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TITLE: Vascular and Skeletal Muscle Function in Gulf War Veterans Illness

PRINCIPAL INVESTIGATOR: Scott Kinlay, MBBS, PhD

CONTRACTING ORGANIZATION: Boston VA Research Institute, Inc. (BVARI)
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Boston, Massachusetts 02130-4817

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14. ABSTRACT
Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, memory loss, and mood changes reported by Gulf War Veterans shortly after their return in 1991. Approximately 40% of Gulf War Veterans (over ¼ million Veterans) have GWI by the Center for Disease Control criteria for GWI (a recommended method for defining GWI). The underlying causes of GWI are poorly understood. The overall goal of our study is to determine if there are differences in blood vessels, skeletal muscle performance, and their controlling proteins and genes in Gulf War Veterans with and without GWI. Abnormalities in these factors may explain the symptoms of fatigue and muscle pain that are major parts of GWI. These insights could lead to new treatments for GWI as well as other illnesses with similar symptoms. Our pilot data show that we can assess blood flow to muscle, muscle strength and fatigue and examine proteins and genes from a specimen of muscle in Gulf War Veterans. We will assess if abnormalities in these factors are potential explanations for GWI. This study is seeking to enroll 70 Veterans (35 with GWI and 35 without GWI) and is currently open to enrollment.

15. SUBJECT TERMS
Gulf War Syndrome, Persian Gulf Syndrome/physiopathology, Veterans

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1. **INTRODUCTION:** Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, and neurocognitive reported by Gulf War Veterans shortly after their return from deployment in 1991. The Center for Disease Control and Prevention (CDC)'s clinical diagnostic criteria for GWI is one of two recommended by an Expert Committee and is based on symptoms in three categories: fatigue, mood/cognition, and musculoskeletal symptoms. Currently, approximately 40% of Gulf War Veterans (over ¼ million Veterans) have GWI by these criteria. The pathophysiological mechanisms underlying GWI are not understood and insights into these mechanisms could lead to new treatment interventions. Furthermore, abnormalities in peripheral blood flow related to endothelial function and muscle bioenergetics due to environmental toxins, such as those present in the Gulf War, are plausible mechanisms that could relate to the musculoskeletal symptoms of GWI. This study will determine the pathophysiology, and related genome and transcriptional mechanisms related to endothelial function and muscle mitochondrial biogenesis in Veterans with and without GWI through a case-control design of 70 Veterans who have severed in the Gulf War and are participants of the ongoing Fort Devens Cohort. Specific aims include comparisons of: (1) microvascular endothelium-dependent and endothelium-independent function of the profunda femoral artery using techniques commonly used for peripheral endovascular interventions, (2) peak oxygen uptake and ventilator anaerobic threshold during cardiopulmonary exercise testing and other muscle functions, (3) expression of genes relevant to endothelial function and mitochondrial function in muscle biopsy samples, and (4) gene polymorphisms related to endothelial and mitochondrial respiratory function.

2. **KEYWORDS:**
Gulf War Syndrome
Persian Gulf Syndrome/physiopathology
Veterans

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Major Tasks	**Timeline (months)**	**Status**
Major Task 1: Institutional Review Board (IRB) Approval		
Modify the current protocol to add new experiments and aim (microarray assays and next-generation RNA sequencing)	0.5	Completed
Submit final protocol to VA Boston Healthcare System (VABHS) IRB	0.5	Completed
Milestone: Achieve local IRB approval of protocol	1	Received VA Boston IRB approval for modified protocol amendment, Protocol Version 2.0 on 11-JAN-2016. Submitted to and received HRPO Initial Approval for protocol (version 2/ dated 23-Dec-2015) on 09-FEB-2016.

Major Task 2: Recruitment of Subjects		
Send batch invitations to 400 Gulf War Veterans who have completed the Fort Devens cohort study	1-24	Dr. Maxine Krengel (Co-Investigator) helped initiate recruitment by sending Gulf War Veterans invitation letters to participate around 19-FEB-2016. As of 31-MAR-2018, we completed mailing at least 3 recruitment letters all 1107 Veterans from Dr. Krengel's Fort Devens Cohort. Additionally, we received approval from the VA Boston IRB committees on 13-Nov-2017 to mail a 4th letter accompanied by an opt-out card and to follow up by telephone call if no response is received. As of 31-MAR-2018, 160 Veterans have received a 4th letter and a follow up telephone call from a member of the study staff. We plan to continue this revised recruitment plan until enrollment is complete. As of 30-JUN-2019 55 subjects had returned a second set of symptom and exposure questionnaires as some of these subjects did not have this data in Dr Krengel's study. Complete
Major Task 3: Endothelial Function Study and Muscle Biopsy		
Schedule Visit 1 (Endothelial function studies and muscle biopsies)	1-24	All visit 1 appointments have been scheduled and completed as of 30-APR-2018
Complete endothelial function studies and muscle biopsies and measurement of intravascular ultrasound and flow data to assess microvascular and conduit endothelial function	1-44	As of 30-APR-2018, 78 Veterans completed Visit 1 which includes endothelial function studies, muscle biopsies and measurement and intravascular ultrasound and flow data. Total of 78 is exclusive of 2 Veterans who

		were unable to complete Visit 1 due to extensive peripheral artery disease. Muscle biopsies for 77 Veterans have been collected. All the intra-arterial IVUS images have been measured (over 2600 images). All of the Doppler blood flow images have been measured. The flow and IVUS measurements will be used to measure endothelial function. We have completed an analysis in 20 patients showing that our measurements are reproducible. This reproducibility data was presented at the Society for Vascular Medicine meeting and was published in PLOS-1 medical journal.
Milestone: Complete endothelial function data and muscle biopsies on 70 subjects	44	Complete
Major Task 4: Exercise and Cardiopulmonary Stress Testing		
Schedule Visit 2 (Exercise and cardiopulmonary stress tests)	3-28	Scheduling for Visit 1 continues to occur at least 2 weeks after completion of Visit 1, limited by Veteran's availability. There is 1 remaining subject who requires scheduling for this visit.
Complete exercise and cardiopulmonary stress studies and interpretation	3-36	As of 30-JUN-2018, 77 Veterans have successfully completed Visit 2. One other subject declined to complete this visit.
Milestone: Complete exercise data on 70 subjects	36	As of 30-JUN-2018, 77 Veterans have successfully completed Visit 2. One subject remains to be scheduled.
Major Task 5: Histopathology and Electron Microscopy		
Prepare muscle biopsy specimens for histopathology and electron microscopy and image	4-54	Pending
Complete data on muscle analysis including histopathology	4-54	Pending
Milestone: Complete histopathological data and	54	Pending

electron microscopy data on representative subjects		
Major Task 6: Electron Microscopy		
Assess mitochondrial number in 10 randomly chosen fields at 5000x and ultrastructure at 25,000x	48-54	Pending
Milestone: Complete analysis of data on 70 subjects	54	Pending
Major Task 7: Gene and protein expression relating to mitochondrial biogenesis		
Isolate DNA, RNA, and protein from muscle tissue samples. Prepare cDNA from RNA samples.	4-54	Pending
Complete qPCR and Western Blot to assess genes and proteins regulating mitochondrial biogenesis.	4-54	Pending
Milestone: Complete data on specific genes and proteins regulating mitochondrial biogenesis on 70 subjects	54	Pending
Major Task 8: Next generation RNA Sequencing comparing cases and controls		
Next generation RNA sequencing from cDNA samples at Dr. McCrae's laboratory	48-54	Pending
Interpret results and identify candidate genes related to Gulf War Illness	48-54	Pending
Milestone: Complete analysis of data on 70 subjects	54	Pending
Major Task 9: Finalize data analysis, present results and meetings, publish results		
Complete statistical analyses including comparisons of cases and controls and prepare for publication, presentation, and public release of de-identified data for other researchers.	54-60	Pending

What was accomplished under these goals? This report summarizes the research progress in the most recently completed budget period from July 1, 2018 to June 30, 2019. This period corresponds to the fourth of this project. The objectives of this study are to investigate the hypothesis that when compared to Veterans without Gulf War Illness (GWI), Veterans with GWI will have differences in arterial endothelial function, muscle function determined by cardiopulmonary exercise testing, and expression of genes responsible for mitochondrial function. This is a case control study of 2 visits looking to enroll 70 participants (35 with GWI and 35 without GWI) from a well characterized cohort of Gulf War Veterans (the Fort Devens study). Study Visit 1 consists of an endothelial function test performed using standard cardiac catheterization techniques used for peripheral artery interventions, and a muscle biopsy of the vastus lateralis muscle. Study Visit 2 consists of cardiopulmonary exercise testing and other tests of muscle strength and endurance.

The second and third budget period was heavily focused on participant recruitment (Major Task 2, 3, and 4). In the last year (fourth year), 78 (100%; of expected 70) Veterans have completed Study Visit 1. More Veterans were recruited for the study as 2 were not able to provide Visit 1 data and 6 Veterans' data were not evaluable. Analysis of endothelial function and exercise tests are 100% completed and 100% of muscle biopsies have been collected. 77 Visit 2 appointments were completed with one patient declining this visit.

In this year we have obtained permission from HRPO and DoD to modify the muscle analysis to include next generation sequencing of all expressed genes which will provide much more information than the initially planned microarray analysis of a limited set of genes expression. We also obtained permission from HRPO and DoD to have the histology, electron microscopy, next generation sequencing, and gene and protein expression of specific genes concerning mitochondrial respiration at Dr. Calum McRae's laboratory at the Brigham and Women's Hospital. Dr. Calum MacRae, has a large laboratory funded by the multimillion-dollar One Great Idea grant to analyze gene expression and genomes in large population studies and has agreed to do the analyses we require for our Gulf War Illness study. This greatly expands the information we can obtain from this study. The contract for this work is expected to be completed at the end of December and we expect all these analyses to be completed in approximately 4-6 months after that. We have also requested a no cost extension to allow this work to proceed and we have informal approval from DoD for this extension.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
We assessed the reproducibility of measuring microvascular function from the IVUS and flow wire measurements in 20 patients measured approximately 6 months apart. This demonstrated excellent measurement reproducibility. A paper on these results was published in the PLOS-1 journal.

What do you plan to do during the next reporting period to accomplish the goals? We are generating the largest dataset of exposures, vascular function, exercise capacity, and genetic studies of Gulf War Veterans, and one of the largest studies to collect data of this type in any disease state. Despite significant delays of about a year in starting this project we are close to completing the data collection for this study at the beginning of last year and completed the measurement of endothelial function and exercise parameters this last year. Approval and negotiating the contract for the microbiology and gene analyses at Dr. McRae's laboratory caused a delay of approximately 9-12 months in starting this part of the project but we plan to start this analysis by August 2019 and complete the analysis by December 2019. In the next reporting period, we will complete the microbiology and gene analysis and start testing the hypotheses related to GWI symptoms and differences in endothelial function, exercise parameters, and microbiological and gene expression. We will also assess the impact of the putative causal exposures during the Gulf War with these endpoints. The histopathological and genetic analyses of the muscle biopsies including the next generation RNA sequencing technology will use a large portion of the original grant budget. Remaining funds in this no-cost extension year will support salary to complete the endothelial function analysis, data management, and statistical analysis. The no-cost extension will allow us to complete these tasks within budget.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Fatigue and musculoskeletal symptoms are major components of GWI and could have an important impact on other symptoms associated with GWI. There are plausible reasons why endothelial function and mitochondrial biogenesis in muscle may be affected by exposure to environmental toxins during the Gulf War and lead to these symptoms.

Pyridostigmine and nerve gases are anticholinesterase agents that potentially have long term effects on the balance of cholinesterases and acetylcholine, which could affect activity at the neuromuscular junction of skeletal muscle, muscarinic receptors affecting vascular smooth muscle tone, and damage mitochondrial structure and electron transport activity in several tissues including muscle.

Insights on the pathogenesis of GWI could lead to new treatments for GWI, but also provide novel mechanistic insights into other exposure-related occupational health illness, such as pesticide exposure in the agricultural industry. Our study may also elucidate mechanisms of interest that require investigation as causes of other illnesses with muscle fatigue, pain, and abnormal muscle metabolism, such as peripheral artery disease and chronic heart failure, and advance our understanding of the pathophysiology of GWI and discover molecular pathways that could elucidate novel treatments for GWI. It may also direct future research into abnormalities of important molecules that could form the basis of an improved diagnostic test, although establishing a diagnostic test is not the focus of this proposal.

As the study is still in progress, there are currently no findings to report.

What was the impact on other disciplines? Nothing to Report.

What was the impact on technology transfer? Nothing to Report.

What was the impact on society beyond science and technology? Nothing to Report.

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change: Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them: There was a delay in approval of the no cost extension. This held up the subcontract with the Brigham and Women's Hospital for muscle biopsy analysis. However, now that the no-cost extension is moving forward and we expect to start this phase of the study shortly.

Changes that had a significant impact on expenditures: Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to Report.

6. **PRODUCTS:**

1. Kinlay S, et al. Reproducibility and validity of a novel invasive method of assessing peripheral microvascular vasomotor function. PLoS ONE 2019; 14: e0211152.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project? Individuals who have worked on this project during the most recent budget period are described below with their efforts and contribution divided by each quarterly reporting period.

July 1, 2016 to September 30, 2016

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1.

Name:	Jacquelyn-My Do, MPH
Project Role:	Assistant Program Manager
Research Identifier:	N/A
Nearest person month worked:	3
Contribution to Project:	Ms. Do is the new Project Manager for this study and has assumed the administrative project management responsibilities of Dr. Sara Jones. She is planning and tracking study recruitment, maintaining regulatory approval, and managing project resources and budget. She oversees mailing of recruitment letter, scheduling of patient appointments, and manages data collection.

Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn performs research activities are described in the study protocol, including informed consent of participants and conduct of Visit 1. She also assisted the Project Manager in administrative duties.

October 1, 2016 to December 31, 2016

Name:	Scott Kinlay, MBBS, PhD

Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1.

Name:	Jacquelyn-My Do, MPH
Project Role:	Assistant Program Manager
Research Identifier:	N/A
Nearest person month worked:	3
Contribution to Project:	Ms. Do continues in her role as Project Manager for this study. She continues to track study progress, maintains regulatory approval, and manages project resources and budget. She also oversees mailing of recruitment letter, scheduling of patient appointments, and manages data collection.

Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn performs research activities are described in the study protocol, including informed consent of participants and conduct of Visit 1. She also assisted the Project Manager in administrative duties.

January 1, 2017 – March 31, 2017

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	2
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. Additionally, he is actively recruiting for a Project Manager to replace Ms. Do.

Name:	Jacquelyn-My Do, MPH
Project Role:	Assistant Program Manager
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Do continues in her role as Project Manager for this

	study until 20-JAN-2017. She continues to track study progress, maintains regulatory approval, and manages project resources and budget. She also oversees mailing of recruitment letter, scheduling of patient appointments, and manages data collection. As of 20-JAN-2017, she has left the Boston VA Research Institute and VA Boston. She continues a temporary assignment with the project to help gather and analyze data from Visit 1, slated to end at 30-APR-2017.

Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn performs research activities are described in the study protocol, including informed consent of participants and conduct of Visit 1. She also assisted the Project Manager in administrative duties.

April 1, 2017 – June 30, 2017

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. He continues to actively recruit for a Project Manager to replace Ms. Do, but has hired a new research assistant to replace Margot Quinn.

Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn continues to perform research activities as described by study protocol, including informed consent of participants and conduct of Visit 1. As of 30-JUN-2017, she will be leaving her appointment with BVARI and will not longer be working on this project.

Name:	Melissa Chin, BS
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1

Contribution to Project:	Ms. Chin has a Bachelor of Science in Biochemistry from Boston College and has extensive bench lab experience. She started on this project on 26-JUN-2017 and will be replacing Ms. Quinn's position as research assistant, responsible for recruitment and activities of study visit 1.

July 1, 2017-September 30, 2017

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. Additionally, he has been actively recruiting for Project Manager

Name:	Melissa Chin, BS
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Chin continues to perform research activities as described by the study protocol, including informed consent of participants and conduct of Visit 1 under the direction of Dr. Kinlay

Name:	Mariah Bundy, BS
Project Role:	Senior Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Bundy was assigned to this project on 01-AUG-2017 to help collect and perform repeated measurement analysis on the intra-arterial IVUS images. She will provide blinded intra-arterial diameter measurements for this study.

Name:	Samantha Ly, MA
Project Role:	Program Manager
Research Identifier:	N/A
Nearest person month worked:	1

Contribution to Project:	Ms. Ly is responsible for training Ms. Chin in study procedures and Ms. Bundy with image analysis. She is also helping Dr. Kinlay with additional personnel recruitment and helping with the project's administrative tasks (e.g. maintenance of study regulatory binders, tracking study equipment inventory, and revising case report forms) until a Project Manager is hired.

October 1, 2017-December 30, 2017

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. Additionally, he has been actively recruiting for Project Manager/Coordinator

Name:	Melissa Chin, BS
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Chin continues to perform research activities as described by the study protocol, including informed consent of participants and conduct of Visit 1 under the direction of Dr. Kinlay

Name:	Mariah Bundy, BS
Project Role:	Senior Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Bundy was assigned to this project on 01-AUG-2017 to help collect and perform repeated measurement analysis on the intra-arterial IVUS images. She will provide blinded intra-arterial diameter measurements for this study.

Name:	Samantha Ly, MA
Project Role:	Program Manager
Research Identifier:	N/A
Nearest person month worked:	1

Contribution to Project:	Ms. Ly continues to assist Mr. Chin with study procedures and Ms. Bundy with image analysis as necessary. She is also helping Dr. Kinlay with additional personnel recruitment and helping with the project's administrative tasks (e.g. maintenance of study regulatory binders, tracking study equipment inventory, and revising case report forms) until a Project Manager/Coordinator is hired.

January 1, 2018-March 30, 2018

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. Additionally, he has been actively recruiting for Project Manager/Coordinator
Name:	Melissa Chin, BS
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Chin continues to perform research activities as described by the study protocol, including informed consent of participants and conduct of Visit 1 under the direction of Dr. Kinlay
Name:	Mariah Bundy, BS
Project Role:	Senior Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Bundy was assigned to this project on 01-AUG-2017 to help collect and perform repeated measurement analysis on the intra-arterial IVUS images. She will provide blinded intra-arterial diameter measurements for this study.
Name:	Samantha Ly, MA
Project Role:	Program Manager
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	As of 13-FEB-2018, Ms. Ly no longer works on this project. The new Project Manager/Coordinator will assume her responsibilities of managing project progress and data analysis
Name:	Desiree Tobin, MPH

Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Tobin was assigned to this project 01-AUG-2017 to help with measuring of the flow-map data blinded to patient details and infusion stage

Name:	Samuel Davis
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Mr. Davis completed the DUAL-X-ray-Absorption (DXA) studies on subjects, which is used to adjust the exercise parameters on aerobic thresholds

April 1, 2018-June 30, 2018

Name:	Scott Kinlay, MBBS, PhD

Name:	Melissa Chin, BS
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Chin continues to perform research activities as described by the study protocol, including informed consent of participants and conduct of Visit 1 under the direction of Dr. Kinlay. Ms. Chin has been helping with the project's administrative tasks (e.g. maintenance of study regulatory binders, and managing project progress) until a Project Manager/Coordinator is hired

Project Role:	Principle Investigator
Name:	Mariah Bundy, BS
Project Role:	Senior Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	As of 27-APR-2018 Ms. Bundy no longer works on this project. Intra-arterial measurements have been completed

Name:	Desiree Tobin, MPH
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Tobin was assigned to this project 01-AUG-2017 to help with measuring of the flow-map data blinded to patient details and infusion stage

Name:	Samuel Davis

Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Mr. Davis completed the DUAL-X-ray-Absorption (DXA) studies on subjects, which is used to adjust the exercise parameters on aerobic thresholds

July 1, 2018-December 31, 2018

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1

Name:	Melissa Chin, BS
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Chin continues to perform research activities as described by the study protocol, including informed consent of participants and conduct of Visit 1 under the direction of Dr. Kinlay. Ms. Chin has managed the project's administrative tasks (e.g. maintenance of study regulatory binders and managing project progress). She is also managing and cleaning up the databases and entering data. As of 31-DEC-2018 Ms. Chin was no longer working on this project
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. Additionally, he has been actively recruiting for Project Manager/Coordinator
Name:	Mariah Bundy, BS
Project Role:	Senior Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	As of 27-APR-2018 Ms. Bundy no longer works on this project. Intra-arterial measurements have been completed

Name:	Desiree Tobin, MPH
Project Role:	Research Assistant

Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Tobin was assigned to this project 01-AUG-2017 to help with measuring of the flow-map data blinded to patient details and infusion stage

Name:	Samuel Davis
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Mr. Davis completed the DUAL-X-ray-Absorption (DXA) studies on subjects, which is used to adjust the exercise parameters on aerobic thresholds. As of 31-DEC-2018 Mr. Davis no longer works on this project.

January 1, 2019-June 31, 2019

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principle Investigator
Research Identifier:	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	Dr. Kinlay is overseeing recruitment, ensuring that all Veterans meet study protocol eligibility criteria. He is also performing the peripheral catheterization and muscle biopsy in Visit 1. He is overseeing the cleaning of the data sets for endothelial function, exercise data, demographic data and is assisting BVARI in the contract negotiation for Dr. McRae's laboratory.

Name:	Rebecca Sherrod, BSc, MPH, MA
Project Role:	Research Manager
Research Identifier:	N/A
Nearest person month worked:	4
Contribution to Project:	Ms. Sherrod was hired at the end of APR 2019 as the Project Manager for this project. She is managing the administrative tasks of the study (e.g. maintenance of study regulatory binders and managing project progress). Managing the datasets including the demographic data and merging data from the beginning of the study and the survey of symptoms and exposures during the Gulf War at the end of the study. She will also manage the transfer of specimens to Dr. McRae's laboratory and the manage the data outputs from this part of the study.

Name:	Desiree Tobin, MPH
Project Role:	Research Assistant

Research Identifier:	N/A
Nearest person month worked:	2
Contribution to Project:	Ms. Tobin was assigned to this project 01-AUG-2017 to help with measuring of the flow-map data blinded to patient details and infusion stage. She also cleaned up the datasheets for the exercise data and demographics. As of 31-DEC-2018 Melissa Chin no longer works on this project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Nothing to Report.

What other organizations were involved as partners? The analysis of muscle samples will include isolation and analysis of RNA and DNA, protein isolation expression, microchip arrays, and next generation RNA generation. We will work with our collaborator, Dr. Calum MacRae at the Brigham and Women's hospital to complete these analyses.

Organization Name: Brigham and Women's Hospital
Location of Organization: Boston, MA
Partner's contribution to the project: Collaboration

Until sample analysis commences, we current do not have any partner organizations.

8. **SPECIAL REPORTING REQUIREMENTS COLLABORATIVE AWARDS:** None.

9. **APPENDICES:** Please see the attached quad chart.

Vascular and Skeletal Muscle Function in Gulf War Veterans Illness

Log Number: GW14003

Award Number: W81XWH-15-1-0216

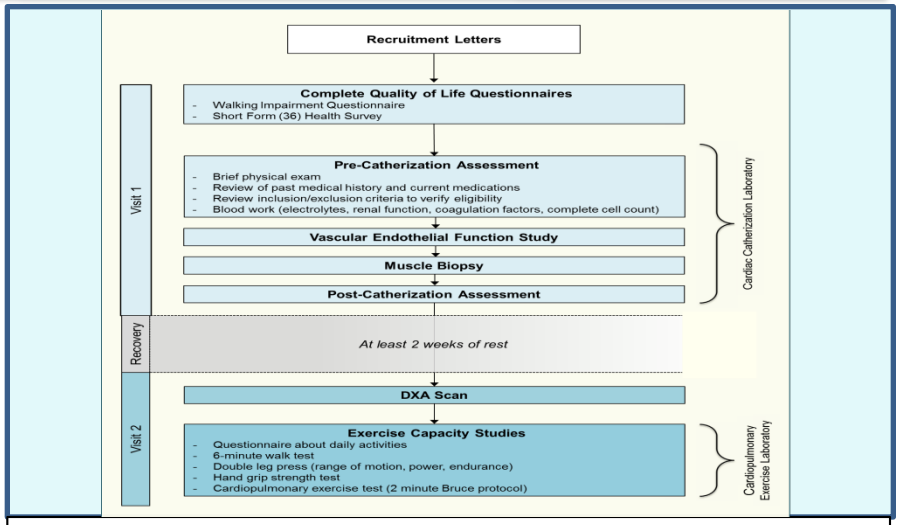
PI: Scott Kinlay, MBBS, PhD **Org:** Boston VA Research Institute, Inc. (BVARI) **Award Amount:** $870,642.00

Study/Product Aim(s)

- To determine if microvascular endothelium-dependent and endothelium-independent function of the profunda femoral artery is impaired in subjects with Gulf War Veterans Illness (GWVI) compared to deployed Veterans without GWVI.
- To determine if peak oxygen uptake and ventilatory anaerobic threshold during cardiopulmonary exercise testing, and other muscle functions are impaired in subjects with GWVI compared to deployed Veterans without GWVI.
- To determine how the expression of genes relevant to endothelial function and mitochondrial function in muscle biopsy samples differs between subjects with GWVI compared to deployed Veterans without GWVI.
- To determine if polymorphisms to genes relating to endothelial function and mitochondrial respiratory function differ between subjects with GWVI compared to deployed Veterans without GWVI.

Approach

Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, and neurocognitive dysfunction reported by Gulf War Veterans shortly after their return from deployment in 1991. There are plausible reasons why endothelial function and mitochondrial biogenesis in muscle may be affected by exposure to environmental toxins during the Gulf War and lead to GWI symptoms. We hypothesize that compared to Veterans without GWI, Veterans with GWI will have differences in arterial endothelial function, muscle function determined by cardiopulmonary exercise testing, and the expression of genes responsible of mitochondrial function.



Accomplishment: This IRB-approved prospective cross-sectional clinical trial will consist of 2 study visits. 70 Gulf War Veterans (35 with GWI and 35 without GWI) will be enrolled.

Timeline and Cost

Updated Timeline / Initial Timeline

Activities CY	15	16	17	18	19
Milestone 1: Achieve local IRB approval of protocol					
Milestone 2: Complete Visit 1 (endothelial function and muscle biopsies) on 70 subjects					
Milestone 3: Complete Visit 2 (exercise and cardiopulmonary stress test) onn70 subjects					
Milestone 4: Complete histopathology and electron microscopy analysis					
Milestone 5: Complete gene and protein analysis					
Milestone 6: Complete analysis on transcriptome microarray data					
Milestone 7: Complete analysis of SNP microarray data					
Finalize data anlysis, present results and meetings, publish results					
Estimated Budget ($K)	**$0**	**$220**	**$361**	**$287**	**$0**

Updated: 26-July-2019

Goals/Milestones

CY15 Goals – Institutional Review Board (IRB)

☑ Achieve local IRB approval

☑ Achieve HRPO approval

CY16/17 Goals – Subject Recruitment

☑ Start recruitment with letters of invitations

☑ Schedule and conduct Visits 1 and 2

CY18 Goals – Complete recruitment and data analysis

☑ Complete Visits 1 and 2 on 70 Subjects

CY19 Goal – Analyze and publish results

☐Complete histopathological data, electronic microscopy data, specific genes and proteins regulating mitochondrial biogenesis, analysis of transcriptome microarray data on samples collected

☐ Analyze, present, and publish results at DoD and scientific meetings

Comments/Challenges/Issues/Concerns: We anticipate that a majority of the expenditures will be used to cover costs of analysis.

Budget Expenditure to Date

Projected Expenditure: $870,642; Actual Expenditure: $343,594