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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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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.									
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Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	7
5. Changes/Problems.....	8
6. Products.....	8
7. Participants & Other Collaborating Organizations.....	8
8. Special Reporting Requirements.....	11
9. Appendices	
Quad Chart.....	12

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 served 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	Completed
Major Task 2: Recruitment of Subjects		
Send batch invitations to 400 Gulf War Veterans who have completed the Fort Devens cohort study	1-24	Completed
Major Task 3: Endothelial Function Study and Muscle Biopsy		
Schedule Visit 1 (Endothelial function studies and muscle biopsies)	1-24	Completed

Complete endothelial function studies and muscle biopsies and measurement of intravascular ultrasound and flow data to assess microvascular and conduit endothelial function	1-28	Completed
Milestone: Complete endothelial function data and muscle biopsies on 70 subjects	28	Completed
Statistical analysis: Data will be entered into a spreadsheet. Microvascular endothelial function between cases with Gulf War Illness versus controls will be compared using repeated measures ANOVA to assess statistical significance in the response to acetylcholine over baseline. Microvascular endothelium-independent function will be assessed between cases and controls using repeated measures ANOVA of the adenosine response over baseline. Conduit artery endothelial function between cases and controls will be assessed by using ANOVA to assess statistical significance in the response to acetylcholine over baseline. Microvascular endothelium-independent function will be assessed between cases and controls using repeated measures ANOVA of the nitroglycerin response over baseline.	28-33	Database completed; data analysis completed
Milestone: Completion of statistical analysis	33-81	Pending
Major Task 4: Exercise and Cardiopulmonary Stress Testing		
Schedule Visit 2 (Exercise and cardiopulmonary stress tests)	3-28	Completed
Complete exercise and cardiopulmonary stress studies and interpretation	3-28	Completed
Milestone: Complete exercise data on 70 subjects	28	Completed
Statistical Analysis: Comparisons of absolute and lean body mass corrected oxygen consumption, anaerobic threshold, power, force, and fatigability will be assessed between Gulf War Illness cases and controls using separate repeated measures ANOVA.	28	Database completed, data analysis pending
Milestone: Complete statistical analysis	33-81	Pending
Major Task 5: Histopathology and Electron Microscopy		
Prepare muscle biopsy specimens for histopathology and electron microscopy and image	45-72	Completed
Complete data on muscle analysis including histopathology	45-81	Pending. <i>This analysis and all subsequent analyses are delayed due to the COVID crisis</i>
Milestone: Complete histopathological data and	45-81	Pending

electron microscopy data on representative subjects		
Major Task 6: Gene and protein expression relating to mitochondrial biogenesis		
Isolate DNA, RNA, and protein from muscle tissue samples. Prepare cDNA from RNA samples.	45-81	Completed
Complete qPCR and Western Blot to assess genes and proteins regulating mitochondrial biogenesis.	45-81	Completed
Milestone: Complete data on specific genes and proteins regulating mitochondrial biogenesis on 70 subjects	45-81	As of 29-JUL-2020, Completed
Major Task 7: Transcriptome microarrays comparing cases and controls		
Run next generation RNA sequencing from cDNA samples for unbiased analysis of genes related to GWI	45-81	Pending
Interpret results and identify candidate genes related to GWI. Data will be quantile normalized. Further analyses will be guided by Dr. MacRae's laboratory to this project.	45-81	Pending
Milestone(s): Complete analysis of transcriptome	45-81	Pending
Major Task 8: SNP Microarray		
Run MVP microarray at Dr. MacRae's Lab	45-81	Pending
Interpret results and identify candidate genes related to GWI	45-81	Pending
Milestone: Complete data analysis of SNP microarray data on 70 subjects	45-81	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.	57-81	Pending

What was accomplished under these goals? This report summarizes the research progress in the most recently completed budget period from July 1, 2019 to June 30, 2020. This time period corresponds to the fifth of this six-year project. The objectives of this study is 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.

In the last year we successfully finished collecting exercise and cardiopulmonary stress testing data for 77 veterans (major task 4). Additionally, with the approval of the subcontract with The Brigham and Women's Hospital, the muscle biopsy samples were prepared and transported to Dr. Calum MacRae's lab for analysis. Despite delays due to COVID and the approval of the

subcontract, Dr. MacRae has successfully completed Major Task 6 at the end of July 2020, which is the analyses of the Gene and protein expression relating to mitochondrial biogenesis.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

We presented our data on the reproducibility of measuring endothelial function in the first 20 patients of this study as a poster at the Society of Vascular Medicine Annual Scientific Meeting in Chicago June 14-16, 2018. The paper “Reproducibility and validity of a novel invasive method of assessing peripheral microvascular vasomotor function” was published in January 2019 in PLOS ONE (<https://doi.org/10.1371/journal.pone.0211152>).

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 the delays caused by COVID-19, in the next reporting year we plan to finish the statistical analysis of survey data. We have already completed the basic descriptive statistics. Dr. Calum MacRae has a large laboratory funded by the multi-million dollar “One Great Idea” grant to analyze gene expression and genomes in large population studies. Although delayed, Dr. MacRae has already completed the analyses of the gene and protein expression relating to mitochondrial biogenesis and continues to work on the other muscle biopsy analyses. Additionally, following the completion of the muscle biopsy analyses we will further statistically analyze those results. We applied and were approved for a No Cost Extension which has allowed us to absorb these delays without impacting our budget. Following the completion of muscle biopsy and analyses, and once able, we plan to disseminate and publish the results in journals and present at conferences.

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.

In particular, 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 were initial delays in completing the subcontract with the Brigham and Women's Hospital for the muscle biopsy analysis, therefore we applied and were approved for a No Cost Extension through 06-30-2019. The subcontract was approved, and the samples were transferred to the lab for analysis. However, the ongoing COVID pandemic delayed the start of the muscle biopsy analysis by Dr. Calum MacRae's lab at the Brigham and Women's Hospital. The lab is now conducting the analyses and anticipates being finished by the end of the year. In response to this delay we applied and were approved for a No Cost Extension, this extension is through 06-30-2021.

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:** Nothing to Report.

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.

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-7687-9136
Nearest person month worked:	1
Contribution to Project:	69

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 a Project Manager/Coordinator.

Name:	Rebecca Sherrod, MPH, MA
Project Role:	Clinical Research Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	Ms. Sherrod oversees data collection and database management, and preliminary statistical analysis. Additionally, she services as the Clinical Research Coordinator and manages the projects finances, IRB submissions, DoD and HRPO reports.

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

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)

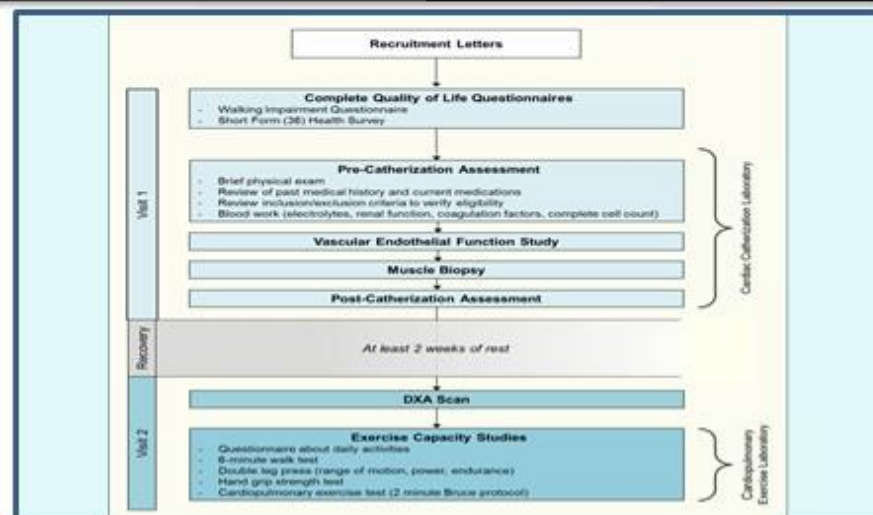
Award Amount: \$870,642.00

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

Activities	15	16	17	18	19	20
Milestone 1: Achieve local IRB approval and protocol	Updated					
Milestone 2: Complete visit 1 (endothelial function and muscle biopsy)		Updated	Updated	Updated		
Milestone 3: Complete visit 2 (exercise and cardiopulmonary stress test) on 70 subjects		Updated	Updated	Updated	Updated	
Milestone 4: Complete histopathology and electron microscopy analysis			Updated	Updated	Updated	Updated
Milestone 5: Complete gene and protein analysis				Updated	Updated	Updated
Milestone 6: Complete analysis on transcriptome microarray data					Updated	Updated
Milestone 7: Complete analysis on SNP microarray data						Updated
Finalize data analysis, present results at meetings, and publish findings						Updated
Estimated Budget (\$K)	50	5220	5361	5287	50	50

Updated: 31-JULY-2020

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

- Transport samples from VA to Brigham and Women's MacRae Lab for analysis.

CY20 Goals – Complete analysis 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: After delays due to COVID-19 the analyses of histopathological data, electronic microscopy data, specific genes and proteins regulating mitochondrial biogenesis, analysis of transcriptome microarray data on samples collected has begun.

Budget Expenditure to Date

Projected Expenditure: \$870,642; Actual Expenditure: \$392,074.35