ethics and Responsibility in Clinical Research); BSC 772 (Fundamentals of Biostatistics for Clinical and Translational Research); BSC 790 (Research Practicum). To better prepare for future human studies, I will take part in the **Collaborative Institutional Training Initiative** (CITI Program) IRB training. This is available free to all UK researchers and has multiple modules that will be chosen by the mentors. Dr. Smyth is also an expert in mouse models of atherosclerosis and **will provide training** in multiple assays related to lesion development. **Justification of need for mentored phase:** The mentored training program described above will uniquely equip me to identify novel biomarkers of pollutant exposure and/or nutrition (e.g., TMAO), and to develop testable mechanistic hypotheses inking pollutant exposure, diet, and cardiometabolic diseases. Increasing my skillset to include stronger knowledge of analytical chemistry and multivariate statistics will make collaborating and publishing with a diverse set of environmental health scientists more obtainable. These added areas of emphasis will allow me to grow and diversify my research portfolio to include a stronger human component. **Evaluation Plan**: I plan to spend two years in the K99 phase; at this point I would have completed 5 years of postdoctoral studies. I will present my research at least twice a year; in the winter (SRP Meeting) and in the summer (Dioxin) as well as at least 4 times a year in lab meetings. My office is adjacent to Dr. Morris' which cultivates efficient mentorship and scientific discussion. I will meet with collaborators in person or via skype as needed. Every 3 months I will organize committee meetings with my entire mentorship team. My committee will evaluate my **training milestones** (table 1) by examining my academic achievement (coursework performance) in combination with scientific success (e.g., research progress, manuscripts). Each proposed study's main goal is to increase my first-author publication record, but certain studies are highlighted for their training and thus will be completed during the K99 phase. I expect to submit one first-author manuscript related to Aim 1, one related to the ACHS-II studies of Aim 3, and prepare for the larger animal studies that occur within the R00. **Plan for transition to independent phase:** I will transition into the independent phase of the award after two years in the mentored phase (approximately Fall 2020). I have obtained a support letter from the Dean of the Medicine outlining resources available to me and UK's strong support of my career advancement. I have also received letters from the directors of the UK-SRC and P30 Environmental Health Sciences Core Center outlining the availability of resources as well as continued assistance with career advancement. After applying for and obtaining a tenure track faculty position at a top-tier research university, I will continue my research progress (R00) while recruiting, teaching, and mentoring lab members. During my transitional phase, Dr. Morris and I will continue to communicate regularly to ensure progress of my work and to assist in preparations for submission of an R01 in the final year of my R00 phase. I will also continue to meet annually with my advising team virtually or in person. Dr. Morris will mediate successful manuscript submissions throughout this current proposed funding cycle as well as ensuring a successful future R01 submission. We expect at least one first author manuscript to be submitted each year during the proposed funding cycle.

SPECIFIC AIMS

Dioxin-like pollutants (DLPs) persist in the environment and, because of their bioaccumulation in adipose tissue can be detected in the blood of most individuals. Exposure to these pollutants causes diabetes and its complications of obesity and cardiovascular disease in animal models. These observations can likely be translated to humans because several large longitudinal epidemiological studies have associated serum levels of these pollutants, for example polychlorinated biphenyls (PCBs) with an increased risk of cardiovascular disease and type 2 diabetes. The variability in inter-individual responses to increased body burdens of these pollutants observed in these epidemiological studies can likely be explained by the additional contributions of genetic and other environmental risk factors, the most powerful of which is the diet. Understanding how overall disease risk is determined by interactions between diet and environmental exposures would lead to new strategies to identify, manage and treat at-risk individuals. Accordingly, the identification of relevant environmental exposure biomarkers would enable the identification of at risk-individuals and the definition of mechanisms linking environmental exposures to disease processes could lead to interventions to mitigate risk. Here, we propose to investigate a mechanism we discovered that links diet and exposure to DLPs to cardiovascular diseases. Metabolomic studies in humans previously associated increased plasma levels of diet-derived trimethylamine-N-oxide (TMAO) with coronary artery disease. TMAO's precursor, trimethylamine (TMA) is generated from dietary methylamines (e.g., choline and carnitine abundantly found in diets rich in meat and dairy products) by the intestinal microbiota. TMA is then oxidized to TMAO by hepatic Flavin-containing monooxygenases, predominantly the FMO3 isoform. While TMAO can accelerate atherosclerosis in mouse models, more recent studies identify additional roles for FMO3 as a regulator of insulin sensitivity and hepatic lipid metabolism. Recent work has shown that inactivation of the FMO3 gene in mice is strongly protective against diabetes and cardiovascular disease. FMO3 and its product TMAO are therefore exciting new targets for the diagnosis and treatment of diabetes and CVD. Our published and preliminary data show that exposure to DLPs (1) strongly increases FMO3 expression in the liver; (2) dramatically increases formation of TMAO from dietary sources; and (3) positively associates with circulating TMAO in an exposed human population. These observations lead us to propose our overarching hypothesis that induction of FMO3 expression is a mechanism linking DLP exposure to cardiometablic diseases and that circulating TMAO levels are a biomarker of DLP exposure in humans. We will test this hypothesis in the following aims.

Aim 1: To test the hypothesis that a diet high in TMAO precursors can exacerbate DLP-induced cardiometabolic disease *in vivo* (K99-R00). We will feed Ldlr -/- mice control diet, high choline, or high L-carnitine diets, treat with PCB 126 and monitor the formation of TMAO, inflammation, and progression of atherosclerosis. We hypothesize that mice fed the experimental diets will show accelerated atherosclerosis and exposure to PCB 126 will exacerbate these effects.

Aim 2: To test the hypothesis that FMO3 and/or gut microbiota are required for DLP-induced cardiometabolic disease *in vivo* (K99-R00). We will breed Ldlr:FMO3 double knockout mice, treat with PCB 126, and monitor the progression of inflammation and atherosclerosis. Separately, we will treat Ldlr -/- mice with broad spectrum antibiotics and examine the effects on PCB 126-initatied inflammation and atherosclerosis. We hypothesize that both models will prevent the formation of TMAO, but loss of FMO3 will be more effective at protecting against the pro-atherogenic effects of dioxins. DLPs upregulate FMO3 and this increased expression may help drive PCB 126-induced cardiometabolic dysfunction through mechanisms that are at least partially TMAO independent.

Aim 3: To test the hypothesis that elevated TMAO levels in DLP exposed individuals result from increased FMO3 activity/expression (K99-R00). Using targeted and untargeted mass spectrometry, we will identify associations between pollutant exposure, CVD risk, and FMO3 substrates/products. We hypothesize that dioxin exposure will be a significant source of inter-individual variability of TMAO.

Completion of these studies will generate important new information about a plausible mechanism that could link environmental exposure to a prevalent persistent pollutant with metabolic and cardiovascular disease risk. Also, these studies are designed as a vehicle for advanced training in analytical chemistry, multivariate statistics, and cardiovascular medicine, and will act as a framework for future hypothesis driven investigations. Proof of our hypotheses would have wide-ranging ramifications for future diagnosis and treatment of pollutant-induced disease. In particular, they would suggest dietary or pharmacological interventions to mitigate the effects of toxicants and identify TMAO as a novel biomarker for pollutant body burden.

SIGNIFICANCE

Genetic, behavioral and environmental factors are major determinants of chronic human disease risk. While the application of large scale genotyping efforts has generated a wealth of information about genetic risk determinants, far less is known about the behavioral and environmental contribution to metabolic diseases, even though this has been estimated to account for as much if not more inter-individual disease risk variability as heritable factors (1). In large part, this is due to challenges in quantitatively monitoring cumulative human environmental exposures, and the use of cell and animal models to discover mechanisms of toxicity that may not extrapolate to human populations (2,3). The most profound behavioral risk factor is diet but again a lack of quantitative information about inter-individual variability in diet composition hampers efforts to understand the association between diet and disease(3). Notwithstanding these considerations, several large epidemiological studies associate both exposure to environmental pollutants and poor dietary habits with increased risk of metabolic and CVDs (4, 5). Emerging experimental evidence now implicates important interactions between dietary and pollutant stressors and accordingly, a major challenge for future environmental health scientists is the identification of credible mechanisms that link environmental exposures and diet to human disease risk (6, 7). We have identified an association between human exposure to important classes of environmental pollutants (dioxin-like pollutants; **DLPs**) and plasma levels of a well-validated biomarker of human cardiovascular and metabolic disease risk (8). We determined, in mice, that exposure to dioxin-like polychlorinated biphenyls increases expression of flavin-containing monooxygenase 3 (FMO3), which drives the production of trimethylamine-N-oxide (**TMAO**) after dietary consumption of methylamine containing precursors. Interestingly, diets from animal sources (e.g., red meat, eggs and dairy) contain the highest levels of these TMAO precursors and are predominant staples of current American diets. In preclinical models, both elevated TMAO and increases in FMO3 promote atherosclerosis and insulin resistance. Our findings support the concept that diet can modulate the toxicity of environmental pollutants. We have recently generated evidence for this association between DLPs and TMAO in a well validated cohort of highly exposed individuals (Anniston, Alabama ACHS-II study (9)-cross sectional cohort for which extensive measurements of multiple DLPs were made by the CDC analytical chemistry laboratory). We propose that continued investigation into the impact of pollutant exposures on the production of this biomarker from dietary precursors will generate new information of relevance to environmental health.

INNOVATION

Our discovery of a mechanism linking environmental pollutants to production of the coronary artery disease risk biomarker TMAO in animal models is, to our knowledge, original. Our new data suggesting that these observations can be translated to humans is similarly novel (*manuscript under review)*. The analytical methods we propose to use in our study are state of the art. Although the proposed metabolomics methodologies are not particularly innovative, our adaptation of these methods to enable monitoring and discovery of additional associations with pollutant exposure and TMAO is an important new avenue for the science of environmental health. Also, the use of low density lipoprotein receptor (Ldlr) deficient mice fed a low-fat atherogenic diet to increase bioavailability of lipophilic environmental chemicals is an original approach for preclinical risk assessment models within environmental health, and the proposed genetic crosses will be a useful shared resource for those investigating mechanisms of pollutant-accelerated atherosclerosis. Overall, the high significance, and conceptual/technical innovation associated with this study should result in important original findings with substantial impact in the fields of environmental health, nutrition, and cardiovascular medicine.

Scientific Premise: **Preliminary data in support of Aims**

AhR activation leads to increased FMO3 expression resulting in increased TMAO formation from dietary precursors. TMAO is a diet-derived biomarker of CVD whose formation is dependent on enzymatic oxidation via FMO3 (10). Previously, it has been shown that DLPs can induce FMO3 expression, but this increase in mRNA is not evident in AhR deficient mice (11). FMO3 has recently been shown to have important signaling roles in diabetes and atherosclerosis (12) so we hypothesized that DLP-induced CVD and diabetes could be attributed to increased FMO3/TMAO. We found using male mice and a model DLP, that PCB 126 strongly upregulates FMO3 mRNA/protein and increases TMAO (8). Although this work was well controlled and with sufficient sample size to observe significant differences, only male mice were used which is a drawback and requires further investigation using both sexes (as proposed within this grant).

FMO3 is a central regulator of insulin signaling, cholesterol homeostasis, and atherosclerosis.

Downregulation of FMO3 can prevent diet-induced atherosclerosis and metabolic disease through modulation

of cholesterol absorption/trafficking and insulin signaling (12, 13). Since exposure to DLPs has been shown in epidemiological and preclinical studies to associate with elevated cholesterol levels, decreased glucose sensitivity, and increased risk of CVD we hypothesize that FMO3 has a key role in these etiologies (5).

Exposure to dioxin-like pollutants is positively associated with circulating TMAO in humans. In collaboration with the CDC and NIH we have determined, in the Anniston, AL ACHS-II cohort, that higher body burden of multiple DLPs (e.g., coplanar PCBs, PCDDs, and PCDFs) significantly associates with higher plasma TMAO (Pearson correlation coefficients shown in **Table 2** using dioxin toxicity equivalents). We next determined, via multivariate linear regression models that even after adjustment for multiple covariates known to alter inter-individual variability in TMAO (e.g., kidney disease, sex, BMI, diabetes status, and race), exposure to DLPs remains significantly associated with TMAO. Interestingly, these associations are more prominent in leaner females (*under review*). Although this work with the ACHS-II cohort was well blinded and had sufficient sample size (n=340), a lack of quantitative measurements of CVD (e.g., lesion size) and diet are a drawback. Within this proposal we will quantitate biomarkers of diet including choline, L-carnitine, and fatty acids found in dairy and fish which, when added in to our multivariate models, will provide stronger evidence to support the hypothesis that TMAO is independently associated with DLP exposure. We have now begun to develop untargeted high resolution methods to discover new metabolites that associate with DLP exposure and which can be examined in future R01 applications (**Figure 1** depicts levels of p-Cresylsulfate in 6 ACHS-II samples).

Methodologies to quantitate biomarkers of diet critical for TMAO formation. It is well established that dietary intake of methylamine-containing foods such as those found in dairy, red meat, eggs, and fish are strongly associated with TMAO formation (14) and that many of these same foods may have higher concentrations of certain lipophilic pollutants. Unfortunately, in the ACHS-II study, only questionnaire information related to diet is available; with a survey relating primarily to local consumption of certain foods. Therefore, for this K99/R00 application we have begun to create robust methodologies to quantitate validated biomarkers of diet including fatty acids specific to bovine digestion of plant materials (e.g. odd and branched chain fatty acids), choline, fish-derived fatty acids (e.g., DHA), and L-carnitine (will measure in ACHS-II) (15)**.**

Dioxin-like PCBs modulate CVD risk factors and accelerate atherosclerosis. Although it is well established that dioxin-like PCBs can promote inflammation (especially hepatic and adipocyte), data are lacking that link DLPs to accelerated atherosclerosis and increased lesion formation. One possible explanation for this is that one of the most common mouse models of atherosclerosis, the Ldlr $^{-/-}$ mouse, is normally fed high fat/high cholesterol diets (e.g., Western Diet) to promote disease phenotypes. *The hyperlipidemia and adipose tissue expansion that is normally associated with these diets is known to limit the bioavailability of lipophilic chemicals to key tissues related to CVD (16).* Similarly, in our ACHS-II studies associations between TMAO and DLPs are evident in leaner individuals. To address these limitations, we examined the effect of a DLP, PCB126, on the development of atherosclerosis in genetically hyperlipidemic Ldl receptor knockout male mice fed a previously described **low-fat** atherogenic diet. PCB 126 robustly accelerated the development of atherosclerosis and hepatic steatosis in these animals through mechanisms that involve increased inflammation and effects on blood and vascular cells (**Figure 2-3;** *Toxicol Sciences-revisions submitted)*. This novel model will be utilized for Aims 1,2 of this proposal to study the role of FMO3/TMAO in DLP-associated acceleration of atherosclerosis and related pathologies. In our published work we observed an increase in pro-inflammatory mediators such as macrophages, thus we have now begun *in vitro* studies investigating the effects of PCB 126 on human-derived monocytes/macrophages and have determined that DLPs elicit macrophage inflammation and increase markers of the Type1, pro-inflammatory state (**Figure 4).**

Exposure to DLPs can modulate the gut microbiota profile of Ldlr $^{-/-}$ mice. It is not yet well established if TMAO by itself is a causative mediator of CVD, or rather a biomarker of FMO3 activity or of sub-optimal gut health. Our focus thus far has been on FMO3's role in these processes, but emerging evidence now links exposure to DLPs and alterations in gut microbiota populations. In a preliminary study using our low-fat fed mouse model using 16S ribosomal RNA sequencing and informatics, we determined that Ldlr $^{-/-}$ mice exposed to PCB 126 developed a shift in gut microbial populations at the phyla and genus levels, decreased alpha diversity, and increased Firmicutes:Bacteroidetes ratio; which is associated with chronic inflammatory diseases (**Figure 5**; *Manuscript submitted)*. Studies within Aim 2 using antibiotics will attempt to elucidate if these observed changes in gut microbiota are necessary or sufficient for DLP-associated cardiometabolic disease.

Measuring individual variability in FMO3 activity and TMAO formation from diet. Our efforts to

investigate the relationship between TMAO levels and environmental pollutant exposures were prompted by observations that PCB 126 increased hepatic FMO3 expression in mice. However, while much less is known about regulation of human FMO3 expression (and much of this comes from studies with cell lines) it is likely that this process is different between humans and rodents and it is also clear that in addition to FMO3 expression/activity, plasma TMAO levels are determined by other factors including the diet(10). Therefore, we have begun to establish and validate methods to monitor FMO3 activity directly in humans by measuring circulating ratios of FMO3 substrates and their products. FMO3 catalyzes the broad specificity N-and S-oxidation of many xenobiotics including nicotine, caffeine and many widely used over the counter prescription drugs (e.g., antihistamines). Targeted/untargeted measurements of FMO3 substrates and products would enable analysis of their associations with TMAO and DLP exposures (17) and will help to provide direct evidence if the observed levels of TMAO in ACHS-II are due to FMO3 activity/expression or another factor.

APPROACH (Please see Table 1 for study timeline)

Specific Aim 1: To test the hypothesis that a diet high in TMAO precursors can exacerbate dioxin-induced cardiometabolic disease *in vivo*. We will feed Ldlr $^{-/-}$ mice control diet, high choline, or high L-carnitine diets, treat with PCB 126 and monitor the formation of TMAO, progression of inflammation, and atherosclerosis **(K99-R00).**

Rationale: The *overall goal* of this aim is to determine the extent that diets high in TMAO precursors can exacerbate DLP-accelerated atherosclerosis in mice. Multiple groups have shown associations between TMAO and inflammatory diseases such as coronary artery disease and diabetes, but more work is needed to elucidate if TMAO itself is causatively linked to inflammation and disease, and if there are exacerbating effects

Table 1: Overview of research design, timeline, and training milestones

Year	Title	Study	Experimental system	Design	Assays/endpoints	Training Milestones/Evaluation
1-5	Interactions between Dioxins and TMAO in mice and cells	1.1 1.2	LDLr $^{-/-}$ mice HUVEC, THP-1	Male and Female +/- PCB 126 +/- TMAO producing diets siRNA studies	-Accelerated atherosclerosis assays, Inflammation assays, TMAO/metabolite quantitation in plasma and urine -qPCR, Western Blots, Magpix, etc.	K99 Phase: **Smyth:** Aortic Root isolation, sectioning, and staining, blood pressures. **Morris:** Plasma and urine extraction of TMAO and proficiency using ABSciex 6500
1-breeding 3-4 Studies	FMO3, gut microbes and acute dioxins exposure	2.1	Wild-type and FMO3 $^{-/-}$ mice -Sacrifice 2 days-2 weeks post PCB	Male and Female +/- PCB 126 +/- antibiotics n=10 per group	-Inflammation – plasma cytokines, liver markers of toxicity -TMAO quantitation -GTT, ITT	**Smyth:** Breeding and organization of mouse colony **Morris:** TMAO quantitation using ABSciex 6500
3-5	Atherosclerosis study	2.2	LDLr $^{-/-}$ mice X FMO3 $^{-/-}$ mice	Male and Female +/- PCB 126 10 week study n=10 per group	-Accelerated atherosclerosis – en face, aorta staining, cholesterol, TMAO. -Inflammation–cytokines, Isoprostanes, GTT, glucose/lipid signaling, etc.	-Oral/Poster presentations -Manuscript submission/acceptances
1-2	Diet, TMAO, and dioxins in ACHS-II Other FMO3 substrates	3.1 3.2	Stored serum samples from ACHS-II (de-identified)	Quantitate diet and drug biomarkers and examine associations with dioxins and TMAO	-Quantitate fatty acids, choline, L-carnitine, nicotine, etc. and use multivariate linear regression modeling to determine if associations with TMAO and dioxins	**Morris:** Proficiency in using the AB Sciex 6500-QTRAP and Agilent 7000C Triple Quadrupole GC/MS System **Charnigo:** Proficiency in stats packages (SPS, JMP)
3-5	Untargeted metabolomics	3.3	Stored serum samples from ACHS-II (de-identified)	Unbiased global metabolomics	Relative levels of amino acids, organic acids, phenolics, etc. in conjunction with biomarker discovery/statistics software packages	**Morris:** Proficiency in using the Thermo QExactive LC/MS **Charnigo and UK SIRM Core:** Proficiency in integrated metabolomics/statistical software

in the presence of dioxins. We will use PCB 126 as a model DLP and our novel low-fat fed ldlr $^{-/-}$ atherogenic mouse model. Our previously published works clearly show that exposure to dioxins can increase inflammation, decrease glucose sensitivity, and increase susceptibility to atherosclerosis in multiple mouse models (18, 19). In our most recent *published data* we examined the effect of PCB 126 on the development of atherosclerosis in Ldlr knockout mice fed a previously described **low-fat** atherogenic diet. Normally, atherosclerosis is promoted in this mouse strain by using high fat/high cholesterol diets, which may increase adipose expansion and subsequently limit bioavailability of lipophilic pollutants (e.g., adipose sequesters lipophilic toxicants). We *hypothesize* that ldlr $^{-/-}$ mice fed the choline or L-carnitine diets will show accelerated atherosclerosis compared to controls, and exposure to PCB 126 will lead to an exacerbated phenotype. To test this hypothesis we will feed ldlr knockout mice diets enriched with choline or L-carnitine, administer PCB 126 and monitor inflammatory, metabolic dysfunction, and atherosclerotic parameters. We *expect* that PCB 126 exposure will increase TMAO production leading to accelerated atherosclerosis in mice. In addition, to examine mechanisms linking increased TMAO production to increased inflammation, human monocytes and endothelial

cells, two cell types involved in CVD, will be treated with TMAO alone or in combination with DLPs and impacts on inflammatory pathways will be examined. Such results would provide evidence for a novel mechanism linking diet and toxicant exposure to inflammatory disorders and would lay the groundwork for future prevention strategies (e.g., dietary/pharmacological manipulations) aimed at limiting the toxic effects of DLPs.

Experimental approach:

Question 1: Does a diet high in TMAO precursors exacerbate PCB-accelerated cardiometabolic disease

Study 1.1: Interactions between diet and toxicant exposure (K99). It is well established that lifestyle-dependent choices such as diet, play a major role in interindividual suceptibility to cardiovascular and other chronic diseases, but emerging data now implicate interactive or exacerbating effects between dietary and chemical stressors. Our preliminary data support the hypothesis that mice exposed to DLPs and concomitantly fed a diet high in TMAO precurors (e.g., L-carnitine, choline) will produce more TMAO and subsequently be more prone to atherosclerosis. We will breed male and female LDLr $^{-/-}$ mice in house and feed the same proatherogenic diet as in my most recent *Toxicol Sci* study (base atherogenic diet with milk based fat). Additional groups will include the base diet supplemented with 1.0% choline and base diet administered 1.5% L-carnitine in the drinking water. Others have shown that these dietary manipulations will increase TMAO and acclerate atherosclerosis (20). Within these dietary groups, mice will be evently distributed to receive either PCB 126 (1 µmol/kg) or vehicle safflower oil at weeks 2, and 4 of a 10 week study (n=10 per group; total of 60 male and 60 female). Blood will be collected via submandibular bleed at weeks 2, 4, and 8 to explore continuous variables of inflammation and atherosclerosis, such as plasma levels of inflammatory cytokines (MAGPIX), triglycerides, TMAO, and cholesterol levels (FPLC). Using metabolic cages, we will also collect urine for isoprostane (marker of oxidative stress) and TMAO, choline, betaine, and L-carnitine analyses. We will monitor body weight weekly and fat/lean mass will be quantified by EchoMRI at week 10. We will determine blood pressures (Kent Coda 8 system; week 10). At completion, blood, plasma, and tissues will be harvested (heart, aorta, liver, fat depots- epididymal, retroperitoneal and subcutaneous) for inflammatory marker investigation by, 1) mRNA extraction, 2) protein extraction, 3) quantification of cytokines by MAGPIX,4) immunostaining of aortas (i.e. VCAM-1), 5) lipoprotein levels and 6) circulating mediators of inflammation by flow cytometry. Atherosclerotic endpoints will include;1) oil red-O stained aortic roots,2) en-face,3) MΦ infiltration by staining of sectioned/fixed tissues.

Table 2. Bivariate associations of dioxin-like pollutant exposures with TMAO

TEQ index of pollutant exposure	Pearson correlation	p-value
TEQ Mixtures		
sum of non-ortho PCBs TEQ	0.157	**0.004**
sum of PCDDs TEQ	0.254	**<.001**
Sum of PCDDs, PCDFs, and coplanar PCBs TEQ	0.215	**<.001**
sum of PCDFs TEQ	0.214	**<.001**
sum of mono-ortho PCBs TEQ	0.174	**0.001**
Total dioxins TEQ	0.207	**<.001**

Question 2: Is TMAO pro-inflammatory by itself or in the presence of dioxin-like pollutants?

Study 1.2: TMAO as a causitive mediator of inflammation (R00). There is some evidence that TMAO can promote inflammation in cell types related to atherosclerosis, but more work needs to be done to elucidate mechanims as well as possible synergistic interactions between pro-inflammatory pollutants and TMAO which circulate in blood together. Our preliminary data support the hypothesis that both TMAO and PCB 126 may elicit NFκB-mediated inflammatory cascades in endothelial (6) and blood cells (**Figure 4**). For this study we will treat endothelial cells (HUVEC) and macrophages (matured THP-1) cells with phsyiologically-relevant concentrations of TMAO (up to100 µM), PCB 126 (10 nM-1 µM), or in combination. Endothelial cell dysfunction markers to be examined include increased adhesion molecule expression (VCAM-1), cytokine production (MCP-1), and increased cellular oxidative stress. Treated cells will be harvested for (1) mRNA extraction with TRIzol reagent, (2) protein extraction, (3) quantification of ROS (superoxide staining)

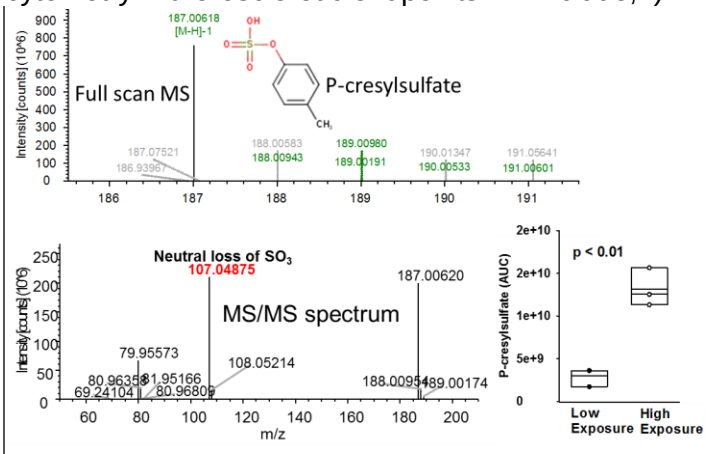

Fig. 1. Proof of concept using untargeted methods to identify novel metabolites that differ between high and low dioxin exposure. 6 ACHS-II (3 high exposure and 3 low exposure) samples were extracted and metabolites were identified using a Full scan with data-dependent MS/MS. P-Cresylsulfate was identified using CompoundDiscover 2.0 (Thermo QExactive).

and (4) quantification of secreted cytokines by Magpix (n = 3 independent replicates for each assay and measurement). For studies involving monocytes, we will examine markers of macrophage polarization (i.e.Type 1 or Type 2) and inflammation (Protein and mRNA levels of TNFα, IL-1β, IL-6, and Arg-1). We will contrast concentration-dependent effects of TMAO and the proposed mixture to identify possible synergistic effects of PCB and TMAO exposure. Follow-up studies will utilize custom made siRNAs (e.g., NFκB) or control scrambled siRNA (transfected via Gene Silencer (Genlantis)). Cells will be exposed to Vehicle DMSO, TMAO, PCB 126, or TMAO+PCB 126 at concentrations determined from the previous experiemnts. Determination of NFκB protein and mRNA will be used to verify successful manipulation. Importantly, it is not yet well estabilished if FMO3 has proinflammatory roles in endothelial cells or macrophages. It is plausible to hypothesize that if TMAO is itself pro-inflammatory, then a feed forward loop ultimately increasing FMO3 expression may exist. To examine this in our available cell systems, FMO3 will also be silenced in separate experiments and the effect of TMAO and/or PCB 126 will be examined as desceobed earlier.

Expected/alternative approaches: Based on our preliminary evidence, we expect PCB exposure will accelerate atherosclerosis in our mouse models as evidenced by increased aortic plaque size and en face lesion area. We also expect that mice fed the diets enriched in TMAO precursors and subsequently treated with PCB 126 will show exacerbated atherosclerosis and inflammation. In our cell culture studies we expect that both TMAO and PCB 126 will elicit inflammatory reactions in endothelial cells and monocyte/ macrophages, and concomitant exposures will result in additive or synergistic toxic effects. We do not expect any technical issues related to the siRNA studies due to past experience using the cell systems, but as an orthogonal approach we can isolate cells from FMO3 or NFκB deficient mice or use chemical inhibitors. Although we believe induction of inflammatory mediators is predominately via an NFκB-mediated mechanism, if we do not see protection in our proposed siRNA studies we will investigate other possibilities (e.g., AhR).

Specific Aim 2: To test the hypothesis that FMO3 and/or gut microbiota are required for dioxin-induced cardiometabolic disease *in vivo*. We will breed Ldlr:FMO3 double knockout mice, treat with PCB 126, and monitor the progression of inflammation and atherosclerosis. Separately, we will treat Ldlr $^{-/-}$ mice with broad spectrum antibiotics and examine the effects on PCB 126-initatied inflammation and atherosclerosis **(K99-R00)**

Rationale: The *overall goal* of this aim is to determine the extent that FMO3 modulates dioxin-induced cardiovascular and metabolic dysfunction. Our most recent *preliminary data* implicate an important role for the FMO3-TMAO pathway in these pathological processes, but more work needs to be completed to elucidate if these effects are due to increased expression of FMO3, or due to increased formation of TMAO by gut microbiota. We *hypothesize* that DLPs upregulate FMO3 via an AhR-mediated mechanism, and this increased expression helps drive PCB 126-induced cardiometabolic dysfunction through mechanisms that are at least partly independent of TMAO or alterations of gut microbiota. To test this hypothesis we will treat wild-type, FMO3 knockout, or Ldlr:FMO3 double knockout mice with PCB 126 and monitor inflammatory and metabolic dysfunction parameters. Since it is known that TMAO formation from dietary precursors is dependent on the gut microbiota, we will also administer antibiotics to our experimental mouse strains to observe FMO3 effects that are independent of TMAO formation. We *expect* that TMAO formation may not be necessary for PCB 126-accelerated atherosclerosis, but increased expression of FMO3 drives the observed phenotypes observed previously. Such results would provide evidence for a novel mechanism of DLP-induced inflammatory disorders and would lay the groundwork for future preventative strategies aimed at limiting the detrimental effects of dioxins.

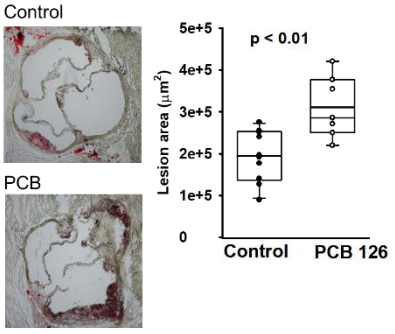

Fig. 2. Exposure to PCB 126 accelerates atherosclerosis in mice fed a low-fat high cholesterol diet. Male Ldlr $^{-/-}$ mice were fed a low fat, 0.15% cholesterol diet and exposed to 1 μmol/kg PCB 126 at weeks 2 and 4. Aortic roots were isolated at week 12 and serially sectioned from the emergence of the 3 valves. Shown are oil red O stained aortic root sections of mice exposed to vehicle control or PCB 126 with associated lesion area quantification (n=10 control, n=7 PCB; p<0.01; Student's t-test).

Experimental approach:

Question 1: Does decreasing FMO3 or the gut microbiota protect against PCB-induced inflammation and cardiometabolic disease?

Study 2.1: Acute PCB 126 exposure to FMO3 $^{-/-}$ mice (C57Bl/6J background) with and without antibiotic administration (R00). *We hypothesize that acute exposure to dioxin-like PCBs will modulate lipid/cholesterol/insulin signaling, and increase inflammation in wild-type mice, and genetic ablation of FMO3*

will prevent these deletrious toxicological effects. Since TMAO itself has been shown to exhibit pro-inflammatory properties, and our preliminary evidence points to increased TMAO production due to pollutant exposure, we also *hypothesize that mice lacking gut microbiota may exhibit some protection from pollutant-induced inflammation and cardiometabolic disease. However, FMO3 $^{-/-}$ mice will exhibit comparably increased protection due to FMO3 functions that are independent of TMAO generation.* To address these questions we will treat half of the wild-type and FMO3 $^{-/-}$ male and female mice with a well-established cocktail of antibiotics that has been shown to eliminate the formation of the TMAO precursor, TMA (21). Antibiotics will be administered in drinking water for one week prior to PCB 126 adminsitration. Drinking water will be measured to ensure dehydration is not occuring. All mice will be fed a standardized low-fat chow diet and half will be exposed to PCB 126 (up to 5 µmol/kg) or vehicle oil control. Our preliminary studies in wild-type male mice show that this dose is sufficient to increase expression of FMO3 protein (48 h post PCB gavage), increase TMAO, modulate hepatic signaling, and increase inflammation (8). 48 h – 2 weeks after acute exposure (oral gavage) of PCB 126, mice will be sacrificed and tissues/blood will be harvested for assays as before (8,19,22).

Study 2.2: Determination of the contribution of FMO3 to PCB-accelerated atherosclerosis in a pro-atherogenic mouse model (K99-R00). Exposure to dioxin-like PCBs can increase CVD risk factors and modulate atherosclerosis using the LDLr$^{-/-}$ mouse model (23). Using the same atherogenic diet described in Aim 1, male and female Ldlr $^{-/-}$ and FMO3:Ldlr double knockout mice will be exposed to PCB 126 (1 µmol/kg) on weeks 2 and 4 of a 10 week study. Additional mice of both genotypes will receive antibiotics as in Study 2.1 in the drinking water throughout the duration of the study. All endpoints in Study 1.1 will be examined. In addition, at the conclusion of the study, cecum contents will be removed and 16s gut microbiota analysis will be completed as before (QIIME software) in collaboration with Dr. Charnigo *(manuscript submitted 2017).*

Expected/alternative approaches: Based on our preliminary evidence, we expect that DLP exposure will accelerate atherosclerosis in our mouse models as evidenced by increased aortic plaque size and en face lesion area. We expect that mice administered antibiotics will lack circulating TMAO and will be moderately protected from PCB-induced inflammation, but this protection will be significantly more pronounced in FMO3 $^{-/-}$ animals. Since FMO3 displays strong dimorphic expression differences (FMO3 may be less inducible in female mice (24)), we expect that male mice may be more prone to FMO3-mediated toxicity.

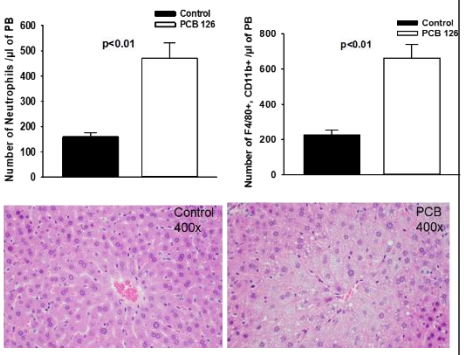

Fig. 3. Exposure to PCB 126 increases circulating levels of macrophages and neutrophils and increases microvesicular fatty change in hepatic centrilobular regions in mice fed a low-fat high cholesterol diet. Male Ldlr $^{-/-}$ mice were fed a low fat, 0.15% cholesterol diet and exposed to 1 µmol/kg PCB 126 at weeks 2 and 4. Peripheral blood and livers were isolated at week 14. Blood cell types were isolated by flow cytometry and liver sections were stained with H&E (n=5 per group).

We also expect that PCB 126 administration will modulate the gut microbiota profile in both genotypes and there may be significant interactions between genotype and exposure (e.g., as determined by 2 Way ANOVA). It is also expected that the dosage of antibiotics used will completely eliminate TMAO, but not totally eradicate the gut microbiota throughout the duration of the 10 week study. Although FMO3's primary function is to detoxify xenobiotics, we do not expect increased PCB-induced wasting or toxicity in our FMO3 $^{-/-}$ mice. There is very little evidence that FMO3 is a critical detoxification enzyme for PCBs, and AhR-mediated upregulation of cytochrome p450s (Cyp1a1) should not be altered by FMO3 ablation. However, if we do observe increased toxicity due to total loss of FMO3, we will use FMO3 targeted 2nd generation antisense oligonucleotide (ASO) technologies to silence, but not eliminate. Although we do not foresee issues crossing the Ldlr $^{-/-}$ and FMO3 $^{-/-}$ mice (Biddinger letter), alternatively we could use the well-established PCSK9 adenovirus method to create FMO3 $^{-/-}$ mice that phenocopy the LDLr $^{-/-}$ model. Mentor Dr. Smyth currently uses this method routinely. On an atherogenic diet these animals become hypercholesterolemic ~21 days post virus administration. During the study, mice would be fed the proposed low-fat, high cholesterol diet.

Specific aim 3. To test the hypothesis that increases in FMO3 activity account for the positive relationship between circulating TMAO and dioxin like pollutant exposure. Using targeted and untargeted mass spectrometry methods in conjunction with multivariate linear regression modeling, we will identify associations between DLPs, CVD risk, and FMO3 substrates/products.

Rationale: The <u>*overall goal*</u> of this aim is to determine the extent that FMO3 expression/activity associates with

DLP exposure and cardiometabolic disease risk in humans. Multiple factors including dietary consumption of TMAO precursors, the gut microbiota, and liver FMO3 levels account for inter and intra individual variability in circulating TMAO. Kidney dysfunction also strongly associates with elevated TMAO probably because of impaired clearance. The major strength of the ACHS-II study is that it combines cross sectional collection of clinical and health outcomes data with extensive measurements of DLPs. However, our evaluation of the relationship between DLPs and TMAO shown in our *published and preliminary data* were made retrospectively. Adjustments for dietary recall and self-reported kidney disease did not alter this association. Because ACHS-II was not designed to measure other determinants of TMAO levels, further analysis of the relationship between DLPs and TMAO in this cohort, via previously collected information, is limited. To address this limitation we propose to use analytical approaches to investigate the roles of diet and variability in FMO3 activity on the relationship between DLPs and TMAO. We *hypothesize* that measurement of circulating biomarkers/ metabolites that report dietary consumption of TMAO precursor-rich foods, like eggs, dairy foods (14) and red meat would enable an adjusted analysis of the relationship between diet, TMAO and DLP exposures (25-27). Also, FMO3 can catalyze N- and S-oxidation of a range of widely distributed xenobiotics including nicotine, caffeine and many drugs. We therefore *hypothesize* that targeted and untargeted measurement of pairs of FMO3 substrates and their oxidation products in plasma samples from the ACHS-II cohort would enable a direct examination of the relationship between DLP exposures and FMO3 activity. This is important because using the ratio of plasma TMA:TMAO is difficult due to TMA's volatility. We *expect* that markers of methylamine-containing foods, will significantly associate with TMAO concentrations in the ACHS-II, but inclusion of these new data to our current multiple linear regression models will not eliminate our observed positive associations between DLP exposure and TMAO. We expect that TMAO formation will strongly positively associate with the ratio between circulating FMO3 substrates, their oxidation products and pollutant body burden. These data would substantiate our overarching hypothesis that DLPs elevate FMO3 activity in exposed individuals, and may provide the rationale for protective FMO3 inhibition strategies.

Experimental approach:

Question 1: Are inter-individual differences in TMAO formation related to diet and pollutant exposure?

Study 3.1: Targeted quantitation of biomarkers of diet (K99). *We hypothesize that a positive association between diets high in TMAO-containing precursors and circulating TMAO will be observed in the ACHS-II population, and inclusion of these new quantitative variables into our multiple linear regression analyses will strengthen the associations of pollutant exposure and TMAO.* Odd and branched chain fatty acids formed by ruminal bacteria in cattle are established circulating biomarkers of dairy food consumption (28, 29). We will measure a panel of these including 15:0, 17:0, iso-14:0, anteiso-15:0, iso-16:0, anteiso-17:0, iso-18:0, iso-20:0 fatty acids as methyl esters using GC MS (SIM or MRM) (15). Appropriate mass labeled standards and a calibration curve will be utilized to determine an absolute concentration for each of the fatty acids. We will also measure levels of choline and L-carnitine which are biomarkers of consumption of eggs and red meat using adaptations of our established methods. All of these markers are stable during storage of plasma and serum. We will then use multivariate statistics to determine if these validated biomarkers of diet significantly associate with TMAO in our population. We will determine if adjustment for dairy or other biomarkers in our current multiple linear regression model impacts on the associations between TMAO and DLP exposures. These nutrients will be measured using a Shimadzu HPLC coupled with an AB Sciex 6500-QTRAP hybrid linear ion trap triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode with data being processed using ABSciex Multiquant software (with mass labeled internal standards). Finally, since there is evidence that consumption of foods derived from animals are associated with CVD risk (25-27), and are possible routes of exposure, we will also determine if increased circulating levels of these biomarkers significantly associate with a) clinical outcomes, and b) levels of DLPs in the ACHS-II cohort.

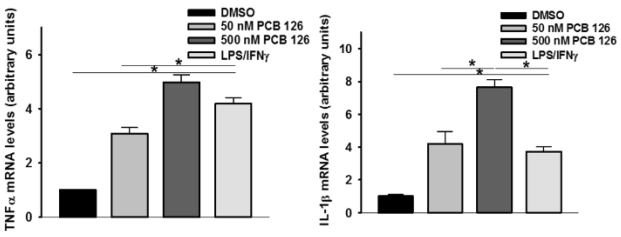

Fig. 4. THP-1 cells exposed to PCB 126 exhibit a pro-inflammatory phenotype. Human THP-1 cells were matured with PMA and subsequently exposed to vehicle DMSO, 50 nM PCB 126, 500 nM PCB 126, or positive control LPS/IFNγ, for 24 hours and mRNA was isolated for q-PCR analyses (Relative ΔΔCT method; normalized to control; β-actin house-keeping gene). PCB 126 dose-dependently increased the mRNA expression of TNFα and IL-1β to an extent greater than or equal to LPS and IFNγ treatment. Data are presented as mean±S.E.M (n=3 per group, One-way ANOVA with Tukey's post-hoc test).

***Study 3.2: Targeted quantitation of other FMO3 substrate/products* (K99).** We and others have observed significant inter-individual differences in formation of TMAO from dietary phosphatidylcholine in human studies which are difficult to interpret given complexity of the process (30). Unfortunately, reproducibly quantitating TMA, which is quite volatile, is difficult in plasma not prepared correctly (TMAO does not have this problem and is stable in plasma for many years). Thus, it is difficult to use the conversion of TMA to TMAO as a surrogate for FMO3 expression/activity. FMO3 can oxidize a number of widely distributed xenobiotics and dietary constituents including caffeine, nicotine (31) as well as commonly used antihistamines (ranitidine). We use targeted mass spectrometry methods to quantitate these substrates and their oxidation products. We will examine associations between DLPs and ratios of these FMO3 substrate and product pairs which, if observed, would support the hypothesis that DLP exposures increase FMO3 activity in these subjects. Importantly, we have medical information related to drug consumption and smoking behavior for this population thus, quantitation of all substrate/product pairs for all ~340 subjects will not be necessary. For example, with the in-depth demographic information available to us we know that approximately 46% of the ACHS-II cohort could be considered smokers and 10 individuals were currently taking ranitidine during the fasted blood draw.

Study 3.3: Untargeted methods to identify additional biomarkers of elevated TMAO and pollutant exposure (R00). Pollutant measurements from plasma may not accurately reflect disease-causing body burden levels in tissues, thus the identification of relevant biomarkers that report systemic exposure to these pollutants might enable early detection or clinical interventions for at-risk individuals. TMAO was discovered to be associated with human disease using untargeted/unbiased metabolomics methods (32-34). We propose to use similar methods to analyze archived samples from the ACHS-II cohort to search for small molecules that associate with DLP exposure. Briefly, this will involve use of UPLC coupled quadrupole orbitrap and quadrupole time of flight instruments as well as GC coupled multistage mass spectrometry approaches using instrumentation available in Dr. Morris's laboratory or through collaboration with the UK Metabolomics Center (detailed in a letter of support). We will work with the metabolomics center to use commercial and proprietary biomarker discovery/statistics software packages to identify molecules that: 1) significantly differ between high and low dioxins exposure (**Figure 1)**; and 2) associate with high TMAO levels. While these studies would proceed empirically, biomarkers discovered could then be validated in animal models to determine mechanisms using the kinds of approaches we propose for TMAO in Aims 1,2.

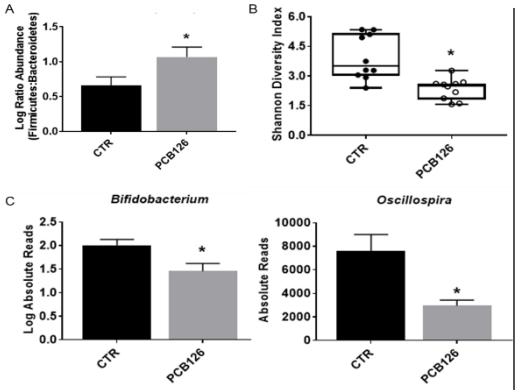

Fig. 5. Exposure to PCB 126 modulates cecum gut microbiota populations in mice fed a low-fat high cholesterol diet. Male Ldlr $^{-/-}$ mice were fed a low fat, 0.15% cholesterol diet and exposed to 1 µmol/kg PCB 126 at weeks 2 and 4. Cecum contents were isolated at week 14, gut microbiota were quantitated by 16S rRNA sequencing, and data was analyzed using QIIME software. **A)** PCB 126 increased the Firmicutes/Bacteroidetes ratio, **B)** decreased the Shannon Diversity Index, and **C)** decreased specific gut microbiota populations (n=10 per group; statistics completed with Dr. Charnigo).

Expected/alternative approaches: The targeted diet biomarker measurements are technically straightforward. We expect that biomarkers of dairy, red meat, fish, and eggs will signifcantly associate with TMAO and pollutant exposure and adjustment for dietary consumption of TMAO precursors might strengthen the already significant association between DLP exposure and TMAO. At a minimum we expect to identify a subset of the FMO3 substrate product pairs which in turn will likely be associated with TMAO and DLP exposure. If we do not observe these expected associations that might focus attention on the possibility that differences in the gut microbiota, in particular species that express the microbial lyases responsible for producing TMA from dietary substrates may be driving our observed associations. While this issue could not be addressed using the ACHS-II cohort, definitive information about the relationship between TMAO, FMO3 and DLP exposures will necessarily require prospective studies in longitudinal cohorts where exposure data and well characterized CVD endpoints are available. Future human studies profiling the gut microbiome in stool samples and FMO3 polymorphism data from subjects might provide useful insights into the basis for variability in plasma TMAO production which can be accomplished using a chip based qPCR array, 16S sequencing, and instrumentation. Finally, it will be necessary to reproduce our observations linking DLP exposure and increased TMAO in other cohorts where exposure data and CVD endpoints are available.

Statistics-We will power all *in vivo* studies to identify a 20% difference in atherosclerosis from control based on previous studies of n=10 per group. For all multivariate models, we will use backward stepwise elimination, to quantify adjusted associations of, for example, pollutant exposure with TMAO in strata defined by covariates (including significant interactions). Statistical analyses will be overseen by Dr. Charnigo.

Training in the Responsible Conduct of Research

Format:

Past training: As a graduate student I completed the semester-long Ethics in Scientific Research (TOX 600) course which focused on didactic and small-group discussions and received a grade of "A". In addition, I have completed the following online courses:

- Environmental Health and Safety Courses on the following subjects:

- Hazardous Waste
- Chemical Hygiene Plan/Laboratory Safety
- Bloodborne Pathogens

- American Association for Laboratory Animal Science Learning Library (http://aalaslearninglibrary.org)

-Euthanasia of Research Animals: AVMA Guidelines
-Avoiding Financial Conflict of Interest in Federal Research
-Common Compliance Issues
-Dose Calculations: Basic
-Introduction to Mice
-Post-Procedure Care of Mice and Rats in Research: Minimizing Pain and distress
-Working with the IACUC

Proposed training:

Along with frequent discussions with my primary postdoctoral mentor, Dr. Andrew Morris, and the entire mentor team, methods for responsible and ethical research have also been established at the University level through the semester-long Ethics in Scientific Research course which focuses on didactic and small-group discussions. As outlined in the training plan, I will also complete the Certificate in Clinical and Translational Science which includes a mandatory Ethical Issues in Clinical Research course (CPH 665). To supplement this active in person training, online training modules including the Collaborative Institutional Training Initiative (CITI) training, and biosafety/bioethics seminars will be attended regularly. For example, UK offers a regular Research Ethics Lecture Series and Bioethics Grand Rounds that invites speakers to discuss topics such as ethical issues in personnel management, successful mentor/mentee relationships, and ethics of using human subjects.

Subject Matter: The CPH 665 course covers the following topics:

- Plagiarism
- Mentoring and Laboratory Supervision
- Research Notebooks and Data Management/Sharing
- Bias in Research
- Authorship and Publication
- Animals in Research
- Peer Review of Manuscripts and Grants
- Human Bioethics including Stem
- Scientific Misconduct
- Vulnerable Populations,
- Subject Recruitment
- Tissue Banking

Faculty Participation: CPH 665 is led by a team of instructors including principal investigators, department chairs, bioethicists, medical professionals, and veterinarians from the Division of Laboratory Animal Research. In addition, the ongoing Bioethics Grand Rounds provides monthly seminars from internal and external speakers. Through conversations with my mentor, Dr. Morris, he has bestowed upon me the ethical comprehension which comes through decades of experience working with various preclinical and clinical models. Our discussions have also included the hazards of unfounded data manipulation, issues with reproducibility, and our responsibility to design experiments appropriately and obtain and present scientifically sound data. Principles taught on the ethics of research are reinforced during lab meetings and journal clubs.

Duration of Instruction: CPH665 is a 3 credit semester-long course and will be taken during the spring of the second year of K99 Training. During the first year of K99 training, I will complete the Group 1 Biomedical Investigators and Key Personnel; Stage 1 CITI curriculum which contains 13 modules and online exams.

Frequency of Instruction: I completed the TOX 600 course in May 2011. Since this was 6 years ago the proposed additional training is imperative to my career goals. The training in occupational health and safety, hazardous waste and blood borne pathogens are required every year. Bioethics Seminars/Grand Rounds presentations occur monthly and will attended during the entirety of K99/R00. CITI training is valid for 3 years.

PLANS AND STATEMENTS OF MENTOR AND CO-MENTORS

Mentoring Team. Dr. Petriello will be mentored by an interactive and collaborative group of established investigators with complementary expertise and interests and strong records of training and mentoring individuals at the advanced post-doctoral and initial independent phases of their careers. Andrew J. Morris will serve as the primary mentor and Dr. Susan S. Smyth enlisted as a co-mentor. Richard Charnigo will serve as an additional mentor to provide training and oversight for multivariate statistics. Drs. Morris and Smyth have an extensive ongoing collaboration in the broad area of genetic and lifestyle determinants of cardiovascular disease risk (97 coauthored publications, 3 active multi PI awards). Dr Morris has additional expertise in analytical chemistry/mass spectrometry that includes a role in NIEHS supported research programs and centers and a particular interest in the impact of environmental chemicals on cardiovascular disease mechanisms. Drs. Morris and Smyth have extensive records of training and mentoring individuals who have successfully transitioned to independence that includes multiple recipients of NIH K series awards and a recent successful K99/R00 awardee. Dr. Charnigo is an experienced researcher and educator who has provided statistics support to Drs. Morris and Smyth (13 coauthored papers and effort on multiple active shared awards).

Interactions with Mentors. Drs. Morris and Smyth share laboratory space. While Dr. Smyth has clinical and administrative responsibilities, Dr. Morris has 85% of his time supported for research and has no administrative duties beyond his role in oversight of the mass spectrometry facility laboratory. Accordingly, Dr. Morris will be available on a daily basis for interactions with Dr. Petriello during the K99 phase and beyond. Beyond these informal meetings, the three Mentors of Dr. Petriello's core mentoring team will meet with Dr. Petriello every three months during an official "committee-meeting" style presentation to review his research and classroom progress, and provide guidance and advice about publications, funding applications and the direction of his research as he advances towards independence. Dr. Petriello will participate in a weekly laboratory meeting and journal club that will provide opportunities for interactions with Dr. Smyth. Because our laboratory is located in the Cardiovascular Research Center, Dr. Petriello also has opportunities for interactions with other experienced CVD researchers with expertise in the mouse models he proposes to use. Dr. Charnigo will be available for consultation and mentoring as needed when opportunities for data analysis arise.

Shared commitment to independence of the candidate. Dr. Morris and the two co-mentors attest that Dr. Petriello will be free to pursue all research observations arising during the K99 phase of this award during the R00 phase and beyond either at the University of Kentucky or at another institution. While Dr. Petriello's research benefits from considerable synergy with that of his mentors, as detailed below, all of these individuals have significant ongoing support for their personal research in areas that are clearly distinct from the work Dr. Petriello proposes to pursue during his K99 training and R00 supported phase. Dr. Petriello's **long-term goal** is to direct an independent research program that employs multidisciplinary approaches to identify and validate mechanisms linking diet and environmental disease with a focus on the cardiovascular system. The mentoring team fully supports this goal and believe that with added expertise in analytical chemistry, multivariate statistics, and cardiovascular medicine, Dr. Petriello will be a leader in the field of environmental health.

Transition to independence. Our expectation is that the program of training and research undertaken during the K99 phase will prepare Dr. Petriello to further develop his R00 research plan into an effective vehicle for his transition to independence. We anticipate that Dr. Petriello's initial independent research will focus on functional validation of associations between diet and environmental exposures identified during the K99 phase of the award. Although we will encourage Dr. Petriello to evaluate his options we hope to retain him at the University of Kentucky which we believe will continue to be a highly supportive and effective environment for his independent research career. A letter of support from Dr. Robert Dipaola, Dean of the University of Kentucky College Of Medicine outlines a plan for transition of Dr. Petriello to a faculty position and a strategy to enable his advancement as an independent investigator.

Components of research available to candidate during independent transition. Dr. Petriello will be able to take with him any of the genetically manipulated mouse strains crossed during the K99 phase (e.g., FMO3,Ldlr double knockouts) which will be a great tool for his independent career. He will also be able to take with him any data collected during the targeted/untargeted mass spectrometry studies of Aim 3 (and any remaining de-identified samples).

Milestones and publications. The mentoring team, and especially Dr. Morris, will oversee successful completion of Dr. Petriello's 3 training objectives. The mentoring team will evaluate Dr. Petriello by examining his academic achievement (coursework performance) in combination with scientific success (e.g., research progress, manuscripts). A main goal of this K99/R00 application is to increase Dr. Petriello's first-author publication record. Dr. Morris will facilitate Dr. Petriello's submission of one first-author manuscript related to Aim 1 and one related to the targeted methodologies of Aim 3 during the K99 phase.

Endorsement of the candidate. Dr. Petriello is a committed environmental disease researcher. He has had a unique education leading up to this K99R00 application with a strong background in understanding the molecular mechanisms of dioxin-like pollutant toxicity. Since the original submission of this proposal Dr Petriello has worked hard to demonstrate academic productivity (two submitted first author papers) and to generate new preliminary data to address the reviewers concerns and to demonstrate feasibility of the work he proposes. He is extremely active in our burgeoning institutional environmental disease research community with roles in organizing training and mentoring opportunities for students and fellows associated with the Superfund Center. As the UK-SRC Training Core Coordinator (5% effort), Dr. Petriello has helped mentor and advise multiple graduate and postdoctoral trainees with a specific focus on building collaborations between the 5 research projects and 5 Cores. The Mentoring Team believes this opportunity to continue as the Training Core Coordinator is a testament to Dr. Petriello's collaborative nature as a scientist and is great training for when Dr. Petriello begins his independent career. This collaborative spirit is also evident with the relationship he has built with Dr. Sudha Biddinger of Boston Children's Hospital. Dr. Petriello wrote and was awarded a Supplement to the P42 Superfund grant that allowed him to work in Boston for approximately 3 months where he was able to help analyze samples from large clinical studies related to type 1 diabetes and also learned new techniques directly applicable to this K99 grant proposal.

He has begun to build a national and international reputation through invited presentations at conferences and meetings, for example the 2016 and 2017 Dioxin Conferences, the NIEHS 50th Anniversary Conference and the Society of Toxicology Annual Meeting where he has presented his research been recognized by multiple travel awards. Drs. Morris and Smyth are currently supporting Dr Petriello with a position on their NIH/NHLBI postdoctoral training grant to provide him with the time and opportunity to develop this K99/R00 application. Dr. Petriello is the driving force behind the science that forms the basis for this proposal which is unique, timely, and well suited to his training and career development. We are all strongly supportive of Dr. Petriello.

Andrew J. Morris, Ph.D., Professor of Cardiovascular Medicine, Primary Mentor.

Role. Dr. Morris will serve as primary mentor with overall responsibility for the planning direction and execution of the K99 phase of the proposed program and a particular role in training Dr. Petriello in mouse models of cardiovascular disease and in analytical chemistry for research at the interface of diet, nutrition and environmental disease. Dr. Morris will then work with Dr. Petriello to review and refine his R00 proposal as he transitions to the independent phase of the training program as well as his initial R01 application.

Research Qualifications. Dr. Morris is an endowed professor of Cardiovascular Medicine at the University of Kentucky who previously held tenured faculty appointments at Stony Brook University and the University of North Carolina. His personal research program broadly concerns roles for lipid metabolism and signaling in cardiovascular and metabolic disease. This research has been continuously supported by grants from the NIH and VA for more than 20 years and has led to more than 240 publications (including papers in most of the more selective journals (Science, Nature, Cell, Developmental Cell, Cell Metabolism, Proceedings of the National Academy of Sciences, Nature Structural Biology) with over 36,000 citations in total including 5 papers that have been cited more than 500 times and 25 papers that have been cited more than 100 times. Dr Morris's H-index (a measure of research productivity and impact over time) is 64 which puts him in the top ~5% of biomedical researchers. To support his interests in lipid metabolism about 10 years ago Dr Morris became invested HPLC and GC coupled multistage mass spectrometry. With support from multiple NIH shared instrument grants and some institutional and programmatic NIH support Dr Morris developed an institution wide facility core for analytical mass spectrometry. Dr. Morris also directs the research support and analytical core components of the UK Superfund and Environmental Diseases Core Centers both of which rely heavily on the staff and instrumentation provided by this facility core. Over the past 8 years Dr. Morris has authored more than 100 publications containing data generated in this facility core using GC and LC coupled mass spectrometry. This includes studies investigating the relationships between diet, nutrition and environmental disease and in particular effects of environmental chemicals on cardiovascular disease processes. Accordingly Dr. Morris is well qualified to oversee training for Dr Petriello in the application of analytical chemistry to research at the interface of diet, nutrition and environmental disease.

Training and mentoring record. Although Dr. Morris's personal research program and the mass spectrometry facility core are now largely run by professional staff his laboratory has been an effective training ground for graduate students, post docs and early career investigators. For example Dr. Morris's first graduate student, Scott Hammond, is now a tenured professor at the University of North Carolina at Chapel Hill. Over the past 20 years, students and fellows receiving training the laboratory have competed successfully for individual support from the American Heart Association (8 pre- or postdoctoral fellowship awards, three

Beginning Grants-in-Aid, and two Scientist Development Grants) and the NIH (T and F32 awards) and gone onto successful careers as independent investigators in academia and industry. Of particular relevance to Dr. Petriello's proposed training, while at the University of Kentucky Dr Morris was a co mentor (with Dr Smyth as the primary mentor) of a successful NIH/NHLBI K99 recipient Prabha Naggareddy (K99 HL122505) who recently obtained a tenure track faculty appointment at the University of Alabama at Birmingham. Dr Morris is also the primary mentor for an NIH/NCI K01 recipient, Fredrick Onono, who will be moving into an independent faculty position in the summer of 2017. At the University of Kentucky Dr. Morris has been a mentor to early career investigators who have been supported by NIH/NIGMS Centers for Biomedical Research Excellence (CORBE) awards that include several individuals who have obtained NIH R01 level funding. Accordingly, Dr. Morris has a sustained record of successful mentoring of individuals during the critical transition between advanced post-doctoral training and early career independence.

Research support. To begin the program of post-doctoral training outlined in this application, Dr. Petriello is currently supported by Dr Smyth's NIH/NHLBI T32 award with Dr. Morris as the primary mentor. Support for Dr. Petriello's research during the K99 phase of the proposed training will come in part from Dr. Morris's active ongoing personal support which includes a recently renewed VA MERIT award and ongoing NIH and VA funding for collaborative research with Dr. Smyth. Of particular relevance to Dr. Petriello's training, the Superfund (NIEHS P42) and Environmental Disease Core Center (NIEHS P30) awards provide substantial support for the Analytical Facility Core instrumentation, staff and operating expenses which will be used by Dr. Petriello during the K99 phase of his training and beyond. Finally, the NIEHS P42 and P30 awards provide funds for career development support that can be used, for example, to pay for travel and accommodation expenses associated attendance at conferences and training events for Dr. Petriello. Accordingly more than sufficient funds are available to support the costs of all aspects of proposed research training project during the K99 and R00 phases of the award. Dr. Petriello will be optimally positioned to take advantage of the infrastructure supported by the NIEHS P42 and P30 awards to advance his personal research program. The P30 center supports pilot grants that might be an additional source of support for Dr. Petriello during the R00.

Supervision and mentoring plan. Dr. Morris will provide evaluations of the Dr. Petriello as required by the annual progress report. In addition to his continued investigations and training using preclinical models, the primary goal of the K99 training phase is to provide Dr. Petriello with experience and expertise in Analytical Chemistry approaches for measurement of environmental pollutants and biomarkers of diet and nutrition. This will position him to apply applied to samples obtained from longitudinal population studies where integrative statistical and informatic analysis can then be applied to discover associations with disease/health outcomes. Dr. Morris's primary responsibility during the K99 phase will therefore be to continue to provide Dr. Petriello with practical training and supervision in the application of GC and HPLC coupled mass spectrometry methods and computational approaches for data analysis to achieve his research goals. As detailed in the research plan, over the past year Dr. Petriello has (under Dr. Morris's supervision and working with the staff in Dr. Morris's facility core) established a collaboration with the NIEHS/CDC Anniston Community Health Survey, obtained a set of serum samples from the second phase of this survey and conducted measurements of Trimethylamine-N-Oxide (TMAO) which is a well validated biomarker of cardiovascular and metabolic disease risk in these samples. These data demonstrate a strong association between exposure to dioxin like environmental pollutants and circulating TMAO levels in this cohort and raise a number of interesting questions that Dr. Petriello can pursue during the K99 phase while also further developing his expertise in analytical chemistry. Specifically, this will include a series of targeted and untargeted measurements of other biomarkers of diet and nutrition that we expect will provide insights into dietary sources of TMAO and the relationship between qualitative (e.g. dietary recall questionnaires) and quantitative measurements of established biomarkers of dietary consumption of particular foods. To accomplish this training in analytical chemistry Dr. Morris and his staff will interact with Dr. Petriello on a daily basis which will include practical training and supervision in the upkeep and operation of analytical instrumentation, assay development and validation and data analysis. Dr. Petriello will attend weekly meetings of Drs. Morris and Smyth's research group as well as focused meetings of the analytical facility personnel. Dr. Petriello will attend workshops and training courses in the use of specialized software for analysis of data generated using untargeted HPLC and GC coupled mass spectrometry methods and (as detailed in the sections below) will participate in formal coursework related to his training in clinical research, multivariate statistics and healthcare informatics to enable integration of these approaches into his personal research program. Dr. Morris will oversee the successful completion of Dr. Petriello's **Objective 1; To develop expertise in analytical chemistry with a focus on quantitating biomarkers of pollutant exposure and diet using HPLC and GC coupled high resolution mass spectrometry.** Along with the hands-on research described above and within the proposal (specifically Aim

3), Dr. Petriello will complete formal didactic training at the University of Kentucky and at the industry leading Agilent Instrumentation's Agilent University. Attending classroom training at Agilent will help to cultivate important industry/academic relationships that will be useful during Dr. Petriello's independent career. At UK, and in collaboration with the Comprehensive Metabolomics Resource Center, Dr. Petriello will take part in their annual 12 day workshop on global metabolomics and present in the Metabolomics monthly seminar series. Although not formally involved in his mentoring, Dr. Petriello will also benefit from an ongoing association with the NIEHS P42 supported University of Kentucky Superfund Research Center and the recently established NIEHS P30 grant supported Center for Research in Environmental Disease. Both of these centers support training and mentoring cores with the P42 center focused on pre- and postdoctoral training and the P30 center focused on early career independent investigators. These Centers and the infrastructure they support for environmental disease research at UK contribute positively to the mentoring environment at the institution. Statements of support from the respective directors (Bernhard Hennig and Xianglin Shi) are included.

Transition to independence. Our expectation is that the program of training and research undertaken during the K99 phase will prepare Dr. Petriello to refine his R00 research plan into an effective vehicle for his transition to independence. To accomplish this goal Dr Morris will review and critique Dr. Petriello's R00 phase application which we expect will focus on discovery and functional validation of associations between diet, nutrition, environmental exposures and human health outcomes identified during the K99 training phase. Dr. Morris will then remain available to assist and support Dr. Petriello during this independent phase of his career.

Susan S. Smyth MD, Ph.D. Professor of Cardiovascular Medicine, Chief of Cardiology, Co-Mentor.

Role. Dr. Smyth will serve as a co-mentor with a particular role in providing training in inpatient clinical research by supervising a small clinical study that Dr. Petriello proposes to undertake during the award and providing oversight for preclinical studies of atherosclerosis in mouse models.

Research qualifications: Dr. Smyth is a physician scientist who combines clinical practice in cardiology with NIH- , VA-, and industry-funded research focused on the interplay between inflammation and thrombosis in vascular biology. Her training and expertise center on the contribution of extracellular mediators and blood and vascular cell-surface signaling receptors. Her team applies genetic and pharmacologic strategies in animal models of cardiovascular disease, in order to define cellular and molecular pathways, and then tests those pathways in clinical studies in humans. Her clinical interests are in arterial and venous thrombosis, and she leads an enterprise-wide effort in thrombosis prevention and management. She has authored more than 150 publications and contributed to over a dozen textbooks and is a member of a number of elected bodies that support biomedical research and scholarship including the American Society for Clinical Investigation.

Training and mentoring record. Dr. Smyth has an extensive record of training that includes leadership roles in institutional training programs for physician scientists and clinical researchers. Over the past ~15 years she has mentored medical and graduate students (>20) and postdoctoral fellows (10), junior faculty (8), and is extensively involved in didactic teaching. At the University of Kentucky Dr. Smyth has had six graduate students perform doctoral dissertation research in her laboratory and all were supported by fellowship awards either from the NIH (F-series) or the American Heart Association. As noted above, one of her most recent post-doctoral trainees recently received K99/R00 and secured an independent tenure track position at the University of Alabama at Birmingham. Twelve of her former trainees have faculty appointments at academic institutions leading research efforts supported by extramural funding. Dr. Smyth devotes approximately 6 hours a week to programmatic direction of training and education in her role as Director of the MD/PhD Program and Co Director of the Center for Clinical and Translational Sciences training component. Her mentoring efforts were recognized by the 2012 Mentor Award from the Center for Clinical and Translational Science. Dr. Smyth is therefore well qualified to provide the training described below.

Research Support. As noted above Dr. Smyth shares several active federally funded research awards with Dr. Morris that broadly support research into studies of bioactive lipid metabolism and signaling. Dr. Smyth will not be personally responsible for supporting Dr. Petriello's research during the K99 phase.

Training and mentoring plan. Dr. Smyth has extensive expertise in preclinical (mouse) models of cardiovascular disease including use of these models to study mechanisms of thrombosis and atherosclerosis. She will provide mentoring and training for Petriello as he uses these models to investigate the role of FMO3 and TMAO in dioxin like pollutant accelerated cardiovascular disease. As a physician scientist Dr. Smyth also has extensive ongoing involvement in clinical research. Although Dr. Petriello only proposes studies using existing samples in the present proposal he seeks to gain additional training in clinical and translational research to eventually enable him to design and implement his own clinical studies to investigate mechanisms linking diet and exposure to environmental chemicals to human disease mechanisms. Dr. Smyth will oversee

the successful completion of Dr. Petriello's **Objective 3; To receive training in clinical, translational, and experimental CVD research.** Dr. Smyth will provide mentoring for Dr. Petriello's by advising him as he completes the certificate in Clinical and Translational Science and Collaborative Institutional Training Initiative (CITI Program) IRB training during the K99 phase. Dr. Smyth will be available to assist with the design and oversight of clinical research components to Dr. Petriello's program at the R00 stage and beyond. Also, as an expert in mouse models of cardiovascular disease, Dr. Smyth will provide training in assays related to atherosclerosis and cardiometabolic disease (e.g., aortic root lesion area quantitation and blood pressures).
Transition to independence. Dr. Smyth will be available to review and critique Dr. Petriello's initial R01 research plan, particularly if this proposes clinical studies, and can continue as a collaborator if warranted.

Richard J. Charnigo Professor and Chair, Biostatistics, Co-Mentor.

Role. Dr. Charnigo will provide mentoring and training in the application of multivariate statistics for analysis of associations between dietary biomarkers, indices of environmental exposures and health outcomes.
Research qualifications. Dr. Charnigo holds a joint appointment in the Department of Biostatistics (College of Public Health) and Department of Statistics (College of Arts and Sciences). He received his Ph.D. in Statistics from Case Western Reserve University in 2003. His research interests include mixture modeling, dimension reduction, nonparametric regression, and cardiovascular health. He has analyzed data generated in a broad range of biomedical research studies including collaborations with Drs. Morris and Smyth.
Training and mentoring record. Dr. Charnigo has been the dissertation advisor to twelve Ph.D. students in Statistics (seven completed) and two Ph.D. students in Epidemiology and Biostatistics (one completed), and a supervisor to twelve graduate and two undergraduate research assistants. Through his extensive involvement as the consulting statistician in collaborative research Dr. Charnigo is involved in informal training and education of researchers at multiple career stages. Indeed Dr. Charngio's value and commitment as an outstanding researcher was recently recognized by the award of a University Research Professorship.
Research Support. Dr. Charnigo will not be responsible for financial support for Dr. Petriello or his research. However, Dr Charnigo receives support from several active research awards including some for which Drs. Morris and Smyth are PIs. The Division of Cardiovascular Medicine provides additional salary support for Dr. Charnigo to participate in and provide statistics consultation and support for clinical research conducted
Training and mentoring plan. Dr. Charnigo has an existing relationship with Dr. Petriello through his involvement in statistical analysis of data generated from the biomarker studies conducted using the Anniston Community Health Survey Cohort and statistical analyses of gut microbiota data that are presented as preliminary data in the accompanying research proposal. Dr. Charnigo will continue his involvement in analysis of data generated from ongoing studies with this cohort during the training phase of this program. Dr. Charnigo will oversee the successful completion of Dr. Petriello's **Objective 2; To obtain formal, in-depth training in multivariate biostatistics and informatics.** During his K99 training Dr. Petriello will take at least 2 graduate level biostatistics courses that will focus on development of skills in multivariate statistics. Dr. Petriello will also receive instruction in alternative approaches including principal component analysis and nonparametric methodology related to Dr. Charnigo's expertise; furthermore, Dr. Petriello has a particular interest in graphical statistical approaches for data visualization. The primary goal here will be to provide Dr. Petriello with expertise in using statistical packages such as R or SAS for analysis of the types of data that he will be generating during his research. Dr. Charnigo will be available to provide consultation and tutoring to Dr Petriello as needed to ensure success of this component of the K99 training plan.
Transition to independence. Dr. Charnigo will be available to review and critique Dr. Petriello's initial R01 research plan and will remain available for continued involvement in his research as a statistical consultant or collaborator.

Andrew J. Morris, Ph.D, Mentor
Professor Cardiovascular Medicine,
University of Kentucky College of Medicine

Susan S. Smyth, MD, Ph.D., Co-Mentor
Professor and Chief, Cardiovascular Medicine,
University of Kentucky College of Medicine

Richard Charnigo, Ph.D. Co-Mentor, Professor of Statistics and Biostatistics, Chair, Biostatistics,
University of Kentucky College of Public Health

HARVARD MEDICAL SCHOOL
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Michael C Petriello
University of Kentucky
900 S. Limestone
CTW Building Room 585
Lexington, KY 40536-0200
10/26/2017

Re: NIH/NIEHS K99/R00

Dear Mike,

This letter is to state the details of our collaboration during you K99 and as you transition to an independent program. **Please note that a separate Reference Letter has been submitted through eRA Commons** to express my support for you as a candidate: you are an outstanding candidate for the K99 award, and I have every expectation that you shall become a leader in the field of environmental health.

We are happy to continue to collaborate with you on your NIH grant application, "TMAO is a biomarker of dioxin-like pollutant exposure and cardiometabolic disease." I think your work linking dioxin-like pollutants to TMAO is interesting and important. We have also been very grateful for your contributions to our ongoing collaborative projects examining the roles of TMAO in diabetes and chronic kidney disease.

As you know, we have a long standing interest in diabetes and insulin resistance, and have been working for some time on the enzyme flavin-containing monooxygenase 3 (FMO3). Specifically for this proposal, I will provide you breeding pairs of *Fmo3* deficient animals (generated via CRISPR/Cas9 system) for use in your *in vivo* studies. I believe these animals, and the proposed cross with *Ldlr* deficient mice, will be a useful tool for your independent research career. In addition, we have other reagents, such as adenoviruses for the overexpression and knockdown of FMO3, and antibodies, that we would be happy to share with you, should you need them.

Good luck with your grant!

Sincerely,

Sudha B. Biddinger, MD/PhD
Associate Professor of Pediatrics
Harvard Medical School

University of Kentucky
Department of Toxicology and
Markey Cancer Center
789 South Limestone
BioPharm Building, Room 523
Lexington, KY 40536-0596
859/218-1028

Teresa W-M Fan, PhD
Professor of Toxicology & Cancer Biology
Member, Markey Cancer Center
Edith D. Gardner Chair in Cancer Research
twmfan@gmail.com

Dear Mike,

I am writing in my capacity as the Outreach and Education Core Director of the UK-Resource Center for Stable Isotope-Resolved Metabolomics (RC-SIRM), to provide a letter in support of the training and career development outlined in your NIH/NIEHS K99/R00 application. As you know, RC-SIRM offers many training opportunities that will help facilitate your transition to an independent academic researcher. These include our annual hands-on 12-day workshop covering practical experiences in experimental design, sample preparation, data acquisitions, and data analysis. This workshop culminates in our annual one-day symposium that fosters collaborations between metabolomics experts at UK and elsewhere. We also run a Pilot Program to assist junior faculty in collecting preliminary metabolomics-related data, which we invite you to apply should you transition to a faculty position at UK or elsewhere during the R00 phase. We also invite you to attend our seminars/journal clubs focused on cutting-edge metabolomics research and expect you will present as part of your K99 training plan.

In your K99/R00 application you propose to examine associations between environmental exposures and diet, and how they relate to risk for cardiovascular diseases. Your primary mentor, Andrew Morris is an expert in targeted quantitation of small molecules and our group can provide assistance and advice regarding your proposed metabolomics studies in Aim 3. Using unbiased methods to identify metabolites that associate with either high TMAO and/or high pollutant exposures in the ACHS-II human cohort is novel and no doubt will provide multiple metabolites of interest to follow up during your R00 phase and future R01 submissions. In addition to the Thermo Q-Exactive instrumentation in your primary mentor's laboratory, RC-SIRM has a wide variety of metabolomics platforms that may be of use to you during your K99/R00 and beyond (e.g. Bruker Daltonics SolariX XR, Thermo Oritrap Fusion™ FT-MS, ITQ 1100 ion trap GC-MS, Agilent 7900 ICP-MS, Agilent 4-channel 600 MHz and Bruker 4-channel 700 MHz NMR, both outfitted with cryogenic probes, and Bruker 400 MHz wide bore NMR with imaging and localized spectroscopy). I wish you good luck with your proposal.

Sincerely,

Teresa W.-M. Fan

UNIVERSITY OF KENTUCKY

UK Superfund Research Center

University of Kentucky
599 Wethington Health Science Building
900 South Limestone Street
Lexington, KY 40536-0200
(859) 218-1343
Fax: (859) 257-1811
www.uky.edu/Research/Superfund/

November 7, 2017

Dear Members of the Selection Committee:

It is my sincere pleasure to write this letter of support for Dr. Michael Petriello's application for the NIH/NIEHS Pathway to Independence Award (K99/R00) entitled *TMAO is a biomarker of dioxin-like pollutant exposure and cardiometabolic disease*. In my capacity as Director of The University of Kentucky Superfund Research Center, a major nexus of environmental health research on the campus of the University of Kentucky, I am committed to facilitating development of promising postdoctoral fellows such as Dr. Petriello to successful transition to faculty status.

The Superfund Center supports a number of highly specialized resources that will be available to him in the proposed work. These include, in particular, the Research Support Core directed by Dr. Petriello's mentor, Dr. Andrew Morris, which, as described in the proposal, contains sophisticated mass spectroscopy instrumentation and employs highly skilled professional staff that will all be invaluable to this K99/R00 project. He will also have full access to all other resources of this center as the need arises.

As Dr. Petriello's training progresses towards independence, his focus on interactions between diet, bioactive nutrients and environmental exposures as determinants of human disease risk fits very well with the overarching goals of the Superfund Research Center, which I expect will lead to further productive interactions with our research translation and community engagement components. As a past trainee of our Superfund Research Center, he has contributed very productively to achievement of our training objectives. Dr. Petriello is our Training Core Coordinator, and we will continue to offer partial support related to his path to independence (e.g., tuition reimbursement and assistance with travel expenses). I expect his contributions to the field of environmental health will continue to grow and we hope he will consider applying for a tenure track faculty position at UK once his R00 phase begins. If so, Dr. Petriello will be a strong candidate for increased involvement with the UK Superfund Research Center as an independent investigator.

In summary, I am enthusiastic in my commitment of these specialized resources to Dr. Petriello's K99/R00 application.

Sincerely,

Bernhard Hennig, PhD
Professor of Nutrition and Toxicology
Director, UK Superfund Research Center

UNIVERSITY OF KENTUCKY

(859) 257 4054
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Xianglin.shi@uky.edu

Xianglin Shi, Ph.D., Professor
William A. Marquard Chair in Cancer Research
Department of Toxicology and Cancer Biology
Director, Center for Research on Environ Disease
Director, NIEHS Core P30 Center
Associate Dean for Research Integration
College of Medicine
University of Kentucky
1095 VA Dr.
Lexington, KY 40536

November 6, 2017

Dear Mike,

I am writing in my capacity as Director of Center for Research on Environmental Disease, College of Medicine to provide a letter in support of the program of research training and career development outlined in your NIH/NIEHS K99/R00 application. As you know, I am also the Director, University of Kentucky Center for Appalachian Research in Environmental Sciences (UK-CARES) (1P30ES026529-01A1). This Center provides a number of research resources that will be available to you. These include the Analytical Core directed by your mentor Andrew Morris which, as described in your proposal, contains instrumentation and employs professional staff that will all be invaluable to you. As your training progresses towards independence your focus on interactions between diet, nutrition and environmental exposures as determinants of human disease risk fits very well with the overarching goals of the Center for Research on Environmental Disease, which will lead to further productive interactions with our research translation focused integrated health sciences facility core and with our community engagement components. The UK-CARES also supports a number of career development resources for early stage investigators which will be valuable to you. Through the support of NIH/NIEHS K99/R00, I am very confident that your career in the area of environment health sciences will be further developed, positioning you very well to play an important role in UK-CARES and in the Center for Research in Environmental Disease as a faculty if you decide to stay at UK.

Sincerely,

Xianglin Shi

Xianglin Shi, Ph.D., Professor

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

UK is one of 115 private and public universities in the country to be classified by the Carnegie Foundation for the Advancement of Teaching among Doctoral Universities: Highest Research Activity (R1). R1 universities represent 2.5% of all institutions in the classification system. UK faculty and staff brought in more than $316.5 million in new sponsored project awards in FY 2016. Of that total, UK was awarded $163.5 million in grants and contracts from federal agencies.

The College of Medicine also maintains a robust **Postdoctoral Resource Office**, established in 2002, that is designed to enhance the postdoctoral training experience through career counseling and professional development resources. Collective efforts to provide quality postdoctoral training have garnered UK a 10th place ranking among all research universities in a “Best Places for Postdocs” survey conducted by *The Scientist*. The ranking was based on assessments of the culture of collaboration, commitment to teaching, and emphasis on training and career counseling. The office provides a range of services including a monthly Postdoctoral Career Development Seminar Series (also open to senior level graduate students), a Postdoctoral Research Seminar Series, and an annual Postdoctoral Poster Session. Career development seminars feature a number of external speakers from academia and industry. Research seminars and poster sessions both provide complementary training in important professional skills. Dr. Petriello will also be able to register as a member of the postdoctoral community to receive communications on further research opportunities and support, career development resources, and information tools. A Postdoctoral Advisory Committee involves interested postdoctoral fellows and faculty members from diverse areas who advise the dean concerning issues relevant to the postdoctoral experience at UK.

Multiple institutionally-supported Core facilities will be available to Dr. Petriello that are directly related to his K99 training. The UK Mass Spectrometry Facility (UKMSF) is a major shared research resource located in the Advanced Science and Technology Commercial Center in the heart of the UK campus. UKMSF operates an array of instrumentation and has expertise in mass analysis involving various methods of sample introduction, ionization, and mass measurement. Available instrumentation includes a Bruker Ultraflextreme MALDI-TOF MS; a JEOL JMS-700T Tandem MStation four sector MS for high resolution EI, CI, FAB, ESI, APCI, and FD ion sources, further equipped with the tunable energy electron monchromator (TEEM) for low-eV EI; a ThermoFinnigan LTQ linear ion trap MS equipped with ESI and nanospray ESI ion sources and a Dionex Ultimate 3000 capillary LC system; a ThermoFinnigan PolarisQ ion trap MS equipped with EI and CI ion sources, GC and direct probe sample introduction; and a Shimadzu GC-MS QP5000 quadrupole instrument equipped with EI. Also, The University of Kentucky Small Molecule Mass Spectrometry Core, which the **primary mentor Dr. Andrew Morris directs**, provides analytical capabilities for measurements of proteins, metabolites, and small molecules. The laboratory is located in the same building as Dr. Petriello’s office and contains separate rooms for the individual mass spectrometer systems, bench space for sample preparation and office space.

A key training component of Dr. Petriello’s K99 phase relates to clinical and population-based studies which the University of Kentucky has a strong focus and background. A key priority of the UK Center for Clinical and Translational Science (CCTS) infrastructure is to ensure sound and ethical study design through a dedicated resource. Faculty from key academic disciplines in biostatistics, statistics, and epidemiology serve the biostatistical, study design, and data analysis needs of CCTS investigators (**Drs. Smyth and Charnigo are affiliated with the CCTS)**. Four specialized services include: 1) data analysis, 2) data management, 3) randomized clinical trial design, and 4) population-based study design. This support is integral to Dr. Petriello’s K99 training. Faculty provide direct investigator assistance during the protocol development phase, assist with scientific review to the scientific review committees within the Clinical Services Core, and coordinate ongoing development of a centralized data coordinating center. Dr. Petriello will have access to a workstation equipped with a computer that has SAS, SPSS, and R statistical software installed. A separate consultation office provides office and computer support. In addition, the facility contains seven secure servers, one for printing, four as virtual server infrastructure, and two for statistical modeling. Dr. Petriello will also have access to the UK Institute of Biomedical Informatics and metabolomics core facility which provides access to biomedical informatics expertise, technologies, and data management platforms for investigators conducting data-intensive research (i.e., “big data”). Dr. Petriello will have access to expertise and tools related to current innovations in computer science, such as knowledge representation, machine learning, and cloud computing.

University of Kentucky | College of Medicine

November 1, 2017

Institutional Commitment Statement: Michael C. Petriello Ph.D. K99/R00 Application.

Dear Colleagues,

I am writing in my capacity as Dean of the College of Medicine to support Michael Petriello's application for an NIH/NIEHS K99/R00 award. To address the requirements for this program:

1. During the K99 phase, Dr Petriello will devote 95% of his effort to the research and training activities detailed in this proposal. He has no administrative, teaching or clinical responsibilities but will continue his active role in the career development component of the Superfund Center.
2. All of the research training opportunities and research facilities detailed in the application will be available to Dr Petriello, in particular the mass spectrometry facility core which is supported through a combination of grant recharge, programmatic grant and institutional support.
3. Time and financial support for Dr Morris and the co-mentors is more than adequate for the research training proposed- Dr Morris has both programmatic and institutional support for training and education related to his role as mass spectrometry facility core director and significant effort and support for research in the broad area of the proposal.
4. Dr Petriello is a native born US Citizen who fulfils all eligibility requirements for this program.

I also want to detail our high institutional enthusiasm and support for Dr Petriello's career advancement. The continued growth of the University of Kentucky as a Biomedical Research Institution is critically dependent on our talented research faculty. Given the expense and uncertainty involved in recruitment and retention of nationally competitive faculty, identification and support of new and early career investigators is a proven strategy for improving our competitiveness by strengthening our biomedical research workforce. Dr Petriello clearly has the aptitude and talent to become an independent investigator. He is already immersed in the culture of environmental disease research through his involvement in our NIEHS supported Superfund Center and we believe that with the appropriate training and support described in this application his transition to becoming an independent investigator is a very feasible goal. Indeed, the lead role Dr Petriello has taken in developing the research presented in the accompanying proposal and his recent selection to present his research at several national and international meetings suggests that he is already well on the way to achieving significant peer recognition for his promise and accomplishments.

At the end of the K99 phase of training, while we expect that Dr Petriello will be competitive for faculty level appointments at other academic institutions, we hope that the University of Kentucky will be an attractive home for him at this next stage of his career. While the specific details would have to be negotiated at the time of his transition from the K99 to the R00 phase and would be contingent on his research progress and ratification through our normal process for academic appointments our intention is to offer Dr Petriello a tenure track faculty position as an Assistant Professor in an appropriate department with an assignment of office and laboratory space and an institutional commitment for laboratory startup costs that are in line with those provided by our peer institutions.

In addition to this support, as an independent investigator, Dr Petriello would continue to benefit from our strong programs and infrastructure for environmental disease research. For example our NIEHS P30 Environmental Diseases Core Center and our NIEHS Superfund Center.

In summary, Dr Petriello is a productive and promising young researcher. Support from this K99/R00 award would achieve the mutually beneficial goal of promoting Dr Petriello's research training, and I therefore support this application without reservation. If I can be of further assistance, please contact me directly.

Sincerely,

Robert S. DiPaola, MD Dean, College of Medicine

Willard Education Building | MN 150 | Lexington, KY 40506 | P: 859-323-6582 | F: 859-323-2039 | med.uky.edu

Protection of Human Subjects

This Human Subjects Research falls under Exemption 4.

This application describes analytical measurements of biomarkers of diet, nutrition, and environmental exposures and analysis of healthcare data in a completed clinical study (Anniston Community Health Survey-II). The analytical work done for these projects will involve analysis of de-identified samples and de-identified healthcare information under arrangements where the PI, mentors and staff will not have access to any identifying information. Accordingly this will likely be determined to be exempt from the regulatory requirements in exempt from the regulatory requirements in 45 CFR 46. Indeed we have local IRB exemption for the studies conducted using the Anniston Community Health Survey samples and data (please see below for letter of exemption). The original ACHS-II study obtained IRB approval from the University of Alabama Birmingham.

VERTEBRATE ANIMALS

1. Description of Procedures: For the proposed *in vivo* studies (Specific Aims 1,2) three experimental mouse strains to be studied are as follows: (1)C57BL/6 wild-type mice will act as a positive control as our past data shows this strain is susceptible to PCB-induced inflammation; (2) C57BL/6 Ldlr -/- mice will act to elucidate the impact of dioxin-like pollutants on accelerated atherosclerosis; (3)Ldlr -/- X FMO3 -/- double knockout mice on a C57BL/6 background will act to elucidate the importance of FMO3 on PCB-induced atherosclerosis and inflammation. Using the Ldlr single KO will allow us to examine the extent of dietary exacerbation (e.g., diets high in TMAO precursors) of PCB-induced acceleration of atherosclerosis. We will include 10 mice in each experimental group, but we will refine this number requirement as more data become available (these numbers are based off of previous experience and biostatistical power calculations. Please see Aims 1,2 for more details related to groups). Due to the importance of FMO3 sexual dimorphism, male and female mice will be used for all studies. Mice fed either a standard control diet or nutrient enriched diet will be administered vehicle or PCB by gavage, with a maximum of 4 doses used. Breeding colonies will be monitored under the supervision of Drs. Morris and Smyth. Adult, 8 week old mice will be used for all studies.

2. Justification for animal use: The broad goal of this study is to test *in vivo* the hypothesis that FMO3/TMAO is a critical mediator of dioxin-like pollutant accelerated atherosclerosis. Mice which lack the Ldlr or FMO3 genes will be used to demonstrate throughout this study that dioxin-like pollutants can accelerate atherosclerosis and that loss of FMO3 is protective. The mouse is the least sentient subject that is appropriate to a study of this nature, though wherever possible we will attempt to us cell cultures rather than living whole animals. For example, in future studies we may silence FMO3 in cell types associated with inflammation and atherosclerosis and subsequently treat with dioxin-like pollutants. However, Specific Aims 1 and 2 absolutely require animals because we will determine atherosclerotic markers which mimic human atherosclerosis and lesion formation, and because of the ability to manipulate the mouse genome for knockouts. Variability in the biological response to vessel injury requires the initial use of 10 animals per treatment group, but we will refine this number as more data become available. Using previous proatherogenic knock-out mice (e.g., Ldlr -/- or ApoE -/-), power analysis estimates indicate that we will detect a 20% change from control values as statistically significant ($p \leq 0.05$) with a power of 0.8 when we use this sample size. Some animals will be purchased, but most animals need to be bred to obtain strains which lack particular genes. The FMO3 -/- animals will be provided by Dr. Biddinger and the wild-type and Ldlr -/- mice will be purchased from Jackson Labs. These mice are not in short supply or cost prohibitive. Our previous success to-date with other knockout strains indicates that we can successfully breed the required number of animals to complete the proposed studies.

3. Minimization of Pain and distress: No animal will experience pain other than the momentary discomfort of handling at the time of euthanasia. Blood samples will be collected at the time of euthanasia. No survival surgery is required for the proposed studies. All personnel handling laboratory animals are required to successfully complete a basic training program (Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs, National Academy Press, 1991). Wasting will not occur with the doses of dioxin-like PCB 126 proposed within Aim 2. Animals will be humanely euthanized by being overdosed with carbon dioxide gas (from a gas cylinder). This method of euthanasia is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. Should any abnormal events occur resulting in distress to any animal, it will be killed immediately in accord with the guidelines of University of Kentucky IACUC.

Veterinary care of the animals: All animals will be housed in the AAALAC accredited Medical Center vivarium at the University of Kentucky and will be cared for by Division of Laboratory Animal Resources (DLAR) animal care technicians. Animals will be fasted (with free access to water) for 6 hours prior to collecting of blood. The Institutional Animal Care and Use Committee must approve experimental protocols requiring the use of animals before any study can begin. Mice will be checked daily by trained animal technicians who will check food, water, room temperature and lighting twice a day. A veterinarian is available at all times for emergencies, and Veterinary clinical care is provided by 3 licensed veterinary technicians and 4 veterinarians, three of which are ACLAM Diplomates. In addition, the DLAR emphasizes preventative medicine that is exemplified by an extensive rodent screening serology program and health monitoring programs for other species.

SELECT AGENTS

NONE

Authentication of Key Biological and/or Chemical Resources

Key Chemical Resources:

1. Polychlorinated biphenyl 126 (PCB 126) will be used as a model dioxin-like toxicant within Aims 1,2 of the proposed study. PCB 126 will be purchased from the highly reputable vendor AccuStandard which has a long track record of high quality, pure analytical grade standards. Each of their PCB standards is Certified Reference Materials (CRMs) grade and comes with a quality control specification sheet. Using the mentor's EPA-certified methods and instrumentation we can also determine the purity of the PCB 126 stock upon arrival. Analytical standards for other environmental pollutants and/or nutrient metabolites will be purchased at the highest purity possible and examined via LC/GC MSMS.

Key Biological Resources:

1. Cell lines are proposed to be used within this K99/R00 proposal and will be purchased through ATCC.

   Genetically altered (i.e., knockout) mouse models will be used in Aim 2 of this grant. To authenticate the genetic deletion we can genotype via common tail snip and PCR methods.