

NEOMED

**Office of Research and
Sponsored Programs'
Student Research
Fellowship Program**

2021

Project Catalog

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PROGRAM GOALS, PARTICIPATION POLICY AND PROGRAM REQUIREMENTS

GOALS

The fellowship projects provide summer experiences for NEOMED's medical and pharmacy students, in a variety of disciplines. The Summer Research Fellowship Program is a mentored research program, designed to provide intensive training in research procedures and principles on projects in basic and clinical disciplines; to enhance students' research horizons; and develop scientific presentation and writing skills. These projects are funded by the Office of Research and Sponsored Programs (ORSP).

PARTICIPATION

Phase 1, M1, M2, P1 and P2 students *in good standing* may participate in the ORSP's Summer Research Fellowship Program.

M3, M4, P3 and P4 students who have completed their clerkships and have no conflict with their electives, may participate in the ORSP's Summer Research Fellowship Program.

A M4 and P4 student must have written documentation of the time permitted to complete the summer project.

If the project is to cross-over into any elective time, the student must obtain written approval of the elective director indicating the time frame that will be allotted to the fellowship project.

The project investigator will have to approve the plan.

Special requests will be considered if it is arranged and approved in advance.

PROGRAM REQUIREMENTS

Prior research experience is not required for research projects. However, research experience may be a factor for selection for a specific project and will be up to the discretion of the individual project investigator.

Students are required to complete all applicable training prior to beginning their research projects. Required training will be determined by the project investigator.

All students are required to complete the online Collaborative Institute Training Initiative (CITI) Human Subjects Research – Social-Behavioral-Educational Module Certificate. (See page 3 for details.) If you have completed this training during the past three years you do not have to repeat it. You can provide a certificate of completion if you are selected for a fellowship.

**Fellowship Stipends and Commitment
Through the Office of Research and Sponsored Programs**

1. All students agree to fulfill a commitment with the project investigator for completion of a summer research fellowship. Each project investigator is volunteering their time and expertise to train the fellow. It is the student's responsibility to be prompt, available for the project for the contracted time and attend to all requirements of the fellowship.

2. The total stipend for the student research fellowship will be \$3,000. Student fellows are considered to be NEOMED employees and will be paid on a monthly basis through NEOMED's payroll system and will be subject to withholding tax and OPERS withholdings. A W-2 will be issued to the summer research fellow the following January for use in filing their tax return.

(A special note – a student can claim exempt on their tax withholdings if they believe they are eligible for a tax refund on their tax return. A student can also apply for a refund for the withholding paid to OPERS after they leave NEOMED's employment. However, the 1.45% withholding for Medicare is not exempt and cannot be refunded after end of employment.)

**Collaborative Institute Training Initiative (CITI) at the University of Miami
Human Subjects Research – Social-Behavioral-Educational Module Certificate**

1. All students who are selected for a summer research fellowship will be required to take the computerized on-line researcher course at:

<https://www.citiprogram.org>

If you have taken this training within the past year you do not need to repeat it. Please provide a copy of the completion certificate to Nona Hose in the Office of Research and Sponsored Programs, Office G-235 if you are selected for a fellowship.

2. Description of course from the CITI Program:

“Basic HSR modules are suitable for all persons involved in research studies involving human subjects, or who have responsibilities for setting policies and procedures with respect to such research, including IRBs. These modules are typically assembled into a basic course, which is the learner's first exposure to the content. Refresher modules, which can be assembled into refresher courses presented to learners at intervals defined by the institution, are designed to provide continuing education in human subject research issues. The standalone courses are intended for institutional/signatory officials, IRB administration (administrators, directors, coordinators, and other support staff), and IRB chairs.

HSR module topics include: basics of IRB regulations and the review process, assessing risk to subjects, avoiding group harms, conflicts of interest, cultural competence, FDA-regulated research, genetic research, HIPAA-regulated research, informed consent, international research, Internet research, IRB member responsibilities, IRB chair responsibilities, records-based research, research in schools, research with protected populations, research with vulnerable subjects, the role of the community member, unanticipated problems and reporting, and students in research.”

3. A certificate of completion will be awarded. Send this certificate to Nona Hose, Executive Administrative Assistant, Office of Research and Sponsored Programs, NEOMED
4. You will not permitted to participate in any research without this certification.



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Collaborative Institutional Training Initiative
at the University of Miami

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Over 7.3 million CITI Program courses have been completed since 2000

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Log in through my institution

Create an account

Access requires registration as an affiliate of a subscribing CITI institution or as an unaffiliated learner.

Student Research Symposium

Friday, November 19, 2021

This will be a virtual event.

All ORSP sponsored fellows are **required** to participate in the Student Research Symposium.

Details as to preparation, deadline, etc., for poster presentations will be provided at a later date.

NEOMED
**Office of Research &
Sponsored Programs**

**Student Research
Fellowship Program**

**APPLICATION
MATERIALS**

APPLICATION AND HIRING PROCEDURES

PLEASE NOTE: The application and interview process begins as soon as the project catalog is distributed. Please submit your applications as soon as possible.

The deadline to apply is Friday, February 19, 2021.

1. Students who are required to complete a summer course remediation are strongly discouraged from participating in any student research fellowship program that overlaps with the remediation exam study period. Please contact Craig Theissen, Director of Academic Support at ctheissen@neomed.edu or (330) 325-6758 for additional information.
2. Application/Interview process:
 - a. Complete the application form online.
 - b. Submit a *curriculum vitae* along with your application online.

3. Hiring Process:

Students and project investigators should approach the fellowships as job opportunities. Students are asked to submit an application and curriculum vitae to the project investigator(s) of their choice. The project investigators will then contact the student(s) in which they are interested and set up an interview. After interviews are conducted, the project investigator will make his/her selection and offer the position to the student of his/her choice. Once a student has accepted the offer of a fellowship, the project investigator will notify Nona Hose in the Office of Research and Sponsored Programs.

Nona Hose will provide Human Resources (HR) the names of the students who have been selected. HR will contact each student to provide the necessary paperwork and to set up an appointment for onboarding if necessary. The project investigators will be asked to fill out a NEOMED Training Checklist form indicating any safety training that will be required (lab safety, animal care and use, etc.). This checklist will be provided to NEOMED's Safety Office.

All onboarding paperwork and applicable safety training must be done before the student can begin working on a project.

You may contact Nona if you have any questions or need additional information.

Nona Hose, Executive Administrative Assistant

Office of Research & Sponsored Programs, Room G-235

Phone: 330-325-6499

E-Mail: nhose@neomed.edu

CONTACT LIST FOR PROJECT APPLICATIONS

Applications may be submitted electronically at the link below:

Project Investigator contact information:

Anatomy and Neurobiology department's main office is located in E-116

Lisa Cooper, Ph.D.

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Natalie Bonfine, PhD
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NEOMED

**Office of Research &
Sponsored Program's
Student Research Fellowship
Program**

**Project
Descriptions**

Submit your application to Dr. Lisa Cooper

1) Title: Age-Related Changes to the Bone of Bowhead Whales

PI: Lisa Cooper and Chris Vinyard, Department of Anatomy and Neurobiology

LOCATION: NEOMED, D-hallway Biomechanics Lab

2) Abstract: Bowhead whales (*Balaena mysticetus*) are one of the longest-lived mammals in the world with a lifespan that exceeds 200 years. The rib bones of the long-lived bowhead whales undergo a unique strategy for mineralization in which the bones of newborns are rich with mineral and have a small medullary cavity. Sexually mature whales display greater bone mineral density and medullary cavities with a larger cross-sectional area. Our previous ontogenetic studies have shown that these shifts in bone phenotype are at least partially due to increased expression of the gene *EZH2*, an epigenetic regulator of bone mineral mass. This study quantifies the biomechanical consequences of these molecular and phenotypic shifts by measuring bone hardness of these ribs throughout the life of bowheads. Rib samples taken from bowhead whales and their close terrestrial relatives (i.e., pigs, cows) will be sampled at defined regions of interest and then dented using a Microindentation apparatus. Previous results from the bowhead data show that with increasing age, hardness of bone also increased, as was expected. These specimens will lay the foundation for a summer fellowship in which a qualified applicant will quantify and compare bone hardness and stiffness between whales, pigs, and cows. Results will be presented at the ORSP Summer Research Fellowship program, and potentially result in co-authorship in a resultant publication.

3) Background and Rationale: Function of ribs in whales is novel compared to that of terrestrial mammals in that these bones are loaded under compressive forces in water. Rarely, if at all, are these same bones exposed to the rigors of sheer and torsional forces associated with life on land in terrestrial taxa. In this comparative study of the tissue-level properties of bone, we aim to quantify how bone functions at the tissue-level in taxa with disparate habitats. Although phenotypic changes with age are well characterized in bowhead whales and pigs, nothing is known of the age-related changes in the bone extracellular matrix in a comparative context. By quantifying age-related changes in the bone extracellular matrix, our study will identify potentially unique biomechanical attributes of the whale rib cage and further our understanding of the requirements to breathe and live in an aquatic habitat.

4) Goals and Objectives: The overall objective of this study is quantifying the biomechanical consequences of differences in phenotype within the rib bones of bowhead whales, pigs, and cows. Based on preliminary data, the working hypothesis for this study is that whales, compared to pigs and cows, display a novel strategy for mineralization that results in hyperdense bone that is more brittle compared to terrestrial mammals.

5) Investigative Methods: Based on results of our previous microindentation analyses, this study will expand our bowhead whale dataset by comparing differences tissue hardness and stiffness in the rib bones of whales, cows, and pigs. Based on our established methods for denting, a summer fellow will characterize inter- and intra-specific differences in bone properties using ANOVA analyses. By characterizing the age-related shifts in bone tissue mechanics, we expect results to establish a critical understanding of the novel phenotypes documented in bowheads.

The fellow will be required to generate and analyze data from sections of frozen ribs. The student will receive fresh tissues that will then undergo a sampling procedure and be mounted on a metal holder and polished on a lapidary wheel. A student will then be expected to dent bones and quantify the dimensions of a dent and analyze the resultant data in a statistics program.

6) Proposed Method of Data Analysis: Significance of hardness and stiffness differences will be analyzed with ANOVA.

7) Significance of Anticipated Findings: The skeleton of whales is loaded through largely compressive forces in an aquatic habitat and this differs from the torsional and shear forces that are standard loading regimes in terrestrial mammals. As such, maintenance of bone mineral density throughout the lifespan of long-lived whales may display an altogether different strategy compared to terrestrial taxa. Curiously, the evolutionary history of rib bone phenotypes in fossil whales is documented along their land-to-sea transition, and shows that increases in bone mineral density were potentially critical to their ability to counteract body buoyancy. In addition, in our recent publication, we note that hyperdense ribs and actions of bone eroding cells suggest that the ribs of bowheads may be acting as a mineral reservoir for the production of keratinous plates of baleen that include calcium in their extracellular matrix. Taken together, these data suggest that rib bones have a historical significance for the evolutionary success of whales, and may also play a unique role in the growth and development of baleen, a hard tissue that is critical for the feeding apparatus of some whales.

Our contribution is expected to further our goal of understanding the unique attributes of whale ribs compared to that of terrestrial mammals. If the concept proves out, the contribution will be significant because data will assist in developing a more nuanced understanding of dynamic shifts in ballast and buoyancy throughout the lifespan of bowhead whales. It is also highly likely that our results will vertically advance our understanding of plasticity within the mammalian skeleton associated with life in novel habitats.

Student Fellow Training/Mentoring Plan (1/2 page): Funding is requested to support one summer research fellow. PI's Cooper and Vinyard are committed to fostering the researcher's development for the summer. This goal will be achieved through a structured mentoring program, as described below. First, the researcher will be trained to participate in every phase of project research, including specimen preparation and analyses. Opportunities for students to gain experience with unusual model organisms are rare, and the skills gained through involvement with this project should substantially broaden the researcher's skill sets. Besides benefiting from working alongside both PI's, the student will be required to attend and present at bi-monthly laboratory meetings. The Musculoskeletal Research Focus Area - a joint effort of the Department of Anatomy and Neurobiology and the Department of Integrated Medical Sciences at NEOMED – also sponsors a regular brown bag seminar and journal club on the general topic of "Evolutionary Morphology", where the fellow would have the opportunity to share and discuss ongoing research findings and pertinent scientific publications. Finally, the student will design and present a poster for the end-of-program poster symposium at NEOMED.

Submit your application to Dr. Rebecca German

1. *The Effect of Preterm Birth and RLN Damage on Airway Protection and Maturation*

Principal Investigator: Rebecca Z. German, Professor

Location: NEOMED, Department of Anatomy and Neurobiology

2. Abstract: The overarching objective of my research group is to understand the biomechanics and neural control in normal and pathophysiologic swallowing (dysphagia) and to develop rehabilitation strategies for infants with this condition. Coordination among the functional components of the aerodigestive system, particularly between swallowing and respiration, is critical for successful airway protection in infants. Disruption of this coordination can produce failure of airway protection, manifest as pulmonary aspiration. These problems may be compounded with damage to the recurrent laryngeal nerve (RLN) resulting from cardiovascular repairs necessitated by prematurity. Because the current understanding of the pathologies in these fragile patients is based largely on non-invasive technologies, the causal relationship between disordered coordination and airway protection, including how development impacts this system, is unknown. This project will determine the kinematic and biomechanical deficits in an animal model of such infants, and how those deficits change longitudinally over the course of development. Such data will change our understanding of the potential for recovery and be the basis for designing intervention strategies for preterm infants.

3. Significance: Preterm infants constitute 11% of all infants born (Martin et al. 2015). In those infants, pulmonary aspiration occurs frequently, and is correlated with pneumonia and associated lung injuries (Hegde and Greenberg 2015). Given the fragile nature of preterm infants, existing data on airway protection is frequently qualitative (Lau and Smith 2012). Even less is known about airway protection in preterm infants with recurrent laryngeal nerve (RLN) damage due to patent ductus arteriosus (PDA) ligation or other cardiovascular surgery (Tashiro et al. 2014), including any interaction of gestational age (GA) and nerve damage.

4. Goals and objectives: We have created an animal model to measure the coordination between respiration and swallowing in infants, and test the impact (1) of prematurity, (2) RLN sensory damage, and (3) the recovery and maturation over time of that coordination. We will be collecting several modalities of data (outlined below), each of which needs to be independently analyzed, and ultimately integrated into a larger picture. A trainee will participate in all aspects of data collection over the summer, but will design and carryout a project that will include hypothesis formulation and testing for a portion of these data. This strategy has been successful with numerous trainees.

5. Research Methods used/learned by students: The methods in this project include electrophysiology and highspeed fluorographic imaging. We will measure (1) performance or the degree of aspiration; (2) the kinematics, movements of oropharyngeal and laryngeal structures, using high-speed bi-planar videofluoroscopy; (3) the kinematics of respiration and airflow. Because we can measure these factors simultaneously in an animal model, we will determine the causal links amongst these levels, and thus be able to translate our results to patients with dysphagia. *Data Collection Methodology:* We will film movements of the bolus and surgically implanted markers in the tongue, hyoid bone, and epiglottis at 100 fps. We will simultaneously measure respiration at both the thorax (using a plethysmograph band) and the nares (using infant thermocouples), which will permit us to infer laryngeal function as a valve controlling respiration.

6. Proposed method of data analysis This project will use the data analyses used in previously published work (Thexton et al. 2009; Gould et al. 2015a; Gould et al. 2015b; Ballester et al. 2018; DeLozier et al. 2018). We will compare the timing of the swallow to the respiratory traces, and determine the precise (msec) coordination for each swallow. Groups of swallows will be compared over time (longitudinal design), as well as to recently collected control infant data. We will also analyze bolus movement, as well as movements of the hyoid, thyroid, epiglottis and tongue. Relative timing of the movement among structures is possible with cross-correlation analyses. For each feeding session, we will calculate the average frequency of sucking, swallowing and the volume per swallow.

7. How the anticipated findings from the summer research fellow will contribute to the success of the overall research being investigated: The results from these experiments will determine how the developmental course of airway protection differs in preterm infants from term controls. The students involved in this research will help collect the primary data to address the aims outlined above. The students will be involved in all phases of this work, from pre-experiment planning, through surgery, animal care, data collection on these infants, data reduction, and data analysis. Given that RLN injury often occurs in preterm infants, this model and design will permit us to understand the interaction between gestational age and iatrogenic insults. The data collected and analyzed by the trainees will provide a physiologic basis for decisions about care and intervention in these delicate patients that are informed by developmental changes.

Summer Research Fellow Training/Mentoring Plan

The student research fellow will be involved in all phases of the project. Initially, the fellow will be exposed to the larger research program through individual meetings with the PI, weekly lab meetings, and weekly Musculoskeletal Journal Club (four PI's plus labs, discussion of ongoing projects). The fellow will be given several suitable papers to review at the start of the project. There are several parts of this project that are appropriate for a summer project. The trainee and PI will discuss these sub-projects, and select one that is appropriate. The trainee will be responsible, working with the PI, for developing a hypothesis, alternatives, and outlining the necessary data to test the hypotheses. Because significant amounts of data for multiple projects are collected within a single "experiment", the trainee will work with other lab members in each stage of data collection. The project selected by the trainee may be sufficient to result in a publication at the end of the summer.

Complete resources for this project are available in the PI's lab which is part of the Anatomy & Neurobiology Biomechanics Laboratory at NEOMED (PI's: German, Vinyard, Young). The PI's lab includes 2 postdoctoral fellows, 2 other trainees and 1 technician. There are likely to be 2-3 summer fellows as part of this project, which is a sub-project of a currently funded NIH R01 grant. There are sufficient funds to cover the experimental costs.

Literature Cited

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- Thexton, A. J., A. W. Crompton, T. Owerkowicz and R. Z. German (2009). "Impact of rhythmic oral activity on the timing of muscle activation in the swallow of the decerebrate pig." J Neurophysiol **101**(3): 1386-1393.

Submit your application to Dr. Tobin Hieronymus

Title: *In-vivo* Study of Smooth Muscle Function

PI: Tobin Hieronymus, Assistant Professor of Anatomy & Neurobiology

Research Location: NEOMED

Abstract: Smooth muscle plays a critical role in the function of many organ systems, but our ability to understand the effects of smooth muscle contraction are limited by our current inability to directly record smooth muscle activity *in-vivo*. Unlike skeletal muscle, smooth muscle does not depend on cell-membrane depolarization to coordinate contraction, so standard techniques such as electromyography (EMG) will not work. Current research in Hieronymus lab is focused on two major aims: (1) applying established methods of measuring brain activity (fiber photometry) to the novel setting of peripheral smooth muscle tissues to directly record activity, and (2) developing selective, localized, and reversible interventions to manipulate smooth muscle contraction for functional studies. This summer research project will involve summer fellows in the current phase of one or both of these aims, where students will contribute to surgeries and behavioral trials using our model systems for dermal smooth muscle: domestic chickens (*Gallus gallus*) and golden pheasants (*Chrysolophus pictus*).

Significance: In addition to the smooth muscle lining the GI tract, airways, and vasculature, avian skin contains bundles of smooth muscle responsible for moving the feathers, similar to the arrector pili muscles in human skin. Because they are abundant and comparatively large, these muscles form a readily accessible experimental model for studies of smooth muscle physiology. My lab is developing a fiber photometry instrument to record smooth muscle activity *in vivo* and investigating means to locally and reversibly knock down smooth muscle function—both of these aims feed into the broader goal of testing hypotheses of smooth muscle function *in vivo* during normal behavior. Current electrophysiology-based methods do not allow for direct measurement of smooth muscle contraction, and instead rely on indirect measures of correlated skeletal muscle contraction, which are only present in some systems. The ability to directly measure and reversibly manipulate smooth muscle function *in vivo* has implications across urogenital, gastrointestinal, and cardiopulmonary physiology.

Goals & Objectives: The goals of this study are (Aim #1) to test the efficacy of calcium-sensitive dyes coupled with fiber-optic photometry to record smooth muscle activity in awake, behaving individuals, and (Aim #2) to assess pharmacological agents for reversible, local, post-synaptic control of smooth muscle contraction.

Research Methods: Aim #1 uses fiber photometry, a technique that is widely applied to measure localized brain activity using fluorescent indicator dyes and fiber-optic light delivery/recovery. This project applies fiber photometry in a novel setting, within peripheral muscle tissue, in an attempt to produce the first direct *in-vivo* recordings of smooth muscle activity. Aim #2 employs cell-permeable pharmacological agents that are known to temporarily disrupt myosin light chain kinase and rho kinase activity during smooth muscle contraction. Work on both aims will be accomplished in a combination of anesthetized trials and awake, behaving trials, by instrumenting and/or manipulating feather muscles that contribute to display in domestic chickens (*Gallus gallus*) and Golden Pheasants (*Chrysolophus pictus*). The

specific focus of student research in this project will depend in part upon our lab's progress on the research in winter and spring of 2021, and in part upon the interests of the student.

Data Analysis: Analyses for both aims will include digitization of videos of *in-vivo* feather movement during display behaviors. Student researchers will gain hands-on experience in training neural networks to track features and landmarks in video of behavioral trials. Analyses of photometry data will include signal processing and time series analyses that are common to a broad range of electrophysiological modalities (*e.g.*, electromyography).

Contribution to Overall Research Effort: Completion of studies under these aims will address different stages of developing two new technical approaches—the feasibility of the technical approaches is itself the focus of the funded research, so any outcome (positive or negative) will advance the project as a whole. Student projects will either form stand-alone papers or be incorporated into manuscripts reporting progress on the project.

Student Fellow Mentoring Plan: Student fellows will take part in weekly lab meetings with the lab PI and technical staff to identify and address lab-wide issues and tasks. Student fellows will also be expected to attend the weekly journal club for the Musculoskeletal Research Focus Area, providing exposure to a broad range of research topics as well as a chance to interact with researchers at different career stages—typical attendance in summer includes 2-4 summer fellows, 2-3 technicians, 1-4 graduate students, 3 postdocs, an assistant professor, two associate professors, and a full professor. Timely completion and reporting of the student fellows' projects will be ensured by weekly one-on-one meetings with the PI, not only to organize work but also to work through main tasks side by side (*e.g.*, worked examples of analysis, drafting, editing and presentation) both during the summer and through the following year as needed. Materials and expenses for the proposed research are supported by and NSF Grant 1838746 to the PI.

Submit your application to Dr. Julia Huyck

1. Project title, Principal Investigator name, title and location

Project: Processes underlying immature auditory perception during adolescence

Principal Investigator: Julia Jones Huyck Ph.D., Associate Professor & Program Coordinator, Speech Pathology and Audiology, Kent State University, and Voluntary Adjunct Assistant Professor, Department of Anatomy and Neurobiology, NEOMED.

Location: Speech Pathology & Audiology Program, Kent State University (1325 Theatre Drive, Kent, OH)

2. Abstract of project

Hearing and listening are critical to how adolescents communicate, learn new information, and engage with technology and culture; however, performance on auditory perceptual tasks takes a long time to become mature. Because few studies of auditory perception have centered on typically developing adolescents, little is known about the mechanisms underlying this immaturity. This project will evaluate the extent to which auditory stimulus encoding and various cognitive processes contribute to immature auditory perception during adolescence, using a combination of perceptual testing, neuropsychological and language testing, eye-tracking, and auditory evoked potentials (electrophysiology).

3. Background and rationale

Despite growing evidence that older children and adolescents have immature auditory perception (Buss et al., 1999; Hartley et al., 2000; Johnson, 2000; Wightman and Kistler, 2005; Bishop and Dawes, 2008; Lutfi et al., 2010; Wightman et al., 2010; Banai et al., 2011; Ross et al., 2011; Buss et al., 2017; Huyck and Wright, 2017; Huyck, 2018; Huyck and Rosen, 2018), most developmental studies only evaluate children up to 9 to 12 years of age and do not span the entire age range from early adolescence to adulthood. Thus, little is known about the processes underlying the prolonged maturation of hearing and listening abilities. Frequently when older children or adolescents perform more poorly on perceptual tasks than do adults, there is some debate as to whether the differences are due to sensory or “nonsensory” factors (Bishop and Dawes, 2008; Wightman et al., 2010b; Halliday et al., 2012; Huyck and Wright, 2013, 2017). The research will evaluate the relative contributions of spectral and temporal (sensory) encoding and a variety of cognitive (a subset of “nonsensory”) functions to immature auditory perception during adolescence using a combination of psychological and physiological measures.

4. Goals and objectives

The goal is to evaluate the extent to which auditory stimulus encoding and various cognitive processes contribute to immature auditory perception during adolescence.

Learning objectives:

- The fellow will become familiar with the medical research environment by actively participating in lab meetings and departmental journal clubs.
- If in-person research is allowed in summer 2021...
 - The fellow will learn to collect data on auditory learning from adolescents and adults using custom computer programs.
 - The fellow will learn to administer neuropsychological tests to assess cognitive skills.
 - The fellow will learn to collect auditory evoked potentials and eye-tracking data.
- If in-person research is NOT allowed in summer 2021 due to the pandemic...
 - The fellow will learn about auditory processing in late childhood and adolescence through a literature review.

- The fellow will learn to analyze perceptual data previously collected both in-person and online.

5. Investigative methods to be used

Human Subjects: All procedures are approved by the Institutional Review Board at Kent State University (IRB #15-355 & #20-299). 10- to 23-year-olds will be recruited from northeast Ohio through flyers and letters sent home from local schools. Participants will have normal hearing as confirmed by a pure tone audiogram. They will be excluded if they (or their parents) report that they have a history of hearing loss, language impairments, learning disabilities, attention deficit/hyperactivity disorder, traumatic brain injury, or other major neurological problems. Participants will be compensated for their time with gift cards. Study procedures pose minimal risk.

Auditory perception, sensory encoding and temporal processing will be measured using combination of perceptual testing, pupillometry and blink-rate to index cognitive processes engaged during active listening, auditory evoked potentials to index temporal and spectral encoding during unattended stimuli, and standardized neuropsychological and language tests.

In the event that Kent State's campus is closed to research with children in summer 2021 due to the pandemic, the project will involve analysis of data collected both in-person and online.

6. Proposed method of data analysis

Most data will be collected via custom computer programs. Some listening tasks and standardized tests will require manual scoring. Data will be analyzed using hierarchical regression and linear mixed models.

7. Significance of anticipated findings

The scarcity of information about auditory perception by typically developing adolescents provides little basis of comparison for adolescents with communication disorders. Thousands of children and adolescents in the United States have normal hearing thresholds but report difficulty on some listening tasks, either due to bottom-up perceptual deficits, language disorders, top-down cognitive issues, or some combination thereof (Loo et al., 2013; Bellis and Bellis, 2015; Moore, 2015). The lack of knowledge of the processes underlying the prolonged maturation of hearing and listening abilities in typical listeners can lead to difficulties in the diagnosis of auditory processing disorders (Loo et al., 2013; Ludwig et al., 2014; Moore, 2015). This project will yield experimental protocols that will be applicable for the development of diagnostic tests regarding auditory processing in adolescents and young adults and provide insights for the development of rehabilitation strategies to treat disorders affecting auditory processing in this population.

Summer Research Fellow Training/Mentoring Plan

Research will be conducted in Dr. Huyck's laboratory, which is part of the Speech Pathology and Audiology Program at Kent State and the Hearing Research Group (HRG) in the Department of Anatomy and Neurobiology at NEOMED. Dr. Huyck's lab emphasizes professionalism, enthusiasm, and scientific rigor. The fellow will receive training from Dr. Huyck and her collaborators in data collection and/or analysis. Lab members meet weekly to develop new projects, address technical concerns, and discuss results and related research.

The fellow will attend regular meetings of the Hearing Research Group (HRG), a highly interactive group composed of members of ten hearing neuroscience laboratories with a wide range of experimental approaches. The fellow is expected to present a summary of the summer project to this group.

For more information on this project please contact:

Julia Jones Huyck, Ph.D.; jhuyck@kent.edu; 330-672-0249

Submit your application to Dr. Jeffrey Mellott

1. **Project Title:** Age-related GABAergic loss in the central auditory circuits. Jeffrey Mellott, PhD. NEOMED; Building E; Rooms 147/153
2. **Abstract:** Age-related hearing loss (ARHL) is one of the most common maladies of industrialized populations. Essentially, as the cochlea (periphery nervous system) ages, excitatory input is lost to the central nervous system. To compensate for the lost excitation, the central nervous system down-regulate inhibition in an attempt to maintain the correct balance of excitation and inhibition. Eventually this inhibitory downregulation becomes problematic as the lost inhibition leads to a variety of hearing deficits. These deficits include difficulty interpreting speech and detecting salient signals from noisy environments. We investigate age-related inhibitory changes in the inferior colliculus (IC) during middle age before ARHL would onset and in old age when hearing deficits are common. The IC is 1) pivotal for the processing of complex acoustic stimuli, 2) receives inhibitory input from a diverse set of nuclei, 3) contains a vast population of inhibitory cells and 3) downregulates inhibition during aging.
3. **Significance:** The project will identify the circuits in the auditory midbrain that undergo age-related changes and whether those changes are occurring prior to the onset of temporal processing deficits.
4. **Goals and objectives:** The student will determine IC regions and circuits that lose inhibitory input during aging.
5. **Methods used:** We will use electron microscopy, immunohistochemistry, fluorescent microscopy, in situ fluorescence hybridization, traditional tract-tracing, neuron reconstruction.
6. **Proposed methods of data analysis:** Most analysis will be conducted with NeuroLucida Explorer (MicroBrightField) for fluorescent microscopy and SerialEM for Electron Microscopy.
7. **Impact of findings:** Our long-term goal is to understand how aging affects auditory midbrain circuits. Findings from this project will help determine the auditory circuitry that attempts to compensate for age-related inhibitory changes. Furthermore, findings will help determine when these changes begin to occur and if these central changes can be related to the changes seen in the periphery.

Student Mentoring Plan

1. The student will be expected to attend weekly lab meetings. As a part of the Hearing Research Group, they will be expected to attend Friday morning Journal Clubs. The student may be asked to present their findings during one of the Journal Clubs.
2. Most of the tissue necessary to conduct the data collection and analysis is already "on slide". Generating the cases needed will be minimal or absent from the 8-week term, which will help maximize the student's time. All needed elements to complete the study

are fully function and routinely used in the lab. Microscopy training will come from the PI. Training on the needed software will be a combination of the PI and Research Assistant.

3. All experiments will be conducted and analyzed in E-147/E153 and E-57.

Submit your application to Dr. Merri Rosen

Project Title, Principal Investigator

How does stress affect hearing? The effects of developmental stress on auditory neural circuits
Merri J. Rosen, Ph.D., Associate Professor; Director, Hearing Research Group
Dept. Anatomy & Neurobiology, NEOMED, D113-115

Abstract of Project

During childhood, impaired sensory experience (such as hearing loss) is known to alter sensory perception later in life, and this is accompanied by changes in sensorineural regions of the brain. In a parallel nature, developmental stress is well known to affect anxiety and cognition, and the related neural circuits. Recently, our lab has been the first to find that early life stress also affects auditory neural regions, and causes impairments in auditory perception in an animal model of hearing. This leaves us in the exciting position of starting to elucidate the neural mechanisms that underlie these perceptual deficits. Our recently-renewed R01 grant from the NIH funds us to study the mechanisms underlying how early-life stress and early hearing loss together and separately impair auditory perception.

This summer project will involve immunohistochemical analysis of neurons in the auditory cortex and thalamus. We will use earplugs to mimic hearing loss during development in gerbils, and will induce stress via intermittent maternal separation. The student will quantify how these manipulations affect specific cellular and molecular elements in the auditory pathway that we have hypothesized could contribute to perceptual deficits and abnormal maturation. These include changes in inhibitory versus excitatory neurons, in glucocorticoid receptors, in molecules related to neural plasticity, in dendritic morphology, and in subtypes of neurons important for developmental refinement of neural connectivity.

Significance of research

Early hearing deprivation, such as recurrent conductive hearing loss from ear infections during childhood, produces deficits in performance that can endure for years after hearing thresholds have returned to normal. Speech processing skills are directly at risk, as both perceptual and productive language deficits correlate with the severity of hearing loss in children. Separately, early life stress (ELS) is a well-known risk factor for children's cognitive and emotional development. But ELS also increases the risk of sustaining long-term speech and language deficits when it co-occurs in children with early hearing loss. This can be a particular problem in low socio-economic populations, where the prevalence of recurrent otitis media is significantly higher than in the general population, and where children are more likely to suffer from social isolation and stressful events.

Ongoing work in our lab has documented several perceptual changes induced by early hearing loss, and has linked them to changes in how auditory cortical neurons respond to sound. We have recently demonstrated that similar perceptual and cortical deficits are induced by ELS (via maternal separation). Further, the perceptual deficits induced by the two insults together are worse than those induced by either one alone. As of yet, it is unknown exactly what types of cellular and molecular changes are responsible for the altered responses of these neurons to sound. Understanding how stress perturbs normal development of the auditory pathway, and how it interacts with neural effects of developmental hearing loss, can provide potential targets

for remediation. This can draw attention to the importance of clinical intervention, particularly in low socio-economic populations.

Goals and objectives

The student will become familiar with the background related to this project. This includes literature regarding 1) the effects of early-life stress on brain regions involved in anxiety and cognition (because there are no studies on the effects of early-life stress on sensory regions), and 2) the effects of early hearing loss on the auditory cortex.

The student will learn to perform quantitative data analysis of immunostained neural tissue. This involves learning how to use anatomical software packages.

Research methods to be used

We will label particular subsets of neurons with specific markers that indicate their role in neural plasticity and/or susceptibility to stress hormones. The student will then quantify the density of specific cell types or cells expressing specific markers, and quantify dendritic morphology. This will involve immunohistochemistry, fluorescent microscopy, tract tracing, neural reconstruction, and stereology.

Proposed method of data analysis

Neurons that are labeled with particular markers will be quantified using Stereo Investigator or NeuroLucida Explorer software.

Relevance of summer project to overall research goals

At present, the mechanisms by which early-life stress alters auditory perception are unexplored. The anticipated findings from the summer fellow will be one step in elucidating mechanisms by which early-life stress affects neural elements in the auditory pathway that could underlie perceptual deficits which our lab has described.

Training / Mentoring Plan

Early on, I will spend time with the student explaining the details of the background and project. My research technician and postdoctoral fellow will be primarily working with the research fellow on the details of conducting the experiments. I will conduct individual research meetings with the research fellow weekly. We also have weekly lab meetings in which the student will participate, and will present her/his results as they are acquired and analyzed. The Hearing Research Focus Group at NEOMED is a robust active group, and we have weekly Group meetings discussing literature in the field and laboratory projects. The fellow will attend these meetings and will be encouraged to engage in our discussions.

Resources: I have an R01 research grant to fund my lab, two technicians, a graduate student, and a postdoc who are available to help in training the student. We are collaborating on this project with Dr. Jeff Mellott, who is available for support. The experimental protocol is worked out and the student can begin collecting data as soon as s/he is comfortable. The research will be conducted in my laboratory or in shared microscope cores on the NEOMED campus.

Submit your application to Dr. Sharad Shanbhag

A. Project Description

1. Project information:

Title: Brain Circuitry Underlying Hearing and Emotions

Principal Investigator: Sharad Shanbhag, Ph.D., Research Associate Professor

Co-Investigator: Jeffrey Wenstrup, Ph.D., Professor and Chair

Location: Department of Anatomy and Neurobiology, NEOMED

2. Abstract

Our work investigates neural mechanisms underlying the process by which emotional centers in the brain assign meaning to social vocalizations. Past experiments in our lab have found neurons in the amygdala that respond selectively to social vocalizations. We have examined how contextual cues associated with a social vocalization alter the interpretation of that vocalization by the individual and by neurons in the amygdala. Recently we have shown that the behavioral and amygdalar response to a vocalization is differentially altered by exposure to olfactory cues associated with either mating or predators. We now propose to examine the mechanisms of selectivity for vocalizations as well as the source of contextual cues in the amygdala. Using optogenetic techniques, we will selectively inactivate cortical or thalamic inputs to the amygdala while assessing the effects on amygdalar neural activity and responses to vocal stimuli. Histological analysis of labeled neurons will help to delineate brain regions involved in the network for vocal signal processing.

3. Background and rationale

Our long-term goal is to improve the understanding of neural mechanisms that underlie acoustic communication. This project focuses on the amygdala, a structure known for its role in auditory fear conditioning. For this role, it receives auditory input from the thalamus and cortex, contributes to identifying a stimulus as aversive, and provides for appropriate emotional responses (e.g., autonomic responses, freezing). Our view is that the amygdala plays a critical role in acoustic communication through participation in several processes. Dysfunction in the amygdala may be involved in abnormal relationships between acoustic inputs and emotional responses in disorders such as autism, schizophrenia, post-traumatic stress, and tinnitus. We are interested in which neural inputs provide vocalization-specific and contextual information necessary for interpretation of acoustic. This is the next step in understanding how these neural inputs act on amygdalar neurons to influence behavior.

4. Goals and objectives

Our long-term goal is to improve the understanding of neural mechanisms that underlie acoustic communication. This summer project aims to identify and quantify the mechanisms of vocalization-selective responses. We hypothesize that discrimination and selectivity in response to social vocalizations arises from projections of secondary auditory cortical areas. We further hypothesize that inputs from the prefrontal cortex, ventral tegmental area and hippocampus underlie contextual modulation of auditory responses.

5. Investigative methods to be used

To identify inputs to the basolateral amygdala, we combine neurophysiological recording with optogenetics. After surgical preparation, injections of recombinant adeno-associated virus (rAAV) coding for light-activated ion pumps or channels are performed into target structures in

the mouse brain. Following a 4-6-week survival period, we record neurophysiological responses to vocal stimuli. Light from a fiber-coupled laser or LED is used to inactivate inputs to the amygdala that originate from the rAAV-injected brain structure. At the completion of recordings, histological preparation and analysis of brain sections will reveal labeled neurons that project to the amygdala. We will evaluate the numbers, types, and distribution of labeled cells that project specific auditory or contextual information to the basolateral amygdala. Data from multielectrode electrophysiology will be analyzed using spike sorting software and custom analysis software.

6. Proposed method of data analysis

Neurophysiology: Electrophysiological responses will be plotted and quantified off-line. Initial steps will include exporting of raw data for spike sorting, processing of data using spike sorting software and then integration of sorted data with stimulus information.

Anatomy: Slide-mounted and cleared tissue sections will be examined using fluorescence microscopy. Sections containing fluorescently labeled neurons or fiber tracts will be digitally photographed and the images stored for further off-line analysis. Analysis will include, but not be limited to, counts of cell bodies in areas of interest and reconstruction of projection pathways to the BLA.

7. Significance of anticipated findings

- The results will provide the description of the convergence of auditory and other sensory information in amygdalar regions responsive to social vocalizations. This helps to establish the neural circuitry underlying contextual modulation of auditory responses.
- The results will identify the regions of the auditory pathway that endow amygdalar neurons with responsiveness to social vocalizations. The results will identify origins of vocalization-selectivity in amygdalar neurons.
- The results will identify brain regions that project to the amygdala to modulate auditory responses. This work will explain how specific inputs to the amygdala contribute to behaviors associated with social communication by sound.

B. Summer Research Fellow Training/Mentoring

All research will be conducted in the Acoustic Communication and Emotions Laboratory, which is part of the Department of Anatomy and Neurobiology at NEOMED. The laboratory includes two faculty, two research associates, and one postdoctoral fellow (Dr. Mahtab Tehrani). The student will work closely with Dr. Shanbhag and Dr. Tehrani and will interact extensively with other laboratory members.

The laboratory emphasizes collaborative interactions, high expectations and enthusiasm. The group meets in weekly laboratory meetings where ideas are developed, and technical issues and results discussed. Our laboratory has an extensive record of mentoring undergraduate and professional student trainees since 2009. The fellow will also attend the weekly journal club of the Hearing Research Group (HRG). The highly interactive HRG is composed of members of nine hearing neuroscience laboratories with a wide range of experimental approaches. The fellow would be expected to present a summary of their summer project to this group. The fellow will be trained in many of the procedures associated with this project, commensurate with their skill and ability. If interested, the student will participate in neural recording experiments, including fabrication of electrode-optical fiber arrays for optogenetic experiments. The student will participate in histological processing to prepare brain sections for

subsequent neural imaging and tracing. The student will also participate in analyzing the results of neural tracing studies and the neural recording data. Experience or expertise in programming (e.g., Python, MATLAB, Java), histology (including processing of tissue and microscopy) or CAD/CAM is desired but not required. From work in our lab, the student will gain experience in identifying brain regions, histological and electrophysiological techniques, and data analysis.

Submit your application to Dr. Chris Vinyard

1. PROJECT TITLE: Bone density in an oversized population of island mice.

Investigator: Chris Vinyard
Department of Anatomy & Neurobiology
NEOMED
Location: NEOMED

2. ABSTRACT OF PROJECT:

This fellowship will support an ongoing project aimed at describing skeletal variation in house mice as it relates to a rapid size increase following an island colonization event. Specifically, we will quantify skeletal bone density of house mice from Gough Island (GI) that have evolved to become the world's largest house mice since populating the island a few hundred years ago. As the largest wild house mice on record, this provides an opportunity to compare Gough Island and smaller mainland mice to see how morphologies have changed in the GI mice. Though not the specific goal of the summer project, these data will ultimately be used to identify the genetic determinants of these morphological changes.

Morphological phenotypes will be collected from the skeleton using a combination of microCT imaging, and image-analysis software. Our primary focus will be on measurement of bone density and integration with other biomechanical parameters that affect skeletal performance. The data collected in this project will facilitate a more complete understanding of 1) variation in skeletal traits between inbred, laboratory and GI mice and 2) how this variation may relate to functional mechanics. These results will also inform evolutionary studies of mammalian skeletal adaptations.

Student responsibilities will include: working with microCT images, measurements of bone density using skeletal cross-sectional images, conducting statistical analyses of measurements, and summarizing and reporting results in summer student poster session.

3. BACKGROUND AND RATIONALE:

Populations often experience rapid evolution after colonizing islands. One example of this phenomenon is observed in island populations of house mice, particularly on Gough Island, where rapid size increases have made them the largest wild house mice on record. These mice display a variety of skeletal changes compared to their mainland relatives. Comparing Gough and mainland mice provides a powerful approach for evaluating the adaptive divergence of island populations as well as the genetic basis of evolutionary change. This project will assess how a rapid increase in body size impacts bone density and related architecture, which has implications for bone strength and function. In the future, these results will be applied in tandem with quantitative trait loci (QTL) mapping to identify genomic regions associated with these morphological changes.

4. GOALS AND OBJECTIVES:

Our proximate goal is to quantitatively assess differences in skeletal density between a large island population of mice and an inbred laboratory strain of mice. Our ultimate goal is to identify genomic regions that have a major effect on the skeletal phenotypes identified in this project. As part of our proximate goal, we have the following specific aim: 1) to characterize phenotypic variation within and between Gough Island mice and a representative mainland mouse.

5. INVESTIGATIVE METHODS TO BE USED:

This project primarily involves making precise, quantitative descriptions of morphology. We initially dissect and skeletonize mouse cadavers. Some are measured using histomorphometrics. Other specimens will be microCT scanned. CT data is output to .tiff files. We will import stacked .tiff files into Avizo Software for virtual reconstruction and re-slicing along desired planes. Slices will be output as new .tiff files and cross-sectional dimensions analyzed using ImageJ. We will phenotype large samples of both inbred and GI mice.

6. PROPOSED METHODS OF DATA ANALYSIS:

We will analyze variation in density and other bone phenotypes by comparing GI and mainland mice. We will also consider variation across an F2 cohort of GI-WSB crosses. We will test hypotheses of morphological and functional variation in bone measures using t-tests.

7. SIGNIFICANCE OF ANTICIPATED FINDINGS:

We are not addressing a particular clinical pathology but rather asking a basic science question about the underlying genetic architecture of skeletal form and function. We anticipate that the basic science information gathered here will further inform our understanding of evolutionary studies of mammalian skeletal adaptations and clinical understanding of bone biology.

STUDENT TRAINING/MENTORING PLAN

The fellow will work closely with Dr. Vinyard to become familiar with and proficient at all relevant techniques. Initially the student will be given relevant background readings that will then be discussed with Dr. Vinyard. The fellow will then be taught the appropriate data collection and analytical techniques. The student will have opportunities to learn about bone biology, technical aspects of microCT, morphological data collection with image-analysis software, data transformation and statistical analysis of the relevant data. The importance of statistical hypothesis testing will be emphasized in this fellowship. Fellows will participate in the weekly biomechanics journal club and the end of summer research presentation at NEOMED. If offered by Dr. Aultman (as in previous years), fellows will have the opportunity to participate in a weekly summer research series mini-course. Furthermore, they can continue in this research after the 8-week term of the fellowship and be part of publications in peer-reviewed journals if so motivated.

All required materials and equipment for data collection and analysis are available in the faculty sponsor's lab facility.

The study will be conducted in the Department of Anatomy & Neurobiology – NEOMED.

Submit your application to Dr. Jesse Young

I. PROJECT TITLE, PRINCIPLE INVESTIGATOR, AND LOCATION

Project Title: Elastic loading of the Achilles tendon: links to lower limb anatomy and tendinopathy

Principle Investigator: Jesse Young, Associate Professor, Dept. of Anatomy and Neurobiology

Location: Comparative Biomechanics Laboratory, D103 – D111

II. ABSTRACT

Achilles tendinopathy (i.e., pain and swelling of the Achilles tendon) is one of the most common sports-related injuries. The Achilles tendon (AT) is a shared tendon for the gastrocnemius and soleus muscles (i.e., the *triceps surae*), which originate on the distal femur and proximal tibia, respectively, and insert on the calcaneal tuberosity in the foot. This muscle group produces plantarflexion at the ankle joint and is important for generating positive work during the latter part of stance phase during both walking and running. Previous studies suggest that excessive tendon strain is a primary factor responsible for tendon damage and that repetitive loading (i.e., overuse) contributes to AT injury and tendinopathy. There are a number of intrinsic factors which play a part in determining how much load is experienced by the AT during locomotion, and thus how much the AT is strained. These variables include kinematic measures (e.g., joint angle, joint moments), tendon morphology (e.g., cross-sectional area), tendon material properties (e.g., elastic modulus - a measure of stiffness), lower limb anatomy (e.g., limb segment lengths), and foot geometry (e.g., ankle moment arm length). In this study, we will use a pre-existing dataset of anatomical and kinesiological data on walking and running humans to create computational models of AT loading, allowing us to precisely explore which variables are the best predictors of AT strain and possible risk for injury.

III. SIGNIFICANCE

We will develop individual computational models derived from *in vivo* kinesiological and morphometric data to understand how anatomical variables like foot geometry (i.e., ankle moment arm) are linked with tendon strain. The results from this study will provide a comprehensive and thorough understanding of how intrinsic anatomical parameters and extrinsic kinematic variables explain how loads are imparted to the AT. Exploring these relationships with this comprehensive methodology offers a novel and transformative approach to validate the use of anthropometrics as potential biomarkers of an individual's risk for injury, perhaps leading to better prevention of chronic overuse injuries.

IV. GOALS AND OBJECTIVES

The primary aim of the proposed study is to use a preexisting dataset of morphometric and kinematic data to build customized subject-specific computational models of AT dynamics during walking and running, allowing us to precisely quantify how anatomical and kinematic variation impact tendon strain during gait and providing validated, easily measured biomarkers of injury risk. We will specifically test the following predictions:

- P1: Controlling for variation in subject body mass, AT strain will be inversely proportional to the length of the calcaneal tuber (a primary determinant of *triceps surae* moment arm length).

- P2: Controlling for variation in subject body mass, AT strain will be directly proportional to the length of the forefoot (a primary determinant of the torque produced by the ground reaction force).
- P3: Controlling for variation in subject body mass, AT strain will be inversely proportional AT cross-sectional area.
- P4: Controlling for variation in subject body mass, AT strain will be inversely proportional to AT stiffness (i.e., elastic modulus).

V. INVESTIGATIVE METHODS

This project is a collaboration between the Young Lab at NEOMED and Dr. Adam Foster's Lab in the Department of Anatomy at Campbell University. Over the past several years, the Foster lab has collected a robust dataset from 24 human subjects, including standard morphological measurements (i.e., limb segment lengths, external measures of foot geometry, and body mass), ultrasound measures of AT cross-sectional geometry, and in vivo biomechanical locomotor data while the subjects moved self-selected speeds ranging from walking to a maximum speed sprint. The Young Lab will leverage these data to develop subject-specific computer models using the proprietary software SIMM (Software for Interactive Musculoskeletal Modeling; Motion Analysis Corporation, Rohnert Park, CA) and the companion open-source program OpenSim (<https://simtk.org/>). Subject-specific musculoskeletal models will be built using SIMM's "Full Body Model" as a starting template. The model geometry will be refined using the scaling features of OpenSim to match model geometry to subject morphometry as closely as possible. The model will then be run throughout a dynamic simulation of walking and running, constrained by the kinesiological data. The results of these constrained dynamic simulations will then be analyzed to quantify how locomotor kinematics and between-subject variation in foot geometry, AT cross-sectional area, and AT material properties influence mean and peak levels of AT strain during locomotion.

VI. DATA ANALYSES

Mean and peak levels of AT strain, as calculated by the computational SIMM/OpenSim model, will be ported for each walking/running stride from each subject in the dataset. These data will be imported into the R Statistical Platform (<https://cran.r-project.org/>) and matched to subject-specific data encapsulating explanatory variables predicted to modulate AT strain (i.e., body mass, ankle moment arm length, forefoot length, AT cross-sectional area, and AT elastic modulus). Dependent and explanatory variables will then be entered into stepwise multiple regression models to calculate which parameters best explain variation in AT loading – and concomitant risk of injury – across subjects.

VII. INTEGRATION WITH OVERALL PROJECT

The data collected here form the bulk of a preliminary dataset that will be used for two purposes: 1) provide data for an upcoming peer-reviewed collaborative manuscript submission from the Young and Foster labs and 2) serve as pilot data for funding applications to federal agencies (e.g., NIH, DoD) and private foundations (e.g., Wenner-Gren Foundation for Anthropological Research).

Summer Research Fellow Training/Mentoring Plan

During my time at NEOMED, I have mentored a total of 39 high school, undergraduate, and medical students and advised five postdoctoral fellows. I am committed to fostering a positive, rewarding research experience for all trainees in my laboratory, including the summer fellow on this project. This goal will be achieved through the mentoring program described below.

First, the fellow will be trained to participate in every phase of project research, including data analysis, interpretation, and dissemination. This involvement will promote mastery of several skills necessary to accomplish holistic biomechanical research, such as the collection and analysis of kinematic and kinetic data and the use of common software packages (e.g., SIMM, OpenSim, and R). Opportunities for medical students to gain experience with *in vivo* biomechanical research are rare, and the skills gained through involvement with this project should substantially broaden the fellow's expertise. Additionally, I will mentor the fellow in a structured literature review, providing the student with the necessary theoretical and empirical background to understand the impetus for our research and the chosen methodology for addressing the research questions. All project findings will eventually be disseminated through presentations at professional conferences, peer-reviewed journal articles, and other scientific media. Where merited, the fellow will be given authorship on all presentations and publications relating to this project, even after the student is no longer actively working in my laboratory.

The student will be given the opportunity to participate in regular brown bag seminars and journals clubs sponsored by the NEOMED Musculoskeletal Biology Research Focus Area. Additionally, the fellow will participate in all laboratory meetings, both with the members of my lab and the members of the larger Comparative Biomechanics Laboratory that I run jointly with Dr. Rebecca German and Dr. Chris Vinyard.

Submit your application to Dr. Rachel Bracken

I. Project Description

1. Project title, Principal Investigator name, title and location

Project title: “Evaluating the Health Humanities as Essential Curriculum”

Co-Principal Investigators: Rachel Bracken, Ph.D., Assistant Professor, Department of Family and Community Medicine; Julie Aultman, Ph.D., Professor, Department of Family and Community Medicine; Rebecca Fischbein, Ph.D., Assistant Professor, Department of Family and Community Medicine

Location: NEOMED, Rootstown campus

2. Abstract of project

In recent years, the Association of American Medical Colleges (AAMC) has placed increased emphasis on the arts and humanities in medical education. NEOMED’s arts and humanities curriculum, including reflective practice, narrative medicine, philosophy of medicine, history of medicine, and disability studies has contributed to students’ moral and professional development, critical thinking and communication skills, and empathic patient care for over 20 years. However, arts and humanities education impacts students and professionals in ways that have yet to be extensively examined and assessed, particularly their impact on lasting habits of mind and practice: professional values, critical thinking skills, and an expansive worldview. Using a mixed-methods design which includes both focus groups and surveys, this project examines the deeper impact of arts and humanities in medical education across the range of professional development, including pre-clinical undergraduates, clinical undergraduates, residents, and practicing physicians. This project is poised to provide much needed evidence that integrated arts and humanities medical education fosters essential knowledge for medical practice in undergraduate education and beyond.

3. Significance of the overall research

For nearly 20 years Northeast Ohio Medical University (NEOMED) has supported longitudinal arts and humanities medical education integrated across the 4-year basic sciences and clinical medicine curriculum. Curricular components such as reflective practice, narrative medicine, philosophy of medicine, history of medicine, and disability studies contribute to NEOMED students’ moral and professional development, critical thinking and communication skills, and empathic patient care.¹ However, arts and humanities medical education impacts students and professionals in ways that have yet to be extensively examined and assessed, particularly the reconciliation of world views and power differentials through processes of textual reading and contextualization, and slow critical thinking to examine the human condition and achieve renewal (rather than resiliency) among professionals. Whereas existing studies demonstrate the utility of arts and health humanities curriculum for developing Inter- and Intrapersonal Competencies (e.g. soft skills, personal and professional values, and professional identity

¹ AAMC Core Competencies for Entering Medical Students: <https://www.aamc.org/services/admissions-lifecycle/competenciesentering-medical-students> Accessed September 13, 2020.

development)², our project is the first to evaluate arts and humanities education as fostering essential cognitive processes (e.g. slow, critical thinking) and knowledge of human behavior crucial to the development of core Science Competencies (per the AAMC). We argue that this material is often introduced or critiqued as optional enrichment or “soft skills” training, while instead it should be considered essential knowledge for the practice of medicine.³

While scholars have designed validated tools to measure reflective writing’s capacity to promote personal and professional virtues,^{4,5} to date, few studies have more comprehensively evaluated the effectiveness of arts and humanities education within medical training.^{6,7} Some scholars warn that the lack of compelling data implies that the goals of the medical humanities are too vague, abstract, and/or overblown.^{5,8} Yet advocates maintain that the learning objectives contained within an arts and humanities curriculum are notably difficult to measure, particularly in quantitative, rubric-oriented assessment modes better suited to measuring technical proficiency than habits of mind or subtle shifts in perception.^{7,9,10} Thus, Lake et al. advocate for “innovative qualitative evaluations with an emphasis on the appreciation of development, growth, creativity and complexity” (p. 770).⁹

4. Goals and objectives

Answering Lake and colleagues’ call for innovative evaluation of arts and humanities curricular interventions within medical education, the aim of this study is to perform a rigorous, mixed methods evaluation of NEOMED’s arts and humanities curriculum that captures the impact of the curriculum on student’s personal and professional development vis-à-vis the clarification of their values, the honing of their critical thinking skills, and the expansion of their worldview to contain a broader, historically and culturally contextualized understanding of medical practice. Specifically, this project will help us:

1. Examine the essential knowledge and habits of mind (e.g. critical thinking skills) fostered by NEOMED’s arts and humanities medical education curriculum;
2. Identify the impact of NEOMED’s arts and humanities medical education curriculum across the academic and professional career spectrum;

² Wald, H.S., McFarland, J. & Markovina, I. (2019) Medical humanities in medical education and training. *Medical Teacher*, 41.5, 492-6. <https://doi.org/10.1080/0142159X.2018.1497151>.

³ Shapiro, J., Coulehan, J., Wear, D., & Montello, M. (2009) Medical Humanities and Their Discontents: Definitions, Critiques, and Implications. *Academic Medicine*, 84(2), 192-98.

⁴ Wald, H.S., Borkan, J.M., Taylor, J.S., Anthony, D., & Reis, S.P. (2012) Fostering and Evaluating Reflective Capacity in Medical Education: Developing the REFLECT Rubric for Assessing Reflective Writing. *Academic Medicine*, 87(1), 41-50. <https://doi.org/10.1097/ACM.0b013e31823b55fa>.

⁵ Liao, H.-C. & Wang, Y. (2016) The application of of heterogeneous cluster grouping to reflective writing for medical humanities literature study to enhance students’ empathy, critical thinking, and reflective writing. *BMC Medical Education*, 16(234).<https://doi.org/10.1186/s12909-016-0758-2>.

⁶ Stern, D.T., Cohen, J.J., Bruder, A., Packer, B., & Sole, A. (2008) Teaching humanism. *Perspectives in Biology and Medicine*, 51(4), 495-507.

⁷ Ousager, J. & Johannessen, H. (2010) Humanities in undergraduate medical education: A literature review. *Academic Medicine*, 85(6), 988-98.

⁸ Bleakley, A. (2015) Seven Types of Ambiguity in Evaluating the Impact of Humanities Provision in Undergraduate Medicine Curricula. *Journal of Medical Humanities*, 36, 337-57. <https://doi.org/10.1007/s10912-015-9337-5>.

⁹ Lake, J., Jackson, L., & Hardman, C. (2015) A fresh perspective on medical education: the lens of the arts. *Medical Education*, 49, 759–772. <https://doi.org/10.1111/medu.12768>.

¹⁰ Belling, C. (2010) Sharper instruments: On defending the humanities in undergraduate medical education. *Academic Medicine*, 85(6), 938-40.

3. Gauge the need for and best approach to implementing arts and humanities medical education curriculum in residency and in continuing medical education; and
4. Inform the development of a validated, empirical methodology to evaluate AHME's impact on essential cognitive processes across the full academic and professional spectrum of medical education and training.

5. Research methods to be used

We are seeking two student Fellows to facilitate development and delivery of a mixed methods survey and to assist in conducting focus groups to identify critical competencies. This research project affords students the unique opportunity to gain familiarity with these complementary qualitative and quantitative methods. We will conduct three focus groups of 6-8 participants based on their level of training: pre-clinical undergraduates, clinical undergraduates, and residents and practicing physicians. Fellows will help recruit participants for focus groups, assist in conducting the focus group session, and then assist with data analysis.

Quantitative data analysis will include a mixed-methods survey with a set of questions specific to their stage of professional development of up to 300 current NEOMED students and alumni who have graduated within the last 15 years. Fellows will assist with developing and launching the online survey in Qualtrics and subsequent data analysis.

6. Proposed method of data analysis

Student Fellows will engage in all aspects of qualitative and quantitative data analysis. Qualitative analysis will include transcription of qualitative data and then thematic analysis, i.e., data will be categorized using labels and emerging themes identified. Quantitative analysis of survey data will include calculation of basic descriptive statistics such as means and frequencies as well as examination of groups differences by training level. Quantitative data analysis will take place in Excel and SPSS. Student Fellows will also participate in developing tables and figures to display findings and summarizing results. Student Fellows will prepare and present a poster for the NEOMED Research Day. If the Fellows wish to continue with the project after the conclusion of the fellowship, they will have the opportunity to participate in preparation of a manuscript for publication.

While it is possible that applicants for the student fellowship may have some experience engaging in qualitative and/or quantitative data analysis, prior knowledge or experience is not required. The co-PIs intend to provide hands-on training and instruction on how to engage in such research in a scientifically rigorous manner.

7. Significance of anticipated findings

Making use of a novel study design and a rich data set pulled from nearly two decades of longitudinal arts and humanities medical education curriculum at NEOMED, this project is poised to provide much needed evidence that integrated arts and humanities medical education fosters essential knowledge for medical practice in undergraduate education and beyond. This study can contribute to the academic literature in a way that will inform current and future curriculum development and evaluation at NEOMED, other medical schools, as well as postgraduate education.

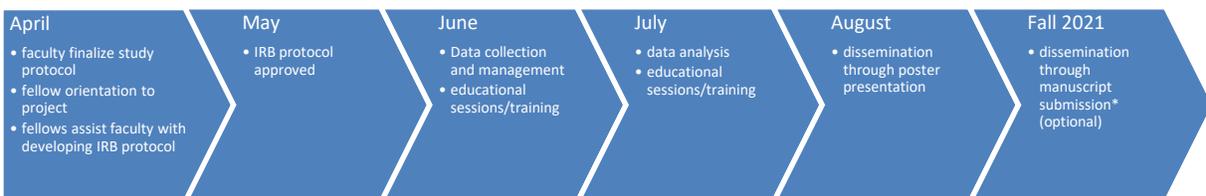
II. Student Fellow Training/Mentoring Plan

Students will learn firsthand how mixed methods models clarify and build on the results of different research methodologies and how the results from one method can impact subsequent methods or inferences drawn from the results to add richness to the final results. The three faculty members will provide project management, guidance and mentoring related to their own areas of expertise while maintaining a highly collaborative, team-based approach to training and research.

As a member of the national Health Humanities Consortium’s Curriculum and Assessment subcommittee, Dr. Bracken will provide overall guidance to the fellows, expertise regarding the study topic, and guidance with dissemination. Dr. Aultman will oversee and provide guidance related to the qualitative methodology, analysis, and dissemination. Dr. Fischbein will oversee and provide guidance related to the quantitative methodology including survey creation and launch using Qualtrics, quantitative data analysis and dissemination.

The co-PI’s will have regular and frequent communication with the summer research Fellows. Given that collaborative relationships are necessary for the successful completion of this project, weekly research team meetings will be scheduled between the co-PIs and summer research fellows. Student Fellows will have access to workspace within the Department of Family and Community Medicine, including access to work stations with computers and appropriate data and software (e.g. Excel, SPSS). All data analyses will be conducted on NEOMED’s campus and will adhere to IRB protocols. Finally, to further enhance research-related knowledge and skills, the fellows will also participate in summer fellowship training seminars in June and early July.

Proposed Project and Mentoring Timeline:



Submit your application to Dr. Amy Lee

Title: Assessing health equity in Portage County

Principal investigators: Amy Lee, MD, MPH, Professor and MPH Program Director; Julie Aultman, PhD, Professor

NOTE: there is capacity for one or two students for this project.

Location: NEOMED, Portage County Health District and locations around Portage County

Abstract: Portage County Health District has identified health equity as a priority in its most recent community health improvement plan. Currently, the only strategy addressing this cross-cutting factor is working to institute implicit bias training. In a recent NEOMED Diversity Council meeting, Portage County Health District's Becky Lehman, MPH, CHES stated Portage County could benefit from understanding where disparities exist so that targeted strategies could be implemented. In this proposal, students would adapt the use of the Health Equity Assessment Tool (HEAT) in Portage County to assess and provide guidance on how Portage County can address health disparities.

Significance of the overall research: Since Portage County has already identified health priorities in the community and strategies to address these priorities, two student investigators will be working with Portage County key stakeholders to adapt and complete the Health Equity Assessment Tool. The tool offers templates on identifying and understanding inequities, providing possible strategies, and offering a framework for evaluation. Findings will be considered by the Portage County Community Health Improvement Plan committees for promoting health equity.

Goals and objectives: This project will be to adapt and follow an existing framework to assess health equity in a community, the Health Equity Assessment Tool (HEAT) with the help of Portage County stakeholders. In addition, students will examine other communities that have had success in promoting health equity e.g., Robert Wood Johnson Culture of Health Prize winners.

- SMART Objective 1: By August 31, 2020, through Portage County, adapt the existing HEAT framework for use in Portage County.
- SMART Objective 2: By August 31, 2020, identify at least three recommendations to promote health equity in Portage County.

Research methods that will be used/learned: Students will be doing a literature search on communities that have successfully implement strategies to address health disparities. With Portage County stakeholders identified by the Portage County Health District, the students will be adapting and using an existing assessment tool. Students will be collecting data from secondary sources as well as speaking to key stakeholders.

Proposed methods of data analysis: The student will use descriptive analysis techniques. In addition, the student will learn how to use infographic software to display their data for their final report.

How the anticipated findings from the summer research fellow contribute to the success of the overall research being investigated: The findings will be used toward helping Portage County to not only define the health disparities in Portage County, but they will also help suggest strategies for improvement. Portage County will be able to make progress in the cross-cutting factor, health equity, in their Community Health Improvement Plan 2020-2022.

Appendix

- *Plan for training/mentoring the summer research fellow—individual, group, lab meetings, journal clubs, seminars, etc.* The student will have regular meetings with the faculty advisors as part of the research “team.” Part of the goal, besides learning research skills, is being able to demonstrate project management techniques, such as creating agendas and meeting summaries and adhering to a project timeline. Students will be expected to take the research course that is offered in the summer. In addition, they will be expected to take the Writing for the Sciences course offered by Coursera (free version) to improve their writing skills.
- *Description of resources available.* The student will have a desk in the Department of Family and Community Medicine. Students will be given guidance by their faculty team and community contacts on who should be contacted and other community sources, such as those listed below.
- *Site where the research will be conducted.* The research will partly be conducted at the NEOMED. The student will also meet with Portage County Health District staff.

Sources

- Ohio Department of Health. The health equity assessment tool: a users guide. <https://odh.ohio.gov/wps/portal/gov/odh/know-our-programs/health-equity/resources/equity-assessment-tool>
- Portage County Combined General Health District. Community Health Improvement Plan. <https://www.portagecounty-oh.gov/portage-county-health-district/health-education-and-promotion/pages/community-health-improvement#:~:text=The%20Community%20Health%20Improvement%20Plan,from%20the%20Community%20Health%20Assessment.&text=The%20Portage%20County%20Community%20Health,released%20on%20December%2019%2C%202019>
- Robert Wood Johnson Foundation. RWJF Culture of Health Prize. <https://www.rwjf.org/en/library/features/culture-of-health-prize.html>

Submit your application to Dr. Yeong-Renn Chen

1. Project title: Mitochondrial heme lyase mediates the cardiac resilience to heart failure

Principal Investigator Name: Yeong-Renn Chen, Ph.D.

Title and location: Professor, RGE344.

2. Abstract of Project:

The objective of this proposed research project is to study the role of mitochondrial health in myocardial adaptation to pathophysiological conditions of chronic ischemia and reperfusion in controlling the disease development of heart failure. The focus of this project will be on one disease model of heart failure caused by chronic myocardial infarction, one major upstream biosynthetic enzyme, cytochrome c heme lyase (HCCS) in the mitochondria of myocardium, and cardiac resilience to ischemia and reperfusion injury in the disease progression of heart failure. The mitochondrial HCCS as the major upstream enzyme of redox signal pathway in heart and a major target of oxidative attack occurred during chronic myocardial infarction and consequent heart failure. Impairment and down-regulation of cardiac HCCS is closely related to myocardial infarction-caused heme defect, associated mitochondrial dysfunction and declining of cardiac resilience to development of heart failure. The disease model of chronic infarction associated heart failure will be created by the animal surgery of in vivo occlusion of mouse heart for 30-min and followed by in vivo chronic reperfusion for 4 weeks. Cardiac-specific HCCS transgenic mice (HCCS-tg) will be employed to evaluate if gain of HCCS function in the myocardium can promote myocardial adaptation to chronic ischemia and reperfusion and enhance myocardial mitochondrial function and preserve mitochondrial health via preserving heme integrity. Mitochondrial function in the mouse heart will be measured using oxygen polarography and kinetic assays of the enzymatic activities from electron transport chain. Echocardiography will be used to measure the cardiac function and determine cardiac resilience to chronic ischemia and reperfusion. The progress of this project will advance our knowledge toward understanding myocardial adaptation to pathological conditions of ischemia and reperfusion and promote cardiac resilience to chronic post-ischemic reperfusion as well as developing therapeutic intervention for heart failure.

3. The significance of overall research:

Myocardial infarction is known colloquially as heart attack. More than 0.7 million Americans suffer a heart attack every year with a 50% mortality rate. Chronic infarction is the leading cause of heart failure. Mitochondria as the major source of energy generation are essential for proper cellular function in heart. There is considerable evidence supporting the key role of mitochondrial dysfunction and decreasing cardiac resilience in the disease pathogenesis of heart failure. At the myocardial level of the post-ischemic heart, there is a marked defect in energy metabolism associated with mitochondrial dysfunction. Downregulation of mitochondrial HCCS has been further detected in the mitochondria of the disease model of acute myocardial infarction. Deregulated HCCS is closely associated to heme impairment and waning ability of myocardial adaptation to chronic post-ischemic reperfusion and eventual vanishing of cardiac resilience in the disease progress of heart failure. This project tests the hypothesis that enhancement of heme biogenesis in the heart via transgenic overexpression of HCCS in the mitochondria in mouse heart mediates myocardial adaptation, promotes cardiac resilience, and alleviates the risk of heart failure in the physiological progress of chronic ischemia and reperfusion.

4. Goals and objectives

The objectives of this research are to assess the role of HCCS and mitochondrial health in myocardial adaptation to ischemia and reperfusion injury and to explore new avenues into promoting cardiac resilience in order to more fully understand the mechanisms of cardioprotection and alleviate the risk of developing heart failure. As mitochondrial dysfunction caused by heme impairment and associated HCCS defect in the mitochondria is likely to have

an impact on myocardial ability to resist pathological stress, it is desirable to obtain further information on how normalizing heme integrity via gaining HCCS function in mitochondria in vivo affects the myocardial adaptation and stimulates cardiac resilience, overall cardiac function, and eases heart remodeling and related pathogenesis of heart failure development. To partially address this question in 8 weeks and optimize the efficacy of the summer fellowship training in biomedical research, the proposed studies narrow the scope of investigation to focus specifically on measuring the phenotypes of cardiac and mitochondrial functions from the disease model of chronic ischemia and reperfusion using echocardiographic and oxygen polarographic approaches.

5. The research methods to be used

In vivo disease model of chronic myocardial infarction using mouse and cardiac function assessed by echocardiography - The procedure for in vivo myocardial ischemia and reperfusion to create the disease model of heart attack will be the same technique reported in our previous publications [Chen, Y-R (2007) *J. Biol. Chem.*, 282, 32640-54, and Chen, C-L (2008) *J. Biol. Chem.* 283, 27991-28003]. The mice of HCCS-tg and wild type control (11-12 weeks old and FBV background) will be anesthetized and subjected to 30-min of in vivo coronary artery ligation followed by 4 weeks reperfusion. At 4 weeks post-ischemic reperfusion the mice will be placed under anesthesia and subjected to measurement of cardiac function with echocardiography [Kang, PT (2015) *Journal of Molecular and Cellular Cardiology*, 88, 14-28]. The mouse hearts will then be excised. The infarct area of heart will be identified by 2,3,5-triphenyltetrazolium chloride (TTC) staining. The risk region of the myocardium will be excised for mitochondrial preparation and analyses with polarographic oxygen consumption.

Mitochondria preparation, oxygen consumption measurements, and enzymatic activity assay of mitochondrial electron transport chain - Mitochondria will be prepared from the non-ischemic and infarct tissues by differential centrifugation described in the published method [Chen, Y-R (2007) *J. Biol. Chem.*, 282, 32640-54; Chen, C-L (2008) *J. Biol. Chem.* 283, 27991-28003; Lee, H-L (2012) *AJP-Heart and Circulatory Physiology* 302, H1410-1422]. Mitochondrial respiration will be measured by the polarographic method using a Clark-type oxygen electrode (Oxytherm, Hansatech Instruments) at 30 °C. The NADH-linked respiration will be induced by malate/glutamate and the enzymatic activity of ETC complexes will be assayed according to published approaches [Kang, PT (2015) *Journal of Molecular and Cellular Cardiology*, 88, 14-28, and Kang PT (2015) *Free Radical Biology and Medicine*, 79: 56-68].

6. The proposed method of data analysis

(1) Data analysis of echocardiographic assessment of cardiac function will be done by measuring the parameters of ejection fraction, fractional shortening, left ventricle internal dimension under systole and diastole, left ventricle volume of systole and diastole, relative wall thickness, ratio of heart weight to body weight, mitral valve E/A ratio based on the m-mode echo image [Kang, PT (2015) *Journal of Molecular and Cellular Cardiology*, 88, 14-28].

(2) Data analysis of mitochondria-mediated oxygen consumption rate and the enzymatic activities of mitochondrial ETC complexes will be accessed by the first derivative of kinetic curve of oxygen consumption rate, ubiquinone 2-mediated DCPIP reduction (complex II), and ubiquinol 2-mediated ferricytochrome c reduction (complex III)[Kang, PT (2015) *Journal of Molecular and Cellular Cardiology*, 88, 14-28, and Kang PT (2015) *Free Radical Biology and Medicine*, 79: 56-68].

(3) All data will be reported as group averages \pm SEM. Statistical analyses and comparison between two groups (sham control hearts vs post-ischemic hearts, and health tissue/mitochondria vs post-ischemic tissue/mitochondria) will be assessed by student's t test.

7. Significance of anticipated findings

Combining the biochemical and echocardiographic approaches with unique genetically modified mice of HCCS-tg and in vivo animal disease model will allow us to gain a new understanding of

the phenotype of mitochondrial health in regulating cardiac resilience in the disease process of heart failure. The project will serve as a pilot study which may establish a useful platform available in the key bioenergetic pathway of heme biogenesis responsible for cardiac resilience in the mitochondria of the cardiac system. Results from these studies will increase the depth of understanding of cardiac adaptation to the stress imposed by ischemia and reperfusion and could potentially translate to clinical intervention for cardioprotection and easing the risk of heart failure.

Summer Research Fellow Training/Monitoring Plan.

1. Requirements and procedure for the student fellow is as follows:

a. First, the student will meet and familiarize themselves with Dr. Chen, and the members of his lab, who will explain project rationale, teach the skills necessary to follow protocol: learning in vivo myocardial ischemia and reperfusion system, how to properly conduct echocardiography, mitochondrial preparation, the assay procedures, data analysis and interpretations.

b. Echocardiography will be conducted in the facility available in the Department of Integrative Medical Sciences/NEOMED under the supervision of Dr. Vahagn Ohanyan.

c. There will be 1:1 meetings between the student fellow and the mentor (Dr. Chen) as well as 2:1 meeting with both Dr. Chen and Dr. Ohanyan (Assistant professor of Integrative Medical Sciences).

d. Disease model training will be completed within the first 3 weeks. Training in polarographic analysis and mitochondrial biology will be completed following first 3 weeks. Training in the final two weeks will be focused on data analysis of echocardiography and bioenergetic analysis.

e. The student trainee will attend weekly lab meetings of the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research including Dr. Chen, Dr. Chilian, Dr. Dong, Dr. Kang, Dr. Ohanyan, Dr. Raman, Dr. Thodeti, Dr. Yin, and Dr. Yun), and will present findings or related research article during meeting.

f. The student will participate in a summer journal club that involves all the summer research students and faculty members. Each summer student will be expected to participate in discussion.

g. The student will be expected to present a poster at the research day when all summer fellows present a synopsis of their summer work.

2. The mouse model and strain of HCCS-tg is available in my lab. All the necessary resources and equipment are available. Furthermore, a research technician is available to assist in training the student fellow.

3. All research will be completed at the RGE and facility of Echocardiography (C building) on NEOMED's campus.

Submit your application to Drs. William Chilian, Vahagn Ohanyan and Arthur Coulton

1. **Title:** Is Heart Failure with Reduced Ejection Fraction (HFrEF) an Outcome of Coronary Microvascular Disease.

Co-Principal Investigator: William M. Chilian, Professor and Chair, Integrative Medical Sciences

Co-Principal Investigator: Vahagn Ohanyan, Assistant Professor, Integrative Medical Sciences.

Co-Principal Investigator: Arthur Coulton, Associate Professor, Integrative Medical Sciences.

Location: NEOMED

2. **Abstract.** The goal of this proposal is to test the hypothesis that impairments in the connection between flow and metabolism are linked to heart failure. In the heart, the connection of flow to aerobic metabolism, metabolotransduction, is essential for our livelihood, because anaerobic metabolism is inadequate to maintain cardiac pump function. Despite the critical dependency of cardiac work on continual delivery of oxygen and nutrients, a complete understanding of mechanisms involved in the coupling of flow to metabolism is evasive. Previously, we found that mice null for Kv1.5 channels rapidly develop heart failure (HFrEF) when subjected to a hemodynamic challenge. These mice show evidence of coronary insufficiency, i.e., inadequate perfusion of the myocardium and impairments in ventricular contraction, minute areas of hypoxic tissue and ST segment changes in the ECG consistent with ischemia. To determine if this heart failure phenotype is a nuance of specific deletion of Kv1.5 channels (or not), we propose to study if mice null for Kv1.2 channels show the same alterations in myocardial blood flow and also show a propensity to develop heart failure. This is done by using a genetically modified mouse, which has smooth muscle specific deletion of Kv1.2 channels. If our results show that Kv1.2 mice show a similar heart failure phenotype to Kv1.5 null mice, this will be a paradigm shift in the understanding of heart failure as a potential form of ischemic heart disease. If reconnecting metabolism to flow halts or reverses the disease, such a result may provide new insights into the treatment of patients with heart failure.

3. **Background and Rationale.** A Medline analysis of heart failure and coronary metabolic dilation/hyperemia yielded 46 “hits,” but none addressed heart failure as caused by inadequate metabolic dilation. What we find interesting is that there is anecdotal literature and clinical practice supporting the role of impaired metabolic dilation in heart failure. Specifically, beta-adrenergic antagonists are now one of the drugs of choice for all but the most severe cases of failure, and *a priori* one would think that a negative inotrope, which reduces pump function, would be contraindicated to treat the myocardium with impaired contractility. Our explanation is that with a reduction in oxygen demands the imbalance between metabolism and flow is lessened, thereby reducing microareas of ischemia and apoptosis, and the progression to failure is slowed. Thus our goal of elucidating the specific ion channels involved in metabolotransduction in the heart may help our understanding of the basis of certain types of heart failure. Within this context, below is a summary of some known concepts that we have elucidated that provide some background into our interrogation of Kv1.2 channels and the potential role they may play in the disassociation of flow from metabolism in heart failure.

- Metabolic coronary dilation is in part mediated by hydrogen peroxide
- Hydrogen peroxide mediated activation of redox sensitive Kv1.5 channels plays a key role in coronary metabolic dilation.
- Heart failure is a progressive condition characterized by microvascular ischemic foci leading to diffuse fibrosis and impaired pump function.
- Kv1.5 null mice show a much higher propensity to develop HFrEF (compared to wild type [WT] mice) when subjected to a hemodynamic stress

Our hypothesis is supported by our preliminary data showing that Kv1.2+/- mice show impaired metabolic dilation; accordingly, we believe they will progress rapidly into HFrEF when subjected to a hemodynamic challenge (high blood pressure).

4. Goals and objectives. The goal of this summer research is to test the hypothesis that expression of the Kv1.5 family channels after the onset of cardiac dysfunction and heart failure during imposition of hypertension will stop the progression of dysfunction or restore normal function. This will be accomplished in a transgenic mouse we have created which has tet-on smooth muscle specific expression of Kv1.5 channels in a Kv1.5 null background. The null mouse shows impaired metabolic dilation and rapidly develops heart failure (within 2 weeks) after imposition of hypertension produced by transaortic constriction. We will express these channels to reconnect myocardial blood flow with metabolism in one group of transgenic mice and compare the results to a group without channel expression. Given that we currently have all the resources necessary for this project, the goal is attainable over the course of the summer. We are not studying wild type animals in this design because we have a plethora of data showing the responses of wild types to the hypertension; importantly these animals do not develop heart failure in response to the heightened cardiac metabolism.

5. Investigative Methods.

METHODS (all studies and surgeries will be performed in anesthetized (isoflurane) mice			
Model	Measurement	Method	Groups and Protocol
Kv1.2 ^{WT} and Kv1.2 ^{-/-} subjected to trans-aortic constriction (TAC)	Myocardial Blood Flow	Contrast Echocardiography	Protocol: 1) Baseline (Hexamethonium), 2) Varying doses of norepinephrine, i.v., to increase cardiac work up to 4 fold. Groups: 1) Kv1.2 ^{-/-} treated with tamoxifen and 2) Kv1.2 ^{WT} , without tamoxifen
	Arterial Pressure, Heart Rate	Solid state catheter in aorta via femoral	
	Stroke Volume	Echocardiography, M-mode	
	Electrocardiogram	Standard limb leads	
	Pulmonary edema	Lung dry/wet wet	
The deletion of the channel is a tamoxifen activated Cre transgenic. If the mice do not receive tamoxifen, they have a WT phenotype, and the channel is expressed. If treated with tamoxifen, the channel is deleted. After the measurements and the end of the protocol, mice will be euthanized by a high dose of isoflurane followed by either i.v. KCl or Fatal Plus.			

6. Proposed method of data analysis. One-way ANOVA and t-tests where appropriate. P<0.05 will be accepted for statistical significance.

7. Significance of anticipated findings. If our hypothesis is correct (we reject the null hypothesis), we will show that re-establishment of the connections between flow and metabolism in the heart may stop, or reverse, the progression of heart failure. To date in clinical medicine, the therapies for heart failure just slow the progression of the disease. Our results may open a new vista in the interrogation of the problem and could lead to a new strategy to control this debilitating condition.

- 8. Appendix: Summer Research Fellow Training/Mentoring Plan.** The plan we have for the student (an M1, Jacqueline Graham, has contacted us about her desire to work on this project) is arranged in a hierarchical manner.
- a. First, Jennifer will interact in a 1:1 manner with Dr. Ohanyan, who will teach her the surgical and experimental procedures, including echocardiographic measurements and data analysis.
Second, Jennifer will interact with Dr. Chilian in a 1:1 manner reviewing the data, the protocols, the rationale and the interpretations. Dr. Chilian and Dr. Ohanyan will serve as co-mentors.
Third, Jennifer will attend the lab meetings of the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Bratz, Raman, Thodeti, Meszaros, Yin, Ohanyan, Jones, and Yun) and will present her results in this weekly meeting.
Fourth, Jennifer will participate in a summer journal club that will involve all the summer research students and faculty. Each summer student will be expected to participate.
Fifth, Jennifer will be expected to present a poster at the research day when all summer fellows present a synopsis of their work.
- b. All the necessary resources (echocardiographs, Millar system to measure arterial pressure, anesthesia machines, computer for measuring evaluating echo images, transgenic mice, surgical instruments, surgical supplies, ultrasonic contrast) and financial resources for completing the research are available.
- c. The research will be completed at NEOMED.

Submit your application to Drs. William Chilian, Vahagn Ohanyan and Parisa Shabani

1. Title: Is HFpEF (HearFailure with Preserved Ejection Fraction) Caused by Coronary Microvascular Disease?

Co-Principal Investigator: William M. Chilian, Professor and Chair, Integrative Medical Sciences

Co-Principal Investigator, Vahagn Ohanyan, Research Assistant Professor, Integrative Medical Sciences

Co-Principal Investigator, Parisa Shabani, Postdoctoral Fellow

Location: NEOMED

2. Abstract. The goal of this proposal is to answer the question, do impairments in the connection between myocardial blood flow and cardiac work (i.e., insufficient dilation to match the metabolic needs of working cardiac muscle) cause a condition known as HFpEF? HFpEF is a type of heart failure that is gaining recent attention in the medical community as there is no consensus as to its cause or its treatment. We believe our work may provide additional insights into the cause of HFpEF, which then may guide towards a therapy. Cogent to this project is our previous work showing that mice null for Kv1.5 channels progress towards a phenotype exhibiting many characteristics of HFpEF, which relate primarily to impairments in diastolic function of the left ventricle. The purpose of this proposal is to unequivocally evaluate whether Kv1.5 null mice are in HFpEF by determining if the left ventricle has stiffened.

3. Background and Rationale. Our results to date show that coronary metabolic dilation is impaired in mice null for Kv1.5 channels. Moreover, the Kv1.5 channels are so critical that when mice null for these channels age they show reduced ventricular diastolic function with aging. We speculate that HFpEF results from this scenario, i.e., deterioration of coronary microvascular control mechanisms render the heart incapable of dilating sufficiently to maintain myocardial perfusion. This leads to apoptosis of cardiac myocytes and “replacement” (replacement of dead myocytes) fibrosis. Based on our preliminary data that support our experiments, we hypothesize that administration of the preferential coronary vasodilator, chromonar, after the onset of HFpEF will increase coronary blood flow and restore normal cardiac diastolic function. If this is true, it will represent a new concept in the treatment of HFpEF; namely, that the condition can be treated by increasing blood flow to the heart.

4. Goals and objectives. The goal of this summer research is to test the hypotheses: 1) The left ventricle of the Kv1.5 null mouse is stiffened, which is consistent with the pathological phenotype of HFpEF. 2) Increases in coronary blood flow after the onset of HFpEF ameliorates the phenotype and partially reverse the stiffening. This will be accomplished in Kv1.5 null mice. The null mouse shows impaired metabolic dilation and develops reduced diastolic function at an age of 6 months. To show left ventricular stiffening, we will insert a pressure volume (P-V) catheter in the left ventricle (via the right carotid), and we will measure LV P-V relations (focusing on diastole) during changes in preload (i.v. volume infusion). The slope of the P-V line is elastance and directly correlates to stiffness. In another group of mice, which will be treated with the drug chromonar (to increase blood flow to the heart), we will measure LV P-V relations. Given that we currently have all the resources necessary for this project (and mice of an appropriate age), the goal is attainable over the course of the summer.

5. Investigative Methods.

METHODS (all studies and surgeries will be performed in anesthetized (isoflurane) mice			
Model	Measurement	Method	Groups and Protocol
Kv1.5 ^{-/-} , Wild Type	Myocardial Blood Flow	Contrast Echocardiography	<u>Protocol:</u> 1) Baseline (Hexamethonium), 2) Varying

	Left Ventricular Pressure, Heart Rate	Solid state catheter in left ventricle via right carotid	amounts of i.v. volume to change preload; 3) 2 will be treated in mice treated with chromonar for 2 weeks.
	Cardiac Function	Echocardiography,	
Groups: Kv1.5 ^{-/-} ; Kv1.5 ^{-/-} + Chromonar (1-4 mg/kg per hr via infusion pump), Wild-Type animal			
The mice we will use are Kv1.5 ^{-/-} , and wild type (WT) mice. To increase blood flow to the heart, we will administer chromonar in a group of Kv1.5 ^{-/-} mice, which will be implanted (i.p.) with an Alzet minipump. Chromonar will be given for 2 weeks after demonstrable decreases in diastolic function. Diastolic cardiac function will be measured in lightly anesthetized mice (isoflurane) and LV P-V loops will be made under the same conditions. After the measurements and the end of the protocol, mice will be euthanized by a high dose of isoflurane followed by either i.v. KCl or Fatal Plus.			

- 6. Proposed method of data analysis.** One-way ANOVA and t-tests with the Bonferroni Inequality to determine intergroup and treatment differences. $P < 0.05$ will be accepted for statistical significance.
- 7. Significance of anticipated findings.** If our hypothesis is correct (we reject the null hypothesis), we will show that an increase in myocardial blood flow will stop, or even reverse, the progression of HFpEF. To date in clinical medicine, there is no treatment for HFpEF, and the treatments used for HFrEF (the typical type of heart failure with reduced ejection fraction) are employed without any evidence. Our results may open a new vista in understanding the basis of HFpEF and could lead to a new strategy to treat this condition.

Appendix: Summer Research Fellow Training/Mentoring Plan.

The plan we have devised is arranged in a hierarchical manner.

- a. First, the student will interact directly with Drs. Ohanyan and Shabani, who will teach the surgical and experimental procedures, including echocardiographic measurements and data analysis. Second, the student will interact with Dr. Chilian in a 1:1 manner reviewing the data, the protocols, the rationale and the interpretations. Some of these meetings will include Drs. Chilian, Shabani, and Ohanyan. Third, the student will attend the lab meetings of the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Chen, Raman, Thodeti, Yin, Ohanyan, Dong, Coulton, and Yun) and will present her results in this weekly meeting. Fourth, the student will participate in a summer journal club that will involve all the summer research students and faculty. Each summer student will be expected to participate. Fifth, the student will be expected to present a poster at the research day when all summer fellows present a synopsis of their work.
- b. All the necessary resources (echocardiographs, Millar system to measure arterial pressure, anesthesia machines, computer for measuring evaluating echo images, transgenic mice, surgical instruments, surgical supplies, ultrasonic contrast) and financial resources for completing the research are available.
- c. The research will be completed at NEOMED.

Submit your application to Dr. Feng Dong

- 1. Project title:** Mechanism and therapy in diabetes and cardiovascular diseases
- 2. Principal Investigator:** Feng Dong, Associate Professor, RGE 200
- 3. Abstract of project:**

Diabetes is a chronic health condition that affects how your body turns food into energy. Patients with diabetes mellitus are at increased risk for acute myocardial infarction (AMI), stroke, and limb loss. Compared to non-diabetics, the overall mortality following MI was 4 and 7 times higher in diabetic male and female patients, respectively. The mechanisms involved in impaired cardiac repair following ischemia injury in diabetes remain unknown.

Diabetic cardiomyopathy (DCM) is a major cardiovascular complication in patients with diabetes and is defined as ventricular dysfunction (in diabetes) independent of coronary artery disease. In diabetic patients with cardiomyopathy, the cumulative probability of death was 18%, development of heart failure was 22%, and the development of death or heart failure was 31% at 9 years. Previously we found blunted stromal cell derived factor-1 (SDF-1):CXCR4 axis in diabetes, and our preliminary results show an increase in chronic cardiac myocyte CXCR4 expression in diabetic murine hearts. Moreover, CXCR4 activation in diabetes produces a profound negative inotropic effect (which may seem counterintuitive, but we think is a key adaptation in the diabetic heart). Furthermore, our preliminary results also demonstrate a significantly increased mortality rate of diabetic (high fat, high sugar [HFHS]) mice null for CXCR4 in cardiac myocytes compared to HFHS diabetic wild type mice. These findings have led us to propose a novel mechanism involved in the development of DCM: diabetes induces persistent up-regulation of cardiac myocyte CXCR4 and down-regulation of SDF-1. This up-regulation of CXCR4 occurs in an attempt to preserve or hibernate the cardiac myocyte through the down-regulation of energy expending cardiac contractility. However, too much of this down-regulation of cardiac contractility results in global myocardial dysfunction or DCM. This proposal leverages novel models of gain and loss of SDF-1 and CXCR4 function to investigate the role of SDF-1: CXCR4 pathway in DCM and define the mechanisms involved.

- 4. The significance of the overall research:**

Upon completion of these studies, we will have determined the importance of SDF-1: CXCR4 axis in DCM. Novel physiology and treatment strategies for DCM will be developed based on the detailed understanding of the mechanisms involved in diabetes associated cardiovascular diseases.

- 5. The goals and objectives for the summer research project what aspect of the overall research will be the focus of the student's summer research experience? What is the specific research question being addressed by the summer research project?**

The goal of our proposed studies is to define the molecular mechanisms and physiology associated with the development of cardiovascular diseases. The focus of the student's summer research experience will be the scientific research procedures and principles on the cardiovascular complication of diabetes.

The specific research question being addressed by the summer research project are:

- A. Determine the role of cardiac CXCR4 in DCM using our cardiac CXCR4 null mouse model.
- B. We will answer an important question: will cardiac CXCR4 deletion rescue or exacerbate cardiac dysfunction in mice with diabetes?

6. The research methods that will be used/learned by the student:

The experiments will expose students to a variety of cellular, molecular, and biochemical techniques such as the culture of cells, western blot, q-PCR. The students will also be exposed to microscopic techniques and animal surgeries such as confocal microscopy, bone marrow transplantation, and echocardiography.

7. The proposed methods of data analysis:

Comparisons between 2 groups will be made with a 2-tailed Student t test. Comparisons among multiple groups will be made with 2-way ANOVA followed by the Tukey post hoc analysis.

8. A statement of how the anticipated findings from the summer research fellow contribute to the success of the overall research being investigated?

The summer research project is a part of an on-going project in the lab. Our preliminary results show significant changes of SDF-1: CXCR4 axis in diet induced diabetic mice. Anticipated findings from the summer research will answer an important question: will cardiac CXCR4 deletion rescue or exacerbate cardiac dysfunction in mice with diabetes? These data are important to study the role of SDF-1: CXCR4 pathway in DCM and define the mechanisms involved.

Student Fellow Training/Mentoring Plan

A. Plan for training/mentoring the summer research fellow – individual, group, lab meetings, journal clubs, seminars, etc.

After proper training, the variety of the experiments (cell biology, molecular biology and microscopy) ensures that each student will have unique and specific tasks relating to the overall completion of the project. The students will be taught troubleshooting methods and encouraged to design alternative strategies and hypotheses based on their findings. Students will present their results and updates on their project in formal lab meetings and informally to the PI. The meetings will focus on discussing the relevant literature, improving critical thinking, and oral presentation skills. The students will present their research at NEOMED (Cardiovascular group, IMS Department).

B. Description of resources available.

PI has lab space for students within the open laboratory of the department (4000 sq. ft.). In addition, PI has access to all core facilities, which includes an animal surgery room equipped with ventilators, surgical instruments, echocardiography systems as well as a station for processing and embedding tissue in paraffin; fully functional tissue culture facilities, dark rooms, FACS, RT-PCR, gel imaging and software for analyses. The laboratory is located in a modern complex that houses the Department of Integrative Medical Sciences and Pharmaceutical Science.

C. Site where the research will be conducted.

Most work will be done in RGE 200 and some work will be done in the room RGE 217.

Submit your application to Dr. Jessica Ferrell

- 1. Title:** TGR5 & bile acid homeostasis
PI: Dr. Jessica Ferrell, PhD; jfrancl@neomed.edu; x6468; Research Assistant Professor
Location: NEOMED, Department of Integrative Medical Sciences, Laboratory F-205A
- 2. Abstract:** Bile acids are amphipathic molecules that are synthesized in the liver, released from the gallbladder, and aid in the digestion of dietary fats and nutrients. Bile acids also bind to cellular receptors with signaling functions that modulate gut metabolism. One of these receptors, Takeda G protein-coupled receptor 1 (TGR5), is anti-diabetic and anti-inflammatory in the liver and intestine and is a potential therapeutic target for obesity and non-alcoholic fatty liver disease (NAFLD). TGR5^{-/-} mice have reduced expression of Cyp7a1, the rate-limiting enzyme that directs bile acid synthesis in hepatocytes, as well as reduced circulating bile acids but the mechanisms by which TGR5 influences bile acid metabolism are unclear. The aim of this project is to further study the regulation of bile acid synthesis and liver homeostasis by TGR5.
- 3. Significance:** Bile acids bind to the G protein-coupled receptor TGR5 to mediate glucose homeostasis through the release of GLP-1 and anti-inflammatory processes via suppression of NF κ B. Our data indicates bile acid and liver homeostasis is altered in TGR5^{-/-} mice. TGR5 is expressed in the ileum and colon, brown adipose tissue, skeletal muscle, and Kupffer cells of the liver (macrophages). However, TGR5 is not expressed in hepatocytes themselves, making the hepatic phenotype of TGR5 knockout mice worthy of further study.
- 4. Goals and Objectives:** The proposed research will further uncover the role of TGR5 in mediating bile acid and liver homeostasis. The goals for the summer research student are to learn scientific technique and experimental design, data analysis and interpretation, and to demonstrate professional presentation of scientific results.
- 5. Research Methods:** Wild type control or TGR5^{-/-} mouse tissue will be collected for: q-PCR to determine changes in gene expression, Western blotting to determine changes in protein expression, lipid and bile acid analyses, and tissue histology.
- 6. Data Analysis:** Appropriate statistical tests (Student's *t*-test, one-way ANOVA, etc.) using GraphPad Prism Software will be performed to determine statistical significance ($p < 0.05$).
- 7. Contribution of Findings:** It is expected that the findings obtained from this project will lead to better understanding of the role of bile acid signaling under normal and pathophysiological conditions and will be an instrumental base in further studying the role of TGR5 in bile acid homeostasis.
- 8. Student Fellow Training/Mentoring Plan:** The training plan for the student encompasses individual and group mentorship from Dr. Ferrell (mentor), as well as a senior lab manager and a lab technician who will be available to help instruct the student learn the techniques necessary to complete this research. The student will become familiar with the research

topic by reading primary and review journal articles. Basic lab techniques will be introduced through one-on-one instruction and will progress to independent work when appropriate. In addition to lab work, the student will be expected to keep records of the experiments and will learn to interpret the data collected. These results will be discussed with the mentor as necessary and during weekly lab meetings. Additionally, lab members participate in biweekly Diabetes, Obesity, and Metabolism Research Focus meetings, which include data and journal article presentations by graduate students, postdocs and staff. The student will attend these meetings and will have the opportunity to present research results at the end of training program. Lastly, the student will prepare and present a poster of their work at the Summer Research Fellow Poster Day. All the resources required to complete this work are available, and the student will receive laboratory safety training. The research will be conducted at NEOMED in F-205A.

Submit your application to Dr. James Hardwick

**Exon-switching of the CYP4F gene produces CYP442b, which functions to prevent immune tumor recognition. 2021 NEOMED Summer Research Fellowship Program
Submit the application to Dr. James P. Hardwick**

1. **Title:** Isolation and characterization of ***CYP4F2b*** in human hepatic tumors that reduces the immune response to tumorigenesis

Dr. James P. Hardwick
Professor of Biochemistry and Molecular Pathology
Department of Integrative Medical Sciences
NEOMED, F141
jph@neomed.edu

2. **Abstract of Project:**

Nonalcoholic fatty liver disease (NAFLD) is a pathological term that represents a broad disease spectrum of liver disease ranging from fatty liver (steatosis), liver inflammation (steatohepatitis), liver fibrosis (NASH), liver cirrhosis, end-stage liver failure, and hepatocellular carcinoma (HCC). Simple fat/triglyceride and cholesterol retention in hepatocytes (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis) NASH. Nonalcoholic fatty liver disease (NAFLD) is a disease with a prevalence of 34% in the United States among adults. It precedes the manifestation of metabolic derangements that include obesity, insulin resistance, atherosclerosis, hypertension, hypertriglyceridemia, and dyslipidemia. Over 30% of the population has NAFLD. It is estimated that by 2030 over 27% of the adult population will have NASH, a 65% increase, with the incidence of decompensated cirrhosis increasing 168%, hepatocellular carcinoma 137%, and liver-related mortality 178%. The progression from steatosis to steatohepatitis suggests that the first hit is fat/triglycerides in hepatocytes.

In contrast, the second hit is initiated by reactive oxygen species (ROS) and lipid peroxidation that promote inflammation. We have shown that a high-fat diet induces *CYP4A* P450s that produce acetate for lipogenesis and uncouple to produce ROS. In contrast, a high-fat diet represses members of the *CYP4F* family that metabolize and eliminate pro-inflammatory eicosanoids. It has been long thought that the ethanol metabolizing *CYP2E1* P450 is the primary source of ROS in hepatic steatohepatitis. However, recent data with *Cyp2e1*-null mice revealed that these mice still develop steatohepatitis with an increase in the expression of the *CYP4A* genes.

Furthermore, in the peroxisomal acyl CoA oxidase-null (*Acox1*-null) and *PPAR α* -null mice, which develop severe steatohepatitis, there is an induction of the *CYP4A* genes. These data suggest that induction of *CYP4A* genes during steatosis increases oxidative stress, *de novo* lipogenesis, and increased production of 20-hydroxyarachidonic acid (20-HETE). Increased 20-HETE production in steatosis may be an important indicator of liver disease progression since increased urinary levels have been seen in patients with liver cirrhosis and HCC. It may serve as a clinical marker of the degree and severity of liver damage. Presently, there are no studies on the regulation of the human CYP4 family member in NAFLD progression. We ***hypothesize*** that induction of the ***CYP4A*** genes by a high-fat diet mediates the production of reactive oxygen species and fatty liver through lipogenesis. In

contrast, the repression of **CYP4F** genes leads to increased hepatic levels of pro-inflammatory LTB₄, which attracts immune cells to the liver in the initiation of steatohepatitis, chronic inflammation, and hepatocellular carcinoma. We also hypothesize variant **CYP4** genes may be the reason for only One-third of human patients with NAFLD progress to NASH and liver cirrhosis.

3. Significance of research:

Nonalcoholic fatty liver disease (NAFLD) is a disease with a prevalence of 30% in the United States among adults. It precedes the manifestation of metabolic derangements that include obesity, insulin resistance, atherosclerosis, hypertension, hypertriglyceridemia, and dyslipidemia. Of concern is recognizing that cirrhosis and liver-related deaths occur in approximately 20% and 8% of these patients, respectively, over ten years. Although over 20 genes have been identified that relate to an individual's chance of obesity, the genes that directly function in increasing a person's susceptibility to fatty liver (steatosis) progressing to the fatty liver with inflammation (steatohepatitis) have not been identified. Identification of the role a high-caloric diet plays in the induction of human CYP4A genes in *de novo* lipogenesis, oxidative stress, and production of 20-HETE. In contrast, high-fat diet repression of the *CYP4F* gene increases pro-inflammatory eicosanoids may provide valuable information on why only 30% of patients with NAFLD progress to NASH. We have identified in human hepatocellular carcinoma the over-expression of the CYP4F2b that metabolizes and inactivates the pro-inflammatory eicosanoid Leukotriene B₄ (LTB₄).

4. Goals and Objectives for the summer research project.

This research aims to determine how the CYP4F2 exon switches from the CYP4F2a P450 that metabolizes arachidonic acid to 20-hydroxycosatrienoic acid (20-HETE) to CYP4F2b that metabolizes and eliminates the pro-inflammatory eicosanoid, LTB₄ and thus prevent immune cell infiltration into tumors. The second goal of this project is to determine what role the CYP4F2b gene has in the transition from a pro-inflammatory environment to an anti-inflammatory environment.

5. Investigative methods to be used:

The human CYP4F2 gene will be used to construct a vector that measures exon switching of the CYP4F2 to produce CYP4F2a or CYP4F2b in human hepatoma cell-line incubated with pro- or anti-inflammatory cytokines. We will also study this phenomenon in different human hepatoma cells that exhibit further proliferation and metastasis abilities.

6. The proposed method of data analysis:

The student will be responsible for:

- Construct an exon-switching vector containing exons three and four of the CYP4F2 gene
- Determine the CYP4F2a and CYP4F2b P450s and mRNA expression in several hepatoma cell lines with different metastatic and proliferation potential.
- Construct CYP4F2b adeno-associated viral vector.

The techniques and procedures the student will learn:

- Isolate RNA and microsome from Human hepatoma cell-line and tumors
- Determine **CYP4** mRNA by RT-PCR qualitative analysis
- Determine CYP4F2a and CYP4F2b protein levels by immunoblot analysis

- Transfect human hepatoma cell-lines with CYP4F gene exon-switching vector determine under what condition switching occurs to produce CYP4F2b.
- Analyze data using prism statistical programs and EXCELL worksheet.

The student will meet daily with the PI to discuss the days' objective and will be taught by the PI and laboratory technician to perform each experiment. The student will be taught how to record experimental results and interpret results. Finally, the student will attend weekly meetings of the liver focus group, and he or she will present results at least once to the group over the eight weeks.

7. Significance of anticipated findings:

These results may lead to novel, innovative diagnostic, and treatments for HCC by targeting the CYP4F2b gene. Exon-switching of the CYP4F2 gene during inflammation may identify a new mechanism involved in switching from a pro- to an anti-inflammatory environment.

Submit your application to Dr. James Hardwick

Over-expression of the Human CYP4A11 P450 in cirrhosis increases portal hypertension and hepatic encephalopathy: Summer fellowship 2021: Submit your application to Dr. James P. Hardwick

1. **Title:** Over-expression of **CYP4A11** in human cirrhosis increases the production of omega hydroxylated arachidonic acid (20-HETE), which causes hepatic vasoconstriction and activation of hepatic metastasis.

Dr. James P. Hardwick
Professor of Biochemistry and Molecular Pathology
Department of Integrative Medical Sciences
NEOMED, F141
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2. **Abstract of Project:**

Nonalcoholic fatty liver disease (NAFLD) is a pathological term representing a broad disease spectrum ranging from the simple fatty liver that progresses to hepatitis, fibrosis, cirrhosis, end-stage liver disease, and hepatic cellular carcinoma. Over 35% of the population has NAFLD. It is estimated that by 2030 over 27% of the adult population will have NASH, a 65% increase, with the incidence of decompensated cirrhosis increasing 168%, hepatocellular carcinoma 137%, and liver-related mortality 178%. Although most NAFLD patients do not progress to end-stage liver disease, about a third of patients with NAFLD progress to NASH and liver cirrhosis. It is presently unknown what genetic or environmental factors trigger patient progression to NASH. In the advancement from steatosis to steatohepatitis, it is believed that the first hit is the accumulation of fats (steatosis) in hepatocytes. In contrast, the second hit is initiated by reactive oxygen species (ROS) and lipid peroxidation that promote inflammation (NASH). We have shown that most ROS is produced in the endoplasmic reticulum (ER) by cytochrome P450 (CYP). It has been long thought that the ethanol metabolizing *CYP2E1* P450 is the primary source of ROS in hepatic steatohepatitis. However, recent data with *Cyp2e1*-null mice revealed that these mice still develop steatohepatitis with an increase in the expression of the *CYP4A* genes. Furthermore, in the peroxisome acyl CoA oxidase-null (*Acox1*-null) and PPAR α -null mice, which develop severe steatohepatitis, there is an induction of the *CYP4A* genes. These data suggest that induction of *CYP4A* genes during steatosis increases oxidative stress and production of 20-hydroxyarachidonic acid (20-HETE). Increased 20-HETE production in steatosis may be an essential indicator of liver disease since increased urinary levels are seen in liver cirrhosis patients. Thus, it may serve as a clinical marker of the degree and severity of liver damage. We **hypothesize** that induction of the **CYP4A** genes during steatosis mediates reactive oxygen species and fatty liver through lipogenesis. In contrast, the repression of **CYP4F** genes leads to increased hepatic levels of pro-inflammatory LTB₄, which attracts immune cells to the liver in the initiation of steatohepatitis, chronic inflammation, and hepatocellular carcinoma.

3. **Significance of overall research:**

Nonalcoholic fatty liver disease (NAFLD) is a disease with a prevalence of 30% in the United States among adults. It precedes the manifestation of metabolic derangements that include

obesity, insulin resistance, atherosclerosis, hypertension, hypertriglyceridemia, and dyslipidemia. By the year 2030, it is estimated that 27% of the population will have NASH and a 137% increase in hepatocellular carcinoma, and a liver-related mortality increase of 178%. Presently, HCC among adults with NAFLD is the fourth leading cause of death. Unfortunately, despite NAFLD's significant health burden, both noninvasive diagnostic tests identifying the progression of NAFLD are lacking and effective treatments for NASH are limited. Although over 20 genes have been identified related to an individual's chance of obese, the genes that directly function in increasing a person's susceptibility to fatty liver (steatosis) progresses to the fatty liver with inflammation (steatohepatitis) have not been identified. We believe that members of the fatty omega hydroxylase gene family play a significant role in human susceptibility and progression of NAFLD by increased acetate production used in *de novo* lipogenesis, repression of CYP4F2a genes increasing pro-inflammatory eicosanoids leading to steatohepatitis (NASH). The number of polymorphic variants of human *CYP4* genes is the underlying reason for only one-third of patients with NAFLD progressing to NASH.

4. Goals and Objectives of the summer research project:

This research aims to determine the functional role of 20-HETE produced by the human CYP4A11 P450 in hepatic hypertension and tumor metastasis.

5. Investigative Research methods:

Mice will be infected with the human CYP4A11 gene adeno-associated virus (AAV), and the degree of hepatic blood flow and systemic hypertension will be measured in mice. Human hepatoma cell lines with different proliferative and metastasis potential will be transduced with CYP4A11 AAV and the degree of proliferation and metastatic potential measured. Finally, an inhibitor of the 20-HETE receptor GPR75 will be used to determine if blocking this pathway inhibits hypertension in mice and cell proliferation in hepatoma cell lines.

The proposed method of data analysis:

The student will be responsible for:

- Infect mice with CYP4A11 AAV and measure hepatic blood flow and hypertension
- CYP4A11 mice will be administered a chemical to block CYP4A11 produced 20-HETE activation of the 20-HETE receptor GPR75 and measure hepatic blood flow and portal hypertension.
- Human hepatoma cell-line will be infected with CYP4A11 AAV and the degree of cell proliferation and metastatic potential determined.

The techniques and procedures the student will learn:

- Measure portal hypertension and hepatic blood flow in mice
- Determine levels of 20-HETE in mice administered CYP4A11 AAV
- Using HPLC-MS to determine 20-HETE levels in hepatic tissue and cell-lines
- Infect human hepatoma cell-lines with CYP4A11 AAV and measure cell proliferation and metastasis.
- Block the 20-HETE GPR75 receptors in cell-lines infected with CYP4A11 AAV and measure proliferation and metastasis.

The student will meet daily with the PI to discuss the days' objective and will be taught by the PI and laboratory technician to perform each experiment. Students will be taught how

to record experimental results and interpret results using prism statistical programs. Finally, the student will attend weekly meetings of the liver focus group, and he or she will present results at least once to the group over the eight weeks.

6. Significance of anticipated findings:

The results from these studies may identify the CYP4A11 as a critical player in hepatic hypertension and hepatic encephalopathy and thus a therapeutic target in the treatment of liver cirrhosis. These data may also provide important insight into how over-expression of CYP4A11 and 20-HETE function in cell proliferation and metastasis in liver cirrhosis transition to hepatocellular carcinoma (HCC).

Submit your application to Dr. James Hardwick

Human CYP4V2 gene role in hepatic steatosis in NAFLD: 2021 NEOMED Summer Research Fellowship Program

Submit the application to Dr. James P. Hardwick

1. Title: The Human CYP4V2 P450 role in hepatic steatosis and progression of NAFLD

Dr. James P. Hardwick

Professor of Biochemistry and Molecular Pathology

Department of Integrative Medical Sciences

NEOMED, F141

jph@neomed.edu

2. Abstract of Project:

Nonalcoholic fatty liver disease (NAFLD) is a pathological term that represents a broad disease spectrum of liver disease ranging from fatty liver (steatosis), liver inflammation (steatohepatitis), liver fibrosis (NASH), liver cirrhosis, end-stage liver failure, and hepatocellular carcinoma (HCC). Simple fat/triglyceride and cholesterol retention in hepatocytes (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis) NASH. Nonalcoholic fatty liver disease (NAFLD) is a disease with a prevalence of 34% in the United States among adults. It precedes the manifestation of metabolic derangements that include obesity, insulin resistance, atherosclerosis, hypertension, hypertriglyceridemia, and dyslipidemia. Over 30% of the population has NAFLD. It is estimated that by 2030 over 27% of the adult population will have NASH, a 65% increase, with the incidence of decompensated cirrhosis increasing 168%, hepatocellular carcinoma 137%, and liver-related mortality 178%. The progression from steatosis to steatohepatitis suggests that the first hit is fat/triglycerides in hepatocytes. In contrast, the second hit is initiated by reactive oxygen species (ROS) and lipid peroxidation that promote inflammation.

The human fatty acid omega hydroxylase CYP4 gene family consists of fourteen members that omega hydroxylates saturated, unsaturated, and polyunsaturated fatty acids to produce metabolites that activate both G-protein and nuclear hormone receptors coupled to several signaling pathways. Some of these pathways include regulation of fatty metabolism, inflammation, cell proliferation, and cancer metastasis. Thus, P450s are acclaimed as pivotal players in the P450-hormone-receptor pathway. Members of the CYP4 family participate in numerous human disease pathways: CYP4A11- hypertension, CYP4F2, and CYP4F3-inflammatory disorders, CYP4F8-ductal arteriosus, CYP4V2-blindness, CYP4X1-breast cancer, CYP4Z1- prostate cancer, CYP4B1-lung cancer, and CYP4F22-dermatitis ichthyosis. Central to human susceptibility to these diseases is the expression of polymorphic variants of CYP4 genes. Unfortunately, It is largely unknown what these P450s produce lipid metabolite to initiate or progress these diseases.

We have shown that a high-fat diet induces *CYP4A* P450s that produce acetate for lipogenesis and uncouple to produce ROS. In contrast, a high-fat diet represses members of the *CYP4F* family that metabolize and eliminate pro-inflammatory eicosanoids. It has been long thought that the ethanol metabolizing *CYP2E1* P450 is the primary source of ROS in hepatic steatohepatitis. However, recent data with *Cyp2e1*-null mice revealed that these mice still develop steatohepatitis with an increase in the expression of the *CYP4A* genes. Furthermore,

in the peroxisomal acyl CoA oxidase-null (Acox1-null) and PPAR α -null mice, which develop severe steatohepatitis, there is an induction of the *CYP4A* genes. These data suggest that induction of *CYP4A* genes during steatosis increases oxidative stress, *de novo* lipogenesis, and increased production of 20-hydroxyarachidonic acid (20-HETE). Increased 20-HETE production in steatosis may be an important indicator of liver disease progression since increased urinary levels have been seen in patients with liver cirrhosis and HCC. It may serve as a clinical marker of the degree and severity of liver damage. Presently, there are no studies on the regulation of the human CYP4 family member in NAFLD progression. We **hypothesize** that induction of the ***CYP4A*** genes by a high-fat diet mediates the production of reactive oxygen species and fatty liver through lipogenesis.

In contrast, the repression of ***CYP4F*** genes leads to increased hepatic levels of pro-inflammatory LTB₄, which attracts immune cells to the liver in the initiation of steatohepatitis, chronic inflammation, and hepatocellular carcinoma. We also hypothesize variant ***CYP4*** genes may be the reason for only one-third of human patients with NAFLD progress to NASH and liver cirrhosis. Recent data from human livers of patients with hepatic steatosis, steatohepatitis, cirrhosis, or hepatocellular carcinoma (HCC) revealed the increased levels of the CYP4V2 mRNA and P450 4V2 protein in hepatic steatosis. We also observed an increased association of the CYP4V2 protein with lipid droplets (LD), suggesting a role in LD formation of dynamics. In humans with Bietti crystalline dystrophy that results in blindness loss, the CYP4V2 gene results in accumulation of cholesterol in the posterior pole of the retina choriorretinal atrophy. In *C. elegans*, mutational loss of the CYP37A1 (ortholog of the human CYP4V2) results in increased lipid droplet size. It is currently believed that loss of CYP37A1 P450 decreases the omega hydroxylation of fatty acid in the synthesis of Ascarosides that activate the dafachronic synthesis pathway resulting in reduced cholesterol levels. This mechanism explains the phenotype associated with loss of function of the CYP37A1 gene and increased cholesterol accumulation in Bietti Crystalline dystrophy. Although the Dafachronic pathway in *C. elegans* is similar to the human bile acid metabolic pathway, the Ascaroside pathway has similarities to the human cytochrome P450 peroxisome beta-oxidation pathway do not know if human produce Ascarosides that regulate the synthesis of bile acids.

3. Significance of research:

We have identified the human CYP4V2 in hepatic steatosis and lipid droplet formation. Over 100 disease-causing mutation in CYP4V2 has been reported. However, no studies are on the role of the CYP4V2 gene in NAFLD or mutations in the CYP4V2 gene concerning NAFLD. Nonalcoholic fatty liver disease (NAFLD) is a disease with a prevalence of 30% in the United States among adults. It precedes the manifestation of metabolic derangements that include obesity, insulin resistance, atherosclerosis, hypertension, hypertriglyceridemia, and dyslipidemia. Of concern is recognizing that cirrhosis and liver-related deaths occur in approximately 20% and 8% of these patients, respectively, over ten years. Although over 20 genes have been identified that relate to an individual's chance of obesity, the genes that directly function in increasing a person's susceptibility to fatty liver (steatosis) progressing to the fatty liver with inflammation (steatohepatitis) have not been identified.

4. Goals and Objectives for the summer research project:

This research aims to determine the function of the CYP4V2 protein in steatosis and LD formation.

Investigative methods to be used:

CYP4V2 AAV and crisper CYP4V2 AAV knock-out vectors are used to over and knock-out the CYP4V2 gene in human hepatoma cells line and determine the role of the CYP4V2 gene in LD droplet formation and size.

5. The proposed method of data analysis:

The student will be responsible for:

- Design and construction of Crispr CYP4V2 AAV knock-out vector
- The CYP4V2 AAV vector is made and functional
- Infection of HepG2 hepatoma cell-line with each vector
- Determine the number and size of lipid droplets in infected cells

The techniques and procedures the student will learn:

- Construction of CRISPR cas9 adenovirus-associated viral vector
- Maintenance of human hepatoma cells
- Oil red staining of cells and determining the number of LD
- Determine the size of LD droplet using morphometric methods
- Analyze data using prism statistical programs and EXCELL worksheet.

The student will meet daily with the PI to discuss the days' objective and will be taught by the PI and laboratory technician to perform each experiment. The student will be taught how to record experimental results and interpret results. Finally, the student will attend weekly meetings of the liver focus group, and he or she will present results at least once to the group over the eight weeks.

6. Significance of anticipated findings:

These results will further our understanding of lipid droplet formation and size in microvesicular and macrovesicular steatosis. The studies may also provide insight into the role of CYP4V2 variants in human susceptibility to NAFLD. We have immunoblot evidence of CYP4V2 P450 polymorphic variants being expressed in NASH patients' liver, suggesting that the CYP4V2 possible mediated LD size and number may be necessary for the progression of steatosis to steatohepatitis.

Submit your application to Dr. Patrick Kang

Project title: Mitochondrial *Disease-in-a-Dish*

Principal Investigator: Dr. Patrick T. Kang

Co-Investigator: Dr. William Chilian

Location: NEOMED main campus, RGE building, 3rd floor.

Project Summary: Mutations in more than 250 genes are known to cause mitochondrial disease. However, the genotype-phenotype association (connection between the mutation and the manifestation of the disease) is complicated; some genetic variants may be counteracted or be aggravated by other genes or environmental factors and result in diverse clinical progressions and outcomes. This genetic diversity complicates the employment of a single preclinical model which is incapable of representing all mitochondrial diseases. Moreover, with this genetic diversity it is likely that the treatment is not a “one size fits all” therapy. An effective treatment for one patient may not be effective in other patients—even those with similar symptoms. Often this complexity, and ineffective treatment does not slow the progression of the disease leading rapid deterioration and the inability of the physician to try another treatment.

This research project focuses on the creation and the validation of *Disease-in-a-Dish* model that is tailored to simulate the complexity of individual patients with mitochondrial disease. Blood samples from patients are retrieved from our collaborator, Dr. Bruce Cohen, in the Akron Children’s Hospital. After harvesting the white blood cells (with nuclei), these nucleated blood cells are reprogramed into induced Pluripotent Stem (iPS) cells for rapid proliferation. Using specific factors and conditions, the iPS cells are differentiated into Cardiomyocytes (CMs), a cell type of high energetic metabolism, which renders it ideal to study the characteristics of the mitochondrial dysfunction, e.g., excessive reactive oxygen species production, inadequate ATP production, and also devise a pharmacological and nutritional therapy to optimize mitochondrial function in each patient. A cocktail of metabolic substrates and drugs can be empirically determined to better mitochondrial function of the particular iPS-CMs. We believe this iPS cell-based platform represents a confluence of evidence-based and precision medicine and will provide in new direction in treating patients with mitochondrial diseases.

The goal is to answer the clinically relevant question: Are iPS-CMs a suitable model to study mitochondrial disease, serving a surrogate for patients with the affliction? The overarching goal is to tailor the best cocktail for each patient.

Research Methods

The blood samples from de-identified patients and healthy donors have been retrieved and preserved with IRB approval. The summer project involves four different modules with each requiring a different approach. (1) Cellular reprogramming and differentiation, (2) Immunophenotyping of cell types. (3) Study of mitochondrial function, and (4) Study of nutritional / pharmacological interventions and followed by statistical analysis. It should be noted that it will take more than two months to complete all four modules for one particular patient. Therefore, we employ a “distributed multithread” approach to break down the task. Multiple cell lines in the pipeline are concurrently running through different modules. Summer students will learn to master cell culture technique and can choose to engage one or more modules according to personal preference.

- (1) Cellular reprogramming and differentiation: Peripheral Blood Mononuclear Cells (PBMCs) will be cultured and reprogrammed into induced Pluripotent Stem (iPS) cells and differentiated into beating Cardiomyocytes (CMs) by cell culture technique on Class II biological safety cabinet and in hypoxia chamber.
- (2) Immuno-phenotyping of cell types: Cultured cell sample prepared on a glass slide will be immuno-labeled with iPS markers (Oct4 or SOX2) or CMs markers (cardiac Troponin T or Sarcomeric Alpha Actinin) and detected by fluorescence microscope.
- (3) Mitochondrial function: Disease and healthy control of iPS and CMs will be compared by Seahorse Bioenergetics Flux analyzer. These measurements allow us to obtain basal oxygen consumption, maximum oxygen consumption, and oxygen consumption due to ATP production.
- (4) The substrate utilization screening of surviving cells will be monitored by light absorbance on Synergy 4 microplate reader. The same microplate reader will also be used to capture fluorescence signals of the reactive oxygen species (ROS) production after different drug interventions.

Students can work in a team to learn basic lab technique, utilize research instruments, and design experiment to find out the best nutritional-pharmacological solution for CMs derived from each patient. The findings may make a difference and directly benefit the donor patient.

Student Fellow Training/Mentoring Plan

Student fellows will be working with experienced lab technicians and Dr. Kang for individually-paced guidance. The student is required to attend weekly Heart and Blood Vessels Diseases group meeting as well as meet with Dr. Kang and Chilian, attend and present at a weekly journal club, and attend IMS department seminars. The student is also encouraged to present research findings during the cardiovascular group meetings. Dr. Kang will work with the student in the preparation of oral presentation and the poster session. In addition to this summer research fellowship program's poster day in NEOMED, a student fellow can also submit the abstract to the annual symposium of United Mitochondrial Disease Foundation (UMDF) Mitochondrial Medicine and have the opportunity to present the research discoveries during this event.

Submit your application to Dr. Vahagn Ohanyan

Title: Chemotherapy related cardiac dysfunction. Combination of chromonar and other FDA approved drugs for prevention and treatment for CRCD

Co-Principal Investigator: Vahagn Ohanyan, Assistant Professor, Integrative Medical Sciences

Location: NEOMED

Abstract. Doxorubicin is an anthracycline class chemotherapeutic agent that is used alone or in conjunction with other medications to treat different types of cancer. Doxorubicin works by slowing or stopping the growth of cancer cells due to its toxic effects mediated through redox cycling that produces oxidative stress. Doxorubicin monotherapy is not enough to treat cancers. Currently combination therapies are used (Doxorubicin plus cisplatin, doxorubicin plus methotrexate, Doxorubicin plus radiation, etc. We are proposing using one of the combination therapies, which is currently used in clinical applications for cancer treatment. Combination therapy has higher chance developing side effects, that's why in many cases the treatment stopped because of this side effect. a form of cardiomyopathy, termed Chemotherapy related cardiac dysfunction (CRCD). CRCD typically has the morphological and functional abnormalities of dilated cardiomyopathy, with all four cardiac chambers being dilated. This dilation occurs because of reductions in ventricular ejection fraction and contractile function, resulting in diastolic and systolic dysfunction. Eventually, congestive heart failure can result, which carries a 50% mortality rate. Currently there is no treatment or prevention for CRCD. The goal of this proposal is to test the hypothesis that chromonar, which is coronary specific vasodilator, will prevent and treat CRCD. We also want to test the differences of chromonar, and other FDA approved drug, which are used for prevention of CRCD. We also want to test the combination of Chromonar and dexrazoxane (FDA approved drug). Previously we have shown, that Chromonar has beneficial effect for treatment of dilated cardiomyopathy caused by coronary microvascular insufficiency, i.e., inadequate blood flow to the heart. We also observed that Chromonar has a beneficial effect for treatment heart failure with non-obstructive coronary artery disease. We speculate that a cause of CRCD is insufficient blood flow to the cardiac myocytes, which causes minute areas of damage that accumulate over time, eventually leading to the development of heart failure. Our goal is to determine, if improvement of myocardial blood flow will prevent and treat Chemotherapy related cardiac dysfunction.

Background and Rationale. There is consensus mechanism explaining the pathophysiology of the doxorubicin-induced cardiomyopathy. Based on our interrogation of other models of heart failure, in which insufficient myocardial perfusion seems to be a causal mechanism, we propose a similar cause for development of CRCD. We postulate that chemotherapy treatment produces coronary microvascular dysfunction and decreased myocardial blood flow. Similar to other types of heart failure, if myocardial blood flow is insufficient, even only a slight degree of insufficiency, that over the time dilated cardiomyopathy will develop. We hypothesize, that improvement of myocardial blood flow by the coronary-specific dilator, Chromonar, prevents and reverses (depending on the time of treatment) chemotherapy -induced cardiomyopathy. We also hypothesize, that combination of chromonar + dexrazoxane will have more beneficial effect on prevention of CRCD. The bases of this this hypothesis are that doxorubicin treatment leads to coronary microvascular dysfunction, micro-areas of ischemia, cardiac dysfunction and heart failure. If our hypothesis is correct, it will represent an explanation, and treatment, for CRCD that is currently has no treatment or prevention method. Also, Dexrazoxane will reduce the level of Reactive oxygen species, so the cardiotoxic effect of doxorubicin will be minimal.

Goals and objectives. The goal of this summer research is to test the hypothesis that heart failure occurring after chemotherapy treatment is caused by microvascular dysfunction. We will test whether Chromonar treatment will prevent and reverse the consequences of chemotherapy treatment. We currently have all the resources necessary for this project, and believe the goal is attainable over the course of the summer.

Investigative Methods.

METHODS (<i>in vivo</i> studies will be performed in anesthetized [isoflurane] mice)			
Model	Measurement	Method	Groups
Wild type mice subjected to i.p. Doxorubicin treatment to induce dilated cardiomyopathy	Hemodynamics: Arterial Pressure, Heart Rate,	Solid state catheter in aorta via femoral artery	<u>Groups 1 WT mice will receive chemotherapy and Chromonar at the same time</u> <u>Group 2 WT mice will receive chemotherapy 4-6 weeks. After the course we will start Chromonar treatment.</u> <u>Group 3 WT mice will receive chemotherapy and Chromonar + dexrazoxane the same time</u> <u>Group 4 WT mice will receive doxorubicin and dexrazoxane the same time for 4 weeks</u>
	Cardiac function	Echocardiography	
	Myocardial blood flow Histology	Contrast echocardiography	
Protocol			
<p>The Chemotherapy related cardiac dysfunction will be approached by injection of Doxorubicin (5mg/kg doxorubicin IP once a week or vehicle for a control (Intraperitoneal bolus 200 ul or, equal volume of sterile saline) for up to 6 weeks. After the development of CRCD we will start Chromonar treatment in Group 1 animals for 4 weeks. At the end of the Chromonar treatment will do contract and transthoracic echocardiography and sacrifice the animals (4% isoflurane to produce deep anesthesia) and excise the heart.</p> <p>The second group of animals will receive chemotherapy and Chromonar the same time. The goal of this treatment is to prevent development of CRCD. At the end of the drugs treatment, we will do contrast and transthoracic echocardiography and sacrifice the animals (4% isoflurane to produce deep anesthesia) and excise the heart.</p>			

The third group of mice will receive doxorubicin, chromonar, and dexrazoxane the same time for 4 weeks. At the end of the drugs treatment, we will do contrast and transthoracic echocardiography and sacrifice the animals (4% isoflurane to produce deep anesthesia) and excise the heart.

The fourth group will receive doxorubicin and dexrazoxane the same time for weeks. At the end of the drugs treatment, we will do contrast and transthoracic echocardiography and sacrifice the animals (4% isoflurane to produce deep anesthesia) and excise the heart.

Proposed method of data analysis. One-way ANOVA and t-tests with the Bonferroni Inequality to determine intergroup and treatment differences. $P < 0.05$ will be accepted for statistical significance.

Significance of anticipated findings. If our hypothesis is correct (we reject the null hypothesis), we will show that the coronary microvascular dysfunction develops after chemotherapy treatment, which leads to development of dilated cardiomyopathy. Treatment with Chromonar, which increases myocardial blood flow will prevent and treat development of CRCD. Our results may lead to a new paradigm for the treatment of this disorder.

Appendix: **Summer Research Fellow Training/Mentoring Plan.** The plan we have devised is arranged in a hierarchical manner.

- a. First, the student will directly interact with Dr. Ohanian (1:1). Dr. Ohanian will teach the in vivo experimental procedures, including echocardiographic measurements and will provide instruction for data analysis, construction of the final poster, and writing the final paper. Second, the student will interact with Drs. Ohanian in a 1:1 manner reviewing the data, the protocols, the rationale and the interpretations. Third, the student will attend the lab meetings of the Heart and Blood Vessel Disease Group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Raman, Thodeti, Meszaros, Yin, Ohanian, Dong, and Yun) and will present the results in this weekly meeting. Fourth, the student will participate in a summer journal club that will involve all the summer research students and faculty. Each summer student will be expected to participate. Fifth, the student will be expected to present a poster at the research day when all summer fellows present a synopsis of their work.
- b. All the necessary resources (echocardiographs, Transonic system to measure arterial pressure, anesthesia machines, computer for measuring evaluating echo images, mice, surgical instruments, surgical supplies, ultrasonic contrast) and financial resources for completing the research are available.
- c. The research will be completed at NEOMED.

Submit your application to Dr. Liya Yin

1. Title: The regulation of mouse coronary collateral growth

Principal Investigator: Liya Yin, Associate Professor, Integrative Medical Sciences

Location: NEOMED

2. Abstract. Ischemic heart disease continues to be a leading cause of death, and ill-health in the United States. The presence of coronary collateral vessels—the naturally occurring vessels that supply flow to an area of the heart to bypass a blocked vessel—confers a significant benefit to patients. The incidence of death decreases. The ability to survive a heart attack is better. And the amount of tissue that dies following a heart attack is less. However, the presence of such collateral vessels occurs in only 10-15% of all patients, so that the vast majority suffer the full consequences of death and ill-health in the event of a blockage in a vessel supplying the heart muscle. Currently, our understanding of coronary collateral growth (also termed coronary arteriogenesis) is based on studies in live animals, in which certain inhibitors are administered to reduce the vascular growth. A limitation of such “loss of function” studies is the cellular “target” of the inhibitor is unknown. The inhibitor could be acting on endothelial cells, smooth muscle cells, cardiac myocytes, inflammatory cells, and/or fibroblasts. Currently there is no way to decipher the cell-based mechanisms of coronary blood vessel growth. Moreover pharmacological inhibitors suffer from the problem on non-specificity. To overcome these deficiencies, we use the transgenic mouse model to interrogate many questions regarding regulation of process of coronary arteriogenesis in normal or diseased model (obesity and diabetes) and which cell types may be involved in this adaptive vascular growth. We hope that these studies will eventually lead to new therapies designed to help patients with ischemic heart disease grow new blood vessels in their hearts.

3. The significance of the overall research

The patients with coronary collaterals have nature bypass during ischemia and have better prognosis after heart attack. If we understand the mechanism of regulation of coronary collateral growth, we can stimulate coronary collateral growth and amplify the effect of coronary collateral growth, especially for the patients who have impaired coronary collateral growth such as patients with metabolic syndrome.

4. Goals and objectives. The goal of this summer research is to study the mechanism of coronary collateral growth and how to stimulate and amplify the effect of CCG.

5. Research Methods. Mice will be anesthetized, intubated, and prepared for sterile surgery (involved areas will be shaved and scrubbed with betadine). In all animals an incision will be made through the sternum, and a special occluder will be situated on the surface of the heart around the left anterior descending artery. The wounds will be repaired and the chest evacuated and closed. Post-operative pain will be treated by injection of an analgesic for the first day post-op, and then as needed (we will defer to the attending veterinarian’s advice). At several points after implantation of occluder, we will perform non-invasive echocardiography to evaluate cardiac function. In some animals, terminal experiments will be made at intervals (days 3, 7, and 14) up to 21 days after implantation of the occluder using contrast echocardiography to measure flow. These measurements will be made while the animals are anesthetized using gas anesthesia. Final measurements blood flow to the heart

and blood pressure will be made in anesthetized mice 21 days following implantation of the occluder using contrast echocardiography. This is done in anesthetized mice (isoflurane) in which arterial pressure is measured from a femoral catheter, and contrast microbubbles and drug infusions are done via a tail vein catheter or a catheter inserted in a jugular vein. After completion of the measurements, the mouse will be euthanized and the hearts will be removed for various in vitro and imaging studies. We also use the microfil to perfuse the heart for micro-CT to analyze the coronary collateral growth. We also use immunohistology and molecular biology techniques to study the genes/proteins involved in CCG.

- 6. Proposed method of data analysis.** The analysis will involve only unpaired t-tests as we will compare shams to animals instrumented with the occluder. $P < 0.05$ will be accepted for statistical significance.
- 7. Significance of anticipated findings.** If the study is successful, it will accelerate our understanding of mechanisms underlying coronary collateral growth as we will be able to use better link particular genes with this adaptive process. This will lead to the novel therapeutic approach to patients with ischemic heart diseases.

Appendix: **Summer Research Fellow Training/Mentoring Plan.** The plan we have devised is arranged in a hierarchical manner.

- a. First, the student will interact in a 1:1 manner with Dr. Liya Yin for experimental design, the protocols, data collection and reviewing and the interpretations.
Second, the student will interact with other lab personnel including graduate students, postdoc, research assistant for surgery, transgenic mouse breeding, phenotyping, contrast echo calculation and image analysis of micro-CT.
Third, the student will present in our lab meetings and the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Bratz, Raman, Thodeti, Meszaros, Yin, Ohanyan, Dong, Mayorga, and Yun) and will present her results in this weekly meeting.
Fourth, the student will participate in a summer journal club that will involve all the summer research students and faculty. Each summer student will be expected to participate.
Fifth, the student will be expected to present a poster at the research day when all summer fellows present a synopsis of their work.
- b. All the necessary resources (echocardiographs, anesthesia machines, computer for measuring evaluating echo images, mice, surgical instruments, surgical supplies, ultrasonic contrast, micro-CT, Fluorescent imaging system including multiphoton and confocal scope) and financial resources for completing the research are available.
- c. The research will be completed at NEOMED.

Submit your application to Dr. Mary E. Fredrickson

Project Description

Start Date: February 1, 2021 **End Date:** July 27, 2021

Project Title: Health professions students' perceptions of philanthropy and the impact of a monthly student giving program

Principal Investigator: Dr. Mary E. Fredrickson, PharmD, BCPS, Assistant Professor of Pharmacy Practice

Location: NEOMED, Department of Pharmacy Practice, B115

Abstract:

Background: Philanthropic support of medical universities is crucial. If students are made more aware of the importance and impact of philanthropic giving, they may be more likely to donate as alumni.¹ The Council for Advancement and Support of Education (CASE) suggests implementation of student philanthropy educational programs may be a successful way in which to enhance student giving.² A 2015 study determined factors associated with student pharmacist philanthropy and found implementation of a senior gift program resulted in an increased number of students who donated to the senior class gift.³ To our knowledge, no studies exist which examine a more widespread giving program at a medical university nor that examine the perceptions of medical, pharmacy, and graduate students with regards to philanthropy.

Objectives: The objectives of this research project are to 1) evaluate the perceptions of philanthropy among students at a medical university and 2) determine the impact of a monthly student philanthropy program on student giving and perceptions of philanthropy

Methods: In the fall of 2021, a monthly student philanthropy program will be implemented in which students will be asked to undertake a five-year commitment to make a monthly donation to the Blue Fund in order to begin their philanthropic connection to NEOMED. Additionally, students will receive education regarding philanthropy and the tradition of giving at NEOMED, starting at the beginning of their first year and continuing throughout their time at NEOMED. In order to determine student perceptions on philanthropy, a survey will be developed and distributed to students currently enrolled in the medical, pharmacy and graduate school programs. Data collected from these surveys will be used to compare perceptions of current students to those of the incoming class of 2025 and will also assist with tailoring the educational component of the giving program. This same survey will be given to the first-year medical, pharmacy, and graduate students during their orientation in the fall of 2021. The survey will then be redistributed to this cohort at the end of academic year 2021-2022 to assess the initial impact of the program. Long-term, student participation in the giving program will be tracked yearly, and the survey will be redistributed to this cohort upon their graduation in 2025.

Significance: As medical, pharmacy, and graduate school programs look to increase funding through philanthropic giving, understanding student perceptions of giving and educating on and promoting the importance of philanthropy will be crucial in growing the number of these individuals who participate in the giving process post-graduation.

Goals and Objectives: The student who assists with this project will complete the following:

- Background literature review
- Completion of IRB proposal
- Development and validation of survey questions
- Development of methods
- Survey distribution and data collection
- Data analysis
- Completion of grant request for project funding

Research Questions: What are the perceptions of philanthropy among students at a medical university, and what impact does implementation of a monthly student philanthropy program have on students' perceptions of giving and willingness to donate as future alumni?

Research Methods:

The research student will use the following research methods:

- Completion of a background literature review utilizing appropriate search strategies
- Development and validation of survey questions
- Distribution of surveys using Qualtrics software
- Determination of appropriate statistical tests
- Data analysis

Proposed methods of data analysis: Data will be analyzed using SPS software. Appropriate statistical tests will be determined with the statistician.

How will anticipated findings contribute to the success of the overall research being investigated?

The research fellow will assist in determining the perceptions of current pharmacy, medical, and graduate students on philanthropy. This will assist in guiding the educational aspect of the monthly philanthropy program and be used as a comparison for data collected from the incoming classes.

Student Fellow Training/Mentoring Plan

The student fellow will:

- Meet regularly with the principal investigator to review aspects of the research process and discuss the ongoing research project
- Complete online educational modules related to the research process.
- Participate in journal clubs utilizing articles uncovered during the literature review.
- Meet with the statistician for a review on data analysis
- Participate in monthly scholarship open houses hosted by the Department of Pharmacy Practice in order to gain further insight the research process
- Attend additional research seminars offered by NEOMED, as applicable

Description of resources available

- Educational materials regarding conducting research/the research process
- Statistician services within Department of Pharmacy Practice

Site where the research will be conducted:

- NEOMED, Department of Pharmacy Practice, B115

References:

1. Bowman N. Alumni giving starts with students. *Diverse*. <http://diverseeducation.com/article/50941>.
2. Paradise A. Schooled in giving. Council for Advancement and Support of Education. http://www.case.org/Publications_and_Products/2014/MayJune_2014/Schooled_in_Giving.html.
3. Chisholm-Burns MA, Spivey CA. Factors Associated with Student Pharmacist Philanthropy to the College Before and After Graduation. *Am J Pharm Educ*. 2015;79(7):102. doi:10.5688/ajpe797102

Submit your application to Dr. Christine Crish

PI: Christine Dengler-Crish, Ph.D., Assistant Professor of Pharmaceutical Sciences Neurodegenerative Disease and Aging Research Group, COP

Location: CCrish Lab, RGE 100, NEOMED campus. *Remote work option available.*

Project title: **Etiology of sex differences in Alzheimer's disease: migraine as a risk factor**

Abstract: Alzheimer's disease is the leading cause of dementia worldwide, affecting 10% of people aged 65 and older. Postmenopausal women are increased risk for Alzheimer's disease (AD), representing 2/3 of all patients diagnosed with this fatal neurodegenerative condition. Numerous hypotheses have been tested regarding the mechanisms of this sex difference in AD incidence, but conclusive data are lacking. This project will explore the possibility that history of a chronic neurological disorder secondary to hormonal sex differences, *migraine*, alters neurobiological mechanisms that increase risk of future AD in women. Critical assessments of published data will be used to link migraine epidemiology and etiology with related factor in AD in order to generate testable hypotheses and design methods for empirically testing these research hypotheses. AD is a critical public health concern because there are currently no disease-modifying treatments available. This project will contribute to an overall body of work identifying early/mid-life risk factors that, if treated or ameliorated, may prevent onset of age-related dementia. This remains one of the most promising avenues for stopping this cruel and fatal disease and reducing the socioeconomic and emotional toll it exerts on patients, caregivers, and their families.

Significance: Migraine incidence is greater in women than men, and peaks during years of reproductive viability (puberty to menopause). Migraine is a neurological condition with mechanisms that involve the neurovasculature, cortical neuron firing, trigeminal signaling, and activation/sensitization of neural pain pathways. One of the earliest neurobiological events that occur before migraine onset is cortical spreading depression (CSD), which is a wave of excessive neuronal excitation in the cerebral cortex that is followed by a dampening (i.e. depression) of neuronal activity. CSD has been correlated with the emergence of sensory aura symptoms in migraine, and research shows that this aberrant cortical activity can cause changes in the permeability of the blood brain barrier (BBB) that make it "leaky," dysregulating the exchange of ions across this important barrier. Leaky BBB is thought to contribute to increased pain signaling in the trigeminovascular/CGRP-pain signaling system that triggers severe migraine headache. In AD, disruptions in BBB have been implicated as early etiological factors that contribute to or permit pathological accumulation of toxic amyloid beta and tau proteins in the brain that eventually cause neuronal death, leading to the devastating loss of cognitive (and eventually, life-sustaining) function in AD.

Project goals/objectives: This is multi-phase project with the potential for the student to continue work on this research after the summer fellowship period. For the summer fellowship period, student is expected to complete **Phase I goals/activities** as described below with the possibility of beginning work on Phase II if time permits. The overall objective of this project is to establish ***whether history of migraine in early-middle adulthood predicts increased risk of Alzheimer's disease in women aged 65+***. This is a seemingly simple question, but it has not been adequately addressed in the literature—mostly because migraine diagnosis standards

were only firmly established in the past ~30 years. The long-term goal of this project is develop it into a multi-tiered translational research study, but first we must establish the feasibility and rationale for addressing this relatively “simple” question.

Research methods/data analysis

Phase 1 (Completion required): Establish feasibility and theoretical foundation for research

question: *Does history of migraine in early-middle adulthood predict increased risk of Alzheimer’s disease in women aged 65+?*

This project will provide the student with the opportunity to develop theoretical basis for investigating migraine as a risk factor for AD in women. It will require the student to review and synthesize empirical literature on the a) epidemiology of migraine, b) neurobiological mechanisms of migraine, and c) both basic and clinical studies on AD etiology. The student will use critical thinking skills to draw comparisons to and identify relationships between factors common to these two diverse topics in order to craft specific, testable research hypotheses. Phase 1 will not include any bench work or testing, but will involve literature review, database exploration, and writing as listed below:

- Significant proficiency in using PubMed and other query-engines/databases to find and obtain relevant articles
- Critical thinking skills to read and summarize articles for their key attributes and findings
- Organizational skills to create searchable Excel database of all obtained articles and their key findings
- Writing proficiency to begin drafting a publication-quality literature review
- Organizational and conceptual skills to create and execute professional presentation of work to peers/faculty

Anticipated outcomes: Ambitious students who achieve these goals will have the potential to co-author a literature review on this topic that will be published in peer-reviewed journal relevant to this field; at minimum, they will be required to present their theoretical basis for investigating migraine in AD at the annual NEOMED Student Research Symposium.

Phase 2. (Completion optional). Design empirical study to address research question using existing published data

- Complete CITI training for human subjects research/working with IRB
- Become proficient in basic experimental design for epidemiological studies (may require coursework)
- Become proficient in quantitative analysis methods for epidemiological/survey data including data preparation, screening, and introductory statistical analysis using SPSS software (may require coursework)
- Identify sources of data (public databases, repositories, etc) that can be used to answer research questions
- Identify variables related to migraine & AD that we need to control for or examine in context of the research question
- Design experiment that includes all target variables and control variables required to answer research question
- Work with lab team members to implement study

Anticipated outcomes: Students who complete Phase 2 are eligible to contribute to additional phases of this project (not described in this document). However, if Phase 2 completion is the student's stopping point, they are eligible to serve as co-author on an empirical manuscript that will be published in a peer-reviewed journal relevant to this field once all phases of study are complete.

Student Fellow Training/Mentoring Plan

*To be eligible for this fellowship, student must be proficient in using PubMed, journals, and other sources to find scientific articles; must know how to read and summarize journal articles

During the fellowship the student is expected to:

- Meet with PI weekly to discuss project goals and report on progress
- Attend weekly lab meetings with entire CCrish lab staff
- Attend weekly/monthly scheduled scientific seminars/journal clubs related to lab research interests.

Resources such as software or library fees needed for this project will be provided by Dr. Christine Crish's research start-up funds. Intellectual resources that may be required (e.g. coursework, additional training/consultation) will be obtained from relevant faculty colleagues in COP and COM.

Research can be conducted from any location where student can access NEOMED library resources including databases, online journals, etc., and Microsoft office software. If additional software is required (i.e. SPSS), a workstation in the RGE building will be made available for student to access on-site.

Submit your application to Dr. Sheila Fleming

1. Title: The effect of exercise in Parkinson's Disease

PI: Sheila Fleming, Ph.D. Assistant Professor in the Department of Pharmaceutical Sciences

Location: Research and Graduate Education Building, RGE-100

2. Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by the loss of dopaminergic neurons in the substantia nigra and the development of alpha-synuclein positive lewy bodies and lewy neurites in the brain and periphery. There are currently no molecular or biological treatments that can slow or stop the progress of the disease. However, preclinical and clinical studies suggest that exercise therapy may have disease-modifying potential Parkinson's disease. Although, it is currently unclear mainly due to two main issues. First, in clinical studies it is difficult to determine whether any improvements observed are due to symptomatic improvement versus the sparing of neurons or slowing pathology in the brain since we have limited ability to quantify neurons and Parkinson's pathology in the living human brain. Second, our ability to turn to preclinical animal models has been limited by the model tools we have had, models that do not accurately reproduce the key pathological feature of the Parkinsonian brain, alpha-synuclein accumulation. In the present project we use an optimized preclinical model of Parkinson's disease to examine whether exercise therapy using treadmill running can protect against this hallmark pathology of Parkinson's disease and the subsequent loss of neurons. We are also examining the mechanisms whereby the effects of exercise may occur and the effect on behaviors impacted in Parkinson's disease including motor, cognitive, and neuropsychiatric function.

3. Background and Rationale: PD belongs to a group of diseases known as synucleinopathies, where the presynaptic protein alpha-synuclein abnormally accumulates in the brain and periphery. Alpha-synuclein is a major component of lewy bodies, the pathological hallmark of synucleinopathies and a key protein in the study of PD. Inherited forms of PD show that mutated or increased alpha-synuclein can lead to the development of PD. Thus, the identification of interventions that can decrease alpha-synuclein accumulation and toxicity could have therapeutic potential. Multiple studies show an inverse correlation between physical activity and PD risk. They suggest that increased physical activity in midlife is associated with an approximately 34% reduced risk of PD. However, it cannot be excluded that there is a reverse causation where a predisposition to PD contributes to less physical activity. Therefore, understanding the biological impact and therapeutic potential of physical activity and exercise in PD would be of considerable value to patients. Preclinical studies have been employed to determine the potential protective effect of exercise in PD. To date though, the results have been mixed with some showing neuroprotection and others showing only symptom improvement and very few actually measuring alpha-synuclein pathology. In the present study we are using the alpha-synuclein preformed fibril (PFF) model of PD and are determining the impact of treadmill exercise at two different stages of pathology. We are testing treadmill exercise early when there is some alpha-synuclein pathology but no neurodegeneration yet and later when neurodegeneration has just started to develop.

4. Goals and Objectives: The goal of these studies is to determine the therapeutic potential of exercise in Parkinson's disease. The objective is to introduce treadmill exercise at different stages of pathology and neurodegeneration to determine whether it can slow or stop the progression of the disease pathology.

5. Investigative Methods: A combination of behavioral, cellular, molecular, and genetic methods will be employed to determine the effect of exercise in the alpha-synuclein preformed fibril model of Parkinson's disease.

Treadmill Training. Separate cohorts of PBS control or PFF-injected rats will be exposed to treadmill training. Rats will be tested during the dark cycle (rats are housed in a reverse light/dark cycle) and will build up to a running rate of 15 meters/min for 30 minutes per day, 5x per week. Rats will then be behaviorally tested to determine the effect of the exercise on sensorimotor, cognitive, and neuropsychiatric function. In the brain alpha-synuclein accumulation and neurodegeneration of the nigrostriatal dopaminergic system will be determined.

Behavioral Testing. All rats will undergo a battery of behavioral testing to determine the impact of treadmill exercise on both motor and non-motor function. The motor tests employed (movement initiation, adhesive removal, and spontaneous activity) have all been shown to be highly sensitive to nigrostriatal cell loss and changes in dopamine in the striatum. These tests can be used repeatedly throughout the experiment. Non-motor tests will also be included because exercise is known to have broad effects in the CNS and the PFF model develops alpha-synuclein pathology outside of the nigrostriatal system including the amygdala, cortex, and olfactory regions. Therefore, tests that measure anxiety-like behavior, cognitive dysfunction, and olfactory dysfunction, impairments also commonly observed in PD, will be included. Since tests that measure fear and cognition can be influenced by repeated testing, these tests will only be included at one time point at the end of the study.

Alpha-Synuclein Accumulation (brain). Soluble and insoluble alpha-synuclein protein will be measured using both immunoblot and immunohistochemistry techniques. For immunoblot fresh frozen tissues will be homogenized and subjected to successive freeze-thaw cycles. Lysates will then be centrifuged and supernatants will be collected as the soluble fraction. The remaining pellet will be resuspended in a SDS-based lysis buffer, boiled and sonicated. Lysates will be centrifuged and the supernatants collected as the insoluble fraction. Protein from each sample is fractionated on gels and then transferred to membranes. The membranes are incubated with primary antibodies for alpha-synuclein. The membranes are developed using ECL Plus Western Blot Detection Kit. For immunohistochemistry, free-floating coronal sections will be collected for analysis. Sections will be processed with primary antibodies and for controls, sections will be incubated with the corresponding IgG at the same concentration as the primary antibody. The avidin-biotin complex method will be used to detect the secondary antibody and the reaction product visualized by DAB.

Neurodegeneration (brain). Neuron counts will be measured using immunohistochemistry in the substantia nigra. Dopamine neurons in substantia nigra pars compacta and dopamine terminals in the striatum will be identified utilizing tyrosine hydroxylase immunohistochemistry protocols routinely used in the lab.

6. Proposed Method of Data Analysis: A combination of parametric and non-parametric statistics will be used to analyze the behavior and tissue data. For parametric statistics, 2X2 randomized ANOVA will be used to analyze treatment (PBS or PFF injected) and exercise (treadmill training or stationary on treadmill). Post hoc comparisons will use the Bonferroni corrected factor when

multiple comparisons are being made. For scores that do not meet the assumptions of ANOVA nonparametric statistics will be used to compare genotypes and treatment.

7. Significance of Anticipated Findings Whether exercise can protect the nigrostriatal system in the context of synucleinopathy remains an open question. Determining the effect of exercise on key pathological mechanisms involved in the progression of PD is an important goal in the field as it could potentially provide a much needed, non-invasive, non-pharmacological therapeutic strategy for Parkinson patients and at risk populations. Results from these experiments will yield essential data on the specific effect of exercise on alpha-synuclein inclusion accumulation, synuclein-triggered neuroinflammation and nigrostriatal degeneration. In addition, this work has high translatability and will help to inform clinical trials and identify optimal intervention strategies for PD patients and at risk populations. Collectively, these studies will provide evidence to support or refute the disease-modifying potential of exercise against synucleinopathy in PD.

Student Fellow Training/Mentoring Plan:

Plan. This is a large project that is ongoing in the lab. The PI will work with the student to determine what aspect of the project best suits his/her interests, abilities, and goals. The student would have the option to work mainly on one aspect of the project (such as behavioral testing and analysis or tissue processing and immunohistochemistry) or multiple aspects of the study. The student will meet with the PI on a weekly basis to discuss project progress and literature in the field. In addition to individual meetings the student will attend regular lab meetings where each person in the lab discusses the project they are working on and the progress or setbacks they have encountered. Short PowerPoint presentations are encouraged during these meetings as they will keep the student on track for the final poster session at the end of the summer.

Resources. This project is funded by the Department of Defense and the lab has all resources necessary for the student to complete a summer project. The rats and treadmills are available and behavioral testing protocols are already established. Supplies and space for tissue processing are also available.

Location. The experiments will be conducted primarily in the laboratory area in RGE-100. There is behavioral testing space in C-133 where motor and cognitive testing will take place. The student will have a desk and access to a computer in the write-up area for data analysis and presentation.

Submit your application to Dr. Sheila Fleming

1. Title: Gene-Environment Interactions in Parkinson's Disease

PI: Sheila Fleming, Ph.D. Assistant Professor in the Department of Pharmaceutical Sciences

Location: Research and Graduate Education Building, RGE-200

2. Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by the loss of dopaminergic neurons in the substantia nigra and the development of lewy bodies and lewy neurites in the brain and periphery. While the cause of the majority of cases is unknown, it is generally considered that gene-environment interactions underlie most cases of PD. Therefore, the identification of gene-environment interactions associated with PD-like pathology and neurodegeneration is an important goal in the field. ATP13A2 is a P₅-ATPase of the P-type ion transport ATPase superfamily and loss of function mutations cause the neurodegenerative condition Kufor-Rakeb Syndrome, an autosomal recessive form of PD. The function of ATP12A2 is unclear but *in vitro* studies suggest it may be involved in the lysosomal degradation of proteins, polyamine and heavy metal transport (manganese and/or zinc), and mitochondrial function, all mechanisms that can overlap with PD. An important next step is to determine how loss of function of ATP13A2 *in vivo* interacts with environmental factors such as heavy metals and toxicants that interfere with cellular transport, protein degradation, and mitochondrial function. It is hypothesized the loss of ATP13A2 function causes an increased vulnerability to the toxic effects of certain heavy metals and pesticides associated with PD. This hypothesis will be tested using Atp13a2-deficient mice that have been shown to develop age-dependent motor impairments, enhanced accumulation of lysosomal storage material, and increased accumulation of the PD protein alpha-synuclein. Wildtype and Atp13a2-deficient mice will be exposed to different metals and toxicants associated with PD (ex. manganese). Sensorimotor function will be measured and in the brain accumulation of the PD protein alpha-synuclein and neurodegeneration will be determined. A combination of behavioral, cellular, and molecular techniques will be employed.

3. Background and Rationale: PD belongs to a group of diseases known as synucleinopathies, where the presynaptic protein alpha-synuclein abnormally accumulates in the brain and periphery. Alpha-synuclein is a major component of lewy bodies, the pathological hallmark of synucleinopathies and a key protein in the study of PD. Inherited forms of PD show that mutated or increased alpha-synuclein can lead to the development of PD. Thus, the identification of genetic and environmental factors that can increase alpha-synuclein accumulation and toxicity could have a major impact on the development of therapeutics for the disease. P-type ATPases are a large family of proteins involved in the transport of cations and other substrates across cell membranes through the utilization of energy from ATP hydrolysis (Schultheis et al., 2004; van Veen et al., 2014). Functionally, they are involved in essential cellular processes including vesicular transport and excitability. ATP13A2 is most abundant in the brain and loss of function mutations in humans causes Kufor-Rakeb Syndrome, an autosomal recessive form of PD. More recently, ATP13A2 polymorphisms have been linked to an enhancement of the neurotoxic effects of manganese in an elderly population. Loss of ATP13A2 function in mice causes age-related sensorimotor impairments, gliosis, enhanced lysosomal storage material, and increased alpha-synuclein accumulation (Schultheis et al., 2013; Kett et al., 2015). This suggests ATP13A2 could be an important factor in gene-environment interactions associated with PD.

4. Goals and Objectives: The goal of these studies is to understand the role of ATP13A2 in cellular dysfunction and neurodegeneration. The objective is to characterize ATP13A2 x environmental exposure interactions and determine the mechanisms by which they contribute to behavioral dysfunction and neurodegeneration *in vivo*.

5. Investigative Methods: A combination of behavioral, cellular, molecular, and genetic methods will be employed to determine the effect of different environmental exposures in Atp13a2-deficient mice.

Environmental Exposures. Separate cohorts of wildtype and Atp13a2-deficient mice will be exposed to manganese. Mice will then be behaviorally tested to determine the effect of the exposures on sensorimotor function and cognition. In the brain alpha-synuclein accumulation, mitochondrial bioenergetics, and neurodegeneration of the nigrostriatal dopaminergic system will be determined.

Behavioral methods. Sensorimotor function will be assessed using a battery of tests shown to be sensitive in genetic mouse models of PD (Schallert et al., 1978; Fleming et al., 2004; Schultheis et al., 2013). Cognitive function will be determined using tests that measure aspects of attention, memory, and executive function.

Alpha-Synuclein Accumulation (brain). Soluble and insoluble alpha-synuclein protein will be measured using both immunoblot and immunohistochemistry techniques.

Mitochondrial Bioenergetics. Mitochondrial bioenergetics will be measured in multiple brain regions using Seahorse analysis.

Neurodegeneration (brain). Neuron counts will be measured using immunohistochemistry in the substantia nigra. Dopamine neurons in substantia nigra pars compacta and dopamine terminals in the striatum will be identified utilizing tyrosine hydroxylase immunohistochemistry protocols routinely used in the lab.

6. Proposed Method of Data Analysis: A combination of parametric and non-parametric statistics will be used to analyze the behavior and tissue data. For parametric statistics, 2X2 randomized ANOVA will be used to analyze genotype (wildtype and Atp13a2-deficient) and treatment (vehicle and manganese). Post hoc comparisons will use the Bonferroni corrected factor when multiple comparisons are being made. For scores that do not meet the assumptions of ANOVA nonparametric statistics will be used to compare genotypes and treatment.

7. Significance of Anticipated Findings: It is anticipated that Atp13a2-deficient mice will be more sensitive to the toxic effects of environmental exposures compared to wildtype mice. It is anticipated that exposed Atp13a2-deficient mice will show more severe alterations in behavior than exposed wildtype mice and vehicle-treated Atp13a2-deficient mice. In the brain it is expected that exposed Atp13a2-deficient mice will have increased alpha-synuclein accumulation, impaired mitochondrial function, and nigrostriatal cell loss compared to exposed wildtype mice and vehicle-treated Atp13a2-deficient mice. These findings will be significant because they will reveal a novel gene-environment interaction that could lead to neurodegeneration in humans. This would also identify ATP13A2 as a potential target for neuroprotection or therapeutic intervention.

8. Appendix:

Fleming SM, Salcedo J, Fernagut P-O, Rockenstein E, Masliah E, Levine MS, Chesselet M-F. Early and progressive motor abnormalities in mice overexpressing wild-type human alpha-synuclein. *J Neurosci*. 2004; 24(42): 9434-9440.

Kett LR, Stiller B, Bernath MM, Tasset I, Blesa J, Jackson-Lewis V, Chan RB, Zhou B, Di Paolo G, Przedborski S, Cuervo AM, Dauer WT. 2015. α -Synuclein-Independent Histopathological and Motor Deficits in Mice Lacking the Endolysosomal Parkinsonism Protein Atp13a2. *J Neurosci*. 2015; 5(14): 5724-542.

Schallert T, Whishaw IQ, Ramirez VD, Teitelbaum P. 1978. 6-hydroxydopamine and anticholinergic drugs. *Science*. 1978; 202(4373): 1216-1217.

Schultheis PJ, Fleming SM, Clippinger AK, Lewis J, Tsunemi T, Giasson B, Dickson DW, Mazzulli JR, Bardgett ME, Haik KL, Ekhaton O, Chava AK, Howard J, Gannon M, Hoffman E, Chen Y, Prasad V, Linn SC, Tamargo RJ, Westbroek W, Sidransky E, Krainc D, Shull GE. Atp13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited α -synuclein accumulation and age-dependent sensorimotor deficits. *Hum Mol Genet*. 2013; 22(10): 2067-2082.

Schultheis PJ, Hagen TT, O'Toole KK, Tachibana A, Burke CR, McGill DL, Okunade GW, Shull GE. Characterization of the P5 subfamily of P-type transport ATPases in mice. *Biochem Biophys Res Commun*. 2004; 323(3): 731-8.

van Veen S, Sørensen DM, Holemans T, Holen HW, Palmgren MG, Vangheluwe P. Cellular function and pathological role of ATP13A2 and related P-type transport ATPases in Parkinson's disease and other neurological disorders. *Front Mol Neurosci*. 2014; 7:48.

Student Fellow Training/Mentoring Plan:

Plan. This is a large project that is ongoing in the lab. The PI will work with the student to determine what aspect of the project best suits his/her interests, abilities, and goals. The student would have the option to work mainly on one aspect of the project (such as behavioral testing and analysis or tissue processing and immunohistochemistry) or multiple aspects of the study. The student will meet with the PI on a weekly basis to discuss project progress and literature in the field. In addition to individual meetings the student will attend regular lab meetings where each person in the lab discusses the project they are working on and the progress or setbacks they have encountered. Short PowerPoint presentations are encouraged during these meetings as they will keep the student on track for the final poster session at the end of the summer.

Resources. The lab has all resources necessary for the student to complete a summer project. Mutant mice are available and behavioral testing protocols are already established. Supplies and space for tissue processing are also available.

Location. The experiments will be conducted primarily in the laboratory area in RGE-200. There is behavioral testing space in C-129 where motor and cognitive testing will take place. The student will have a desk and access to a computer in the write-up area for data analysis and presentation.

Submit your application to Dr. Takhar Kasumov

1. Project title:

Temporal changes in hepatic proteome and acetylome in response to alcohol.

Principal Investigator:

Takhar Kasumov, Ph.D.

Associate Professor, Department of Pharmaceutical Sciences

College of Pharmacy, NEOMED

E-mail: tkasumov@neomed.edu

2. Abstract:

As a major site of alcohol metabolism, the liver is susceptible to alcohol-induced injury including protein damage. Currently, methods that have been used to study protein metabolism in alcoholic liver disease (ALD) rely on static measurements. Studies examining the role of alcohol on protein turnover have focused on the total liver protein instead of individual proteins with distinct functions¹⁻⁴. These studies yielded inconsistent data on ethanol-induced alterations in hepatic protein metabolism, with decreased, increased, or unaltered rates of protein synthesis. In addition, it is unknown how ethanol-induced acetylation, the footprint of ethanol-induced post-translational modification (PTM)⁵⁻⁷, affects the turnover rates of hepatic proteins.

Recently we developed a ²H₂O labeling approach for global proteome dynamics studies^{8,9}. We advanced this method to assess the effect of post-translational glycation in the stability of plasma proteins in patients with diabetes *in vivo*^{10,11}. We further determined the role of alcohol-induced acetylation in the stability of histones in the developing rat brain¹². Here we will apply the ²H₂O method to assess the role of global acetylome dynamics in the underlying pathophysiology of ALD. Protein acetylation at the lysine site alters the charge state of lysine that impacts protein structure, conformation, protein-protein interactions, protein-DNA interactions, subcellular localization, stability, and function. We will test the **hypothesis** that in ALD, hyperacetylation of proteins affects their functions through alcohol-induced alterations in protein stability. We will compare the synthesis and degradation rates for native and acetylated proteins in the normal liver and livers with an early and advanced alcoholic injury. Experiments will be conducted in collaboration with the Alcohol Research Center P50 core using an established mice model of ALD.

3. Background and Rationale:

Acetylation is one of the most important post-translational modifications (PTMs) involved in cellular transcriptional regulation and mitochondrial fidelity¹³. Excessive acetate production during ethanol metabolism modifies cellular proteins through post-translational acetylation. In addition to acetyl-CoA supply, acetylation is also regulated by NAD⁺, a co-substrate for a family deacetylase sirtuins, including mitochondrial Sirt3. Oxidative ethanol metabolism also utilizes NAD⁺ and decreases NAD⁺/NADH ratio. It is unknown whether ethanol-induced acetylation affects protein stability. Recently, we and others applied ²H₂O to assess the life-span of proteins. We demonstrated reduced stability of acetylated histones in high fat containing Western type diet (WD)-fed mice. Immuno-assay using anti-acetyl Lys anti-body revealed that a WD led to hyperacetylation of mitochondrial proteins, which was associated with suppressed expression and activity of mitochondrial Sirt3, the major mitochondrial deacetylase, and reduced hepatic NAD⁺ levels, suggesting a link between a diet-induced NAD⁺ depletion and acetylation of mitochondrial proteins. Here we propose to advance this minimally-invasive technology to assess

the effect of ethanol-induced acetylation on protein stability and investigate the underlying hepatic mitochondrial dysfunction in ALD.

4. Goals and Objectives:

We will apply mass spectrometry-based immunoprecipitation proteomics to determine whether alcohol-induced acetylation of hepatic mitochondrial proteins leads to their reduced stability and dysfunction in an alcohol-fed mice, a mouse model of ALD. The objective of this project is to assess the role of ethanol consumption on hepatic protein homeostasis via efficient enrichment of acetylated mitochondrial proteins from mice liver, quantification of acetylation and NAD⁺ levels in hepatic subcellar compartments, and investigate the effect of acetylation on stability of metabolic enzymes. We will achieve these objectives through studies in the following Specific Aims:

Aim 1. Determine if ALD increases the synthesis of hepatic proteins involved in inflammation and fibrosis, but decreases the synthesis of proteins involved in oxidative defense and energy production. The livers from pair-fed and alcohol-treated mice will be evaluated for oxidative stress, inflammation proteome dynamics using ²H₂O and LC-MS/MS.

Aim 2. Assess the effect of ethanol-induced acetylation on the stability of nuclear, cytosolic and mitochondrial proteins in ALD. We will determine the role of acetylation on the stability and functions of proteins, including transcriptional factors and enzymes involved in the altered substrate metabolism in ALD.

5. Investigative Methods:

We will enrich acetylated peptides using anti-acetyl immunoprecipitation method and analyze them by proteomics approach with data independent acquisition and parallel reaction monitoring mass spectrometry. To assess the effect of acetylation on protein's function and stability, the half-lives of mitochondrial proteins will be determined in control and ALD mice and correlated with ATP synthase enzymatic activity and ATP production. We also will use Q-Exactive Plus hybrid quadrupole-Orbitrap mass spectrometry to analyze acetylated peptides in positive ion mode to acquire the MS spectra in a data-dependent manner from the survey scan (380-1800 m/z) for subsequent high-energy collision dissociation (HCD) fragmentation.

6. Proposed Methods for Data Analysis

MaxQuant, a proteomics software developed for interpreting large-scale proteomic data from tandem mass spectrometry (MS/MS), will be used for the analysis of post-translational modification including acetylation. Raw MS data files including peptide ion masses and fragment spectra obtained from the Q-Exactive Plus mass spectrometry will be processed using MaxQuant against SwissProt mouse protein database. Trypsin-digested peptide sequences will be searched at up to a maximum of two missed cleavages. Carbamidomethylation of cysteine is set as fixed modification while methionine oxidation, nitration and acetylation, will be set as variable modifications. Database searching was performed with 6 ppm mass tolerance for precursor ions and 20 ppm for fragment ions. The false discovery rate (FDR) will be set to a maximum of 1% false identifications from a reversed sequence database. ATP synthase activity will be assessed by colorimetry-based spectrometry using 96 well plate. We will compare at least 7 liver samples per patient group to determine statistical significance between NAFLD, and healthy control.

7. Significance of anticipated findings:

This study will establish the feasibility of the acetylome dynamics method for investigating ALD *in vivo*, which then can be used to study tissue injury in a broad range of disorders. Since protein acetylation links ethanol and nutrient metabolism, and enzymes involved in these processes, in the future we will evaluate the role of alcohol in altered lipid and protein metabolism and

determine if altered metabolism of nicotinamide adenine dinucleotide (NAD⁺), the key co-substrate for sirtuin (Sirt) deacetylase regulates protein acetylation status.

8. Student fellow training/mentoring plan:

The goal of the mentoring program is to provide skills, knowledge and experience to prepare a student fellow to excel in mass spectrometry and bioinformatics technology. To accomplish this goal, the mentoring plan will follow the guidance of the National Academies of Science and Engineering on how to enhance the research experience, by providing a structured mentoring plan, career planning assistance, and opportunities to learn a number of career skills such as developing scientific presentation and writing skill.

Mutual expectations will be discussed and agreed upon in advance. The plan topics will include (a) interaction with coworkers, (b) work habits, and (c) documentation of research methodologies and experimental details so that work can be continued by other researchers and colleagues.

Participation in the journal clubs in which graduate students and postdocs meet weekly, along with a faculty facilitator, to discuss and critique recent journal articles and to discuss how to write and submit papers.

Instruction in Professional Practices will be provided on a regular bases in the context of the research work and will include fundamentals of the scientific method in the design of research question, formulation of a hypothesis and description of defined approaches to test the hypothesis. She/he will learn to identify research questions, definition of objectives, description of approach and rationale and construction of a work plan and timeline.

Success of this mentoring will be assessed by tracking the student fellow's progress through interviews to assess satisfaction with the mentoring program, and tracking of progress through weekly discussions in group meeting. At the end of the internship, the results of the summer research will be presented in a poster session.

References

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Submit your application to Dr. Erin Reed-Geaghan

TITLE: Identification of developmental factors contributing to Alzheimer's disease sex differences

PI: Erin Reed-Geaghan, Assistant Professor, Department of Pharmaceutical Sciences

SITE: NEOMED, Rootstown campus

ABSTRACT: Women are disproportionately affected by Alzheimer's disease (AD), a neurodegenerative disorder characterized by robust inflammation within the brain that accompanies the pathological hallmarks of plaques and tangles. Microglia, the resident immune cells of the brain, play fundamental roles in both brain development and AD pathogenesis, and exhibit inherent sex differences in their phenotype and function. In addition, microglia signal to other cells, including astrocytes and peripheral immune cells, to instruct their function. These other cells also play important roles in brain development and AD, and sex differences in their phenotype and function have been observed as well. Despite advances in understanding the neuroinflammatory processes in AD, the biology underpinning the sex differences in AD has not been resolved. We hypothesize sex differences in the brain's immune cells are established during development by sex chromosomes and gonadal steroid hormones, resulting in sexually dimorphic neuroinflammatory processes and therefore AD onset and progression. We propose to determine the contribution of sex chromosomes and hormones in establishing and modulating the inflammatory response in the AD brain. If successful, we will have identified novel roles for sex chromosomes and hormones in these processes.

SIGNIFICANCE: Successful completion of this research project will identify novel mechanisms leading to sex differences in AD incidence and progression. Understanding the fundamental differences in immune dysregulation and neuroinflammation would provide potential new targets for the development of disease modifying therapeutics. In addition, understanding whether the neuroinflammation differentially affects neuroanatomy and neurochemistry permits for more targeted, and therefore efficacious, therapeutic approaches.

GOALS & OBJECTIVES: The student working on this project will focus on identifying the role of the sex chromosomes in AD sex differences, specifically how they contribute to the microglial response over the course of AD.

RESEARCH METHODS: In order to determine the role sex chromosomes play in microglial activation in AD, the student working on this project will use brain tissue from transgenic mice that model AD. These mice have been genetically modified such that chromosomal and gonadal sex have been separated. The student will perform PCR to genotype mice for the transgene driving AD processes, and the X and Y chromosomes. The student will perform histology and immunohistochemistry on the brain to assess microglial number, shape, and proliferation, as well as western blot and quantitative PCR analyses for the expression of inflammatory cytokines and proteins related to microglial immune function.

DATA ANALYSIS: The student will be taught how to use ImageJ for the counting of microglia, the analysis of microglial shape, and densitometry of western blots. They will also be taught how to use the program for qPCR data analysis. Microsoft Excel will be used to perform statistical analyses of all data generated.

STUDENT CONTRIBUTION TO OVERALL INVESTIGATION: The overall investigation of developmental factors contributing to AD sex differences will be aided immensely by successful contributions of the student in examining the effects of sex chromosomes on microglia. To date, there has been no investigation of sex chromosomes in microglial function, let alone in the context of the neuroinflammatory response in AD. As microglia are the primary drivers of various disease processes, the student contribution stands to be significant in advancing the project.

STUDENT TRAINING/MENTORING PLAN

Training/mentoring: The student joining this project will be mentored and trained by both the PI and the postdoc in the laboratory working on the project. The PI will meet regularly with the student and postdoc to discuss the goals and progress of the project, review data, etc. The postdoc will provide technical oversight and mentorship. The group (PI, postdoc, and student) will also read and discuss papers related to the project on a regular (weekly?) basis as time permits.

Available resources: The laboratory is fully equipped and/or has access to all of the equipment necessary to carry out these experiments. Funding for this project comes from PI start-up funds, and two grant applications have been submitted for foundational support for this project. Notice of those grant applications are expected this spring, with funding available for summer 2021. The mice are currently being generated by the postdoc overseeing this project.

Site: This research project will be carried out within the Department of Pharmaceutical Sciences at NEOMED. The laboratory is located within the RGE building.

Submit your application to Dr. Woo Shik Shin

Project title: Novel combination antibacterial therapy against beta-lactam drug resistance pathogens

Principal Investigator: Woo Shik Shin, Ph.D.

Title: Assistant Professor

Department: Pharmaceutical Sciences

Laboratory: RGE building #101

ABSTRACT

Since the discovery of penicillin more than five decades ago, β -lactam antibiotics have been the primary therapeutic treatment used to combat both gram-positive and negative bacterial infections. However, the emergence of β -lactam drug resistance bacterial pathogens has become a major public health threat against immune-compromised individuals, post-surgical patients, and the elderly in hospitals. The major goal of the project is to develop a novel class of potent β -lactamase inhibitors to rescue existing β -lactam antibiotic activities for combination antibacterial therapy. The ability to preserve the efficacy of existing β -lactam antibiotics arsenal provides maximum opportunity for combination antimicrobial therapy development. To achieve this objective, we will use a state-of-the-art drug design methodology involving computational molecular modeling, cell/enzyme-based study, and chemical synthesis to generate inhibitors of a β -lactamase protein. Estimating first-time novel class of compounds as potent irreversible/reversible inhibitors against β -lactamase with low micromolar inhibition activities comparable to established FDA approved β -lactamase inhibitors drugs.

SIGNIFICANCE

β -lactam drug resistance remains a major public health threat. Developments of a single new β -lactamase inhibitor that resuscitate existing β -lactam antibiotic arsenals provide the maximum opportunity for novel combination antimicrobial therapy for combating drug resistance pathogens.

GOALS AND OBJECTIVES FOR SUMMER RESEARCH PROJECT

The long-term goal of the project is to develop novel classes of potent β -lactamase inhibitors to rescue existing β -lactam antibiotic activities. The ability to preserve the efficacy of existing β -lactam antibiotics arsenal provide maximum opportunity for combination antimicrobial therapy development. As part of these long-term research projects, the short-term goals of the summer research project are:

Aim 1 Find a new potent lead compound structure that can be developed as a new drug candidate by using computer-aided drug design approaches.

Aim 2 Determine the structural activity relationships (SAR) of a novel class of non- β -lactam β -lactamase inhibitors in both biochemical and bacterial cell-based assay to examine the molecular basis for bioactivity in combination therapy.

RESEARCH METHODS FOR SUMMER PROJECT

- Molecular modeling and visualization
- Structure-based virtual screening
- Protein-ligand docking
- multi-sequence alignment and analysis
- Enzyme kinetics
- Bacteria cell proliferation assay
- Human cell toxicity assay

PROPOSED METHODS OF DATA ANALYSIS

Drug design and discovery is an interdisciplinary, expensive and time-consuming process. Modern drug discovery owes to the scientific advancements during the past two decades thereby computers and computational methods became indispensable tools in the pipeline which could lead to a reduction of up to 50% in the cost of drug design.

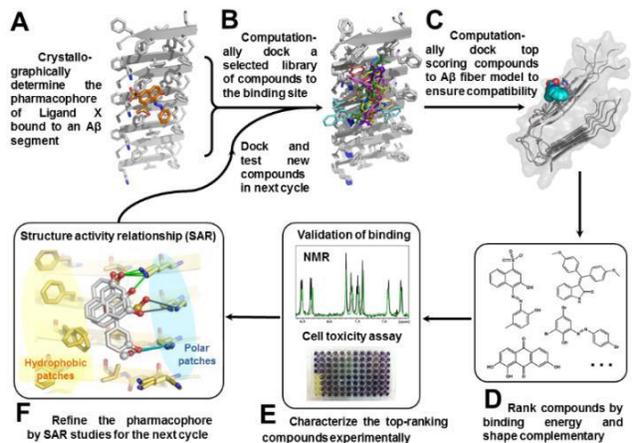


Figure 1. Computational sequence and structure-based identification of small compound inhibitors.

Recently, our laboratory has been successfully established the pipeline of motif-guided drug screening and drug carrier protein design using CADD to *in vitro* & *in vivo* verification (Fig 1). It focuses on the development and optimization of well-balanced methods with computational sequence/structure-based design and molecular cell biology experiment targets to the infectious disease, and neurological disorder.

As early lead identification and lead optimization are our initial goals of the drug discovery process, advances in computational techniques have enabled *in-silico* methods, and in the particular structure-based drug design methods, to speed up new target selection through the identification of hits to the optimization of lead compounds.

CONTRIBUTION OF SUMMER RESEARCH FELLOW

The new potential lead compound findings with its structural activity relationships (SAR) from the summer research fellow will contribute to elucidate the exact mechanism of inhibition and the specific protein-ligand interactions using state-of-the-art computer-aided drug design techniques.

And we will further demonstrate the therapeutic potential of this new potent β -lactamase inhibitor with broad-spectrum activity and identify the stability with pharmacokinetics (pK) against human/animal body system.

This short-term summer project that uses computing power to rapidly find lead compounds and validate the *in-silico* data with simple experiments are exactly aligned with our laboratory's goal of drug discovery and will provide a great research experience to students.

Student Fellow Training/Mentoring Plan

- Plan for training/mentoring the summer research fellow
- Description of resources available.
- Site where the research will be conducted

The research timeline for summer students will be divided into computer-aided drug design and wet lab biology research sessions according to their respective research interests and background expertise.

The students will learn the principles of drug design starting with basic training, participating in the subscale study, research poster presentation, writing research manuscript, and prepare of the grant proposal.

The students will rotate in different research areas within a period of two to three weeks, learn various research approaches, and participate in the project. The topics of each research field that students will learn and participate in are as follows.

Research area rotation

Computer-aided Drug Design

- Bioinformatics (ClustalW, Schrodinger)
- Molecular modeling and visualization (Maestro, VMD, SYBYL, Pymol)
- 3D Structure motif-based virtual screening (Phase)
- Protein-ligand docking and binding free energy calculation (Glide, AutoDock)
- Molecular dynamic simulation and analysis (NAMD, VMD)
- Prediction of protein structure (Prime, SWISS-Model)
- Pharmacophore modeling (PHASE)
- Huge scale multi-sequence data analysis (ClustalW, Schrodinger)

Biological Experiment

- Basic cell culture (human, bacteria)
- Cell Lysis and protein extraction
- SDS/Native Gel PAGE (Western Blot / Silver/ Coomassie blue staining)
- Human cell proliferation assay and toxicity testing
- Bacterial cell viability assay (MIC)
- Protein crystallization study
- Enzyme kinetics
- Protein concentration measurement (BCA/ELIZA)

Available Resource and Laboratory location

- Computer-aided Drug Design (RGE #101 write-up area) – Schrodinger package
- BSL 1 area (RGE #101) – Enzyme kinetics assay / inhibitor preparation / Proteomics study / SDS & Native Gel Page / Protein crystallization
- BSL 2 area (RGE #104 / RGE #108) – Human & bacteria culture environment / Toxicity test / Cell proliferation assay

Submit your application to Dr. Natalie Bonfine

1. Project title: Exploring the Impact of COVID-19 on Community Mental Health Services, the Criminal Justice System and People with Serious Mental Illness

Co-Principal Investigators: Natalie Bonfine, Ph.D., Associate Professor, Department of Psychiatry and Stacey Barrenger, Ph.D., Assistant Professor, Department of Psychiatry

Location of research: NEOMED campus, Department of Psychiatry

2. Abstract of project

People with serious mental illness who have criminal justice system involvement have a complex set of needs that place them at elevated risk for poor health outcomes due to the COVID-19 pandemic. At the same time, the COVID-19 pandemic has been a disruptive force for many social institutions serving this population, including health care systems, social services and the criminal justice system. Approximately one year into the pandemic, there is much to learn about how it has affected people with serious mental illness, and how mental health service providers and the criminal justice system have adapted their practices and policies in response to the COVID-19 pandemic. This project will be part of a broader study that is exploring the perceived impact of COVID-19 on the mental health and substance use services system, the criminal justice system, and adults with serious mental illness who have contact with the criminal justice system.

3. Significance of the overall research

The COVID-19 pandemic has exacerbated many challenges to providing mental health services for people with serious mental illness (National Association of State Mental Health Program Directors Research Institute, 2020). The pandemic has forced community-based mental health service providers to adapt their delivery of services resulting in the development of new or modify existing policies and practices to meet new and emerging restrictions, while simultaneously facing staffing and financial challenges. These service disruptions are especially concerning as people with serious mental illness face a complex array of inter-related problems that may place them at elevated risk for COVID-19 morbidity and mortality (Bartels et al., 2020). The risk factors related to poor COVID-19 outcomes include poverty, housing instability or homelessness, food insecurity, substance use, co-occurring chronic health problems like cardiovascular disease, diabetes, and COPD, and access to care (Bartels, et al. 2020; Moreno et al., 2020). People with serious mental illness are also overrepresented in the criminal justice system. Fifteen percent of men and 31% of women entering jail meet criteria for serious mental illness- prevalence rates that are about three times higher than the general population (Osher et al., 2021; Skeem, Manchak & Peterson, 2011; Steadman et al., 2009). Furthermore, institutionalized settings, like jails and prisons, are known epicenters of COVID-19 infection, placing incarcerated individuals at increased risk for exposure and illness (Moreno et al., 2020).

Efforts to divert people with serious mental illness from the criminal justice system and connect those individuals to the mental health service system are not new (Munetz & Griffin, 2006). However, stakeholders at the intersection of the criminal justice and mental health service systems must now do this work within the context of the COVID-19 pandemic. While emerging studies are examining mental health service system responses to COVID-19, as well as criminal

justice system responses, no known study to date has examined cross-systems perspectives about these processes.

4. Goals and objectives

The aim of this study is to explore stakeholder perspectives on the impact that the COVID-19 pandemic has had on community-based mental health services, the criminal justice system, and people with serious mental illness. While the student Fellow will work with the PIs (Drs. Bonfine and Barringer) to develop and define specific, conceptually driven research questions, the general, guiding research objectives for this study are to:

- 1) describe and summarize current literature on the perceived impact of COVID-19 on community based mental health services, providers, and consumers of services, as well as the impact of the pandemic on the criminal justice system
- 2) analyze quantitative data on the association among key measures of interest to this study, including participant attitudes the impact of COVID-19 on the mental health system, criminal justice system, and on people with serious mental illness who have criminal justice involvement.

5. Research methods to be used/learned

The student Fellow will conduct a review of the literature, participate in the development of research questions, assist in analyzing quantitative survey data, and summarize findings to an academic and general audience. The Fellow may also assist with collating existing contextual data (e.g., Census data).

6. Proposed method of data analysis

The student Fellow will engage in several aspects of quantitative data analysis, including developing research questions, conducting and interpreting descriptives analyses, means comparisons and multivariate analyses (e.g., ordinary least squares and logistic regression). Data analyses will be completed using SPSS. The student Fellow will also participate in other aspects of the project, including literature review and summary, developing tables and figures to display findings, and summarizing results. The Fellow will also gain experience preparing research results for public dissemination. Prior experience doing quantitative data analysis is not required. The Faculty co-PIs will provide hands-on training and instruction on how to engage in such research in a scientifically rigorous manner.

7. Significance of anticipated findings

This study will contribute to an improved understand of how community-based mental health provider agencies in Ohio have responded to the challenges associated with the COVID-19 pandemic. We also want to explore how this pandemic has affected the criminal justice system in local communities in Ohio, as well as the impact on people with serious mental illness. During this project, students will meet with collaborators at the Ohio Criminal Justice Coordinating Center of Excellence, social science researchers associated with the Center for Integrated Primary and Mental Health Care, and community-based stakeholders who are working in a cross-systems, collaborative approach to meet the needs of people with serious mental illness who have criminal justice system involvement.

II. Student Fellow Training/Mentoring Plan

This Summer Fellowship experience will involve collaborative, team-based research. It will result in a student-led independent investigation into issues surrounding COVID-19, mental health services, and the needs of people with serious mental illness. The co-PIs will have regular and frequent communication with the summer research Fellow where the Fellow will be mentored in various aspects of research, including conducting a literature review, developing a research question and hypotheses, data collection and management, data analysis, and presentation of findings to a broad audience. Given that collaborative relationships are necessary for the successful completion of this project, weekly research team meetings will be scheduled between the co-PIs and summer research fellow. These meetings will occur via Zoom or in person with appropriate social distancing. The student Fellow will have access to appropriate data analysis software (e.g. SPSS), and if not working virtually, will have access to work space within the Department of Psychiatry Research Lab. This project will adhere to IRB requirements.

In addition to attending weekly meetings with the co-PIs, the student Fellow will also meet with leadership from the Ohio Criminal Justice Coordinating Center of Excellence in the Department of Psychiatry. Attending these meetings will allow the student Fellow to discuss and explore current statewide activities related to community-based mental health and substance use services and criminal justice involvement of adults with serious mental illness in Ohio. In this way, students can experience some of the practical applications and approaches to understand the phenomenon of the overrepresentation of people with serious mental illness in the criminal justice system. They will also share their research with the Criminal Justice Coordinating Center of Excellence staff and partners.