

AWARD NUMBER: W81XWH-20-1-0128

TITLE: Sex Differences in Stress-Related Cardiometabolic Risk in PTSD

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REPORT DATE: MARCH 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

*Form Approved*²
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE MARCH 2021		2. REPORT TYPE Annual		3. DATES COVERED 1 MARCH 2020 – 28 FEB 2021	
4. TITLE AND SUBTITLE Sex Differences in Stress-Related Cardiometabolic Risk in PTSD				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-20-1-0128	
				PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Sabra Inslicht, PhD E-Mail: sabra.inslicht@va.gov				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Northern California Institute for Research and Education 4150 Clement Street 151NC San Francisco, CA 94121-1563				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT: Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Post-traumatic stress (PTS) has been associated with biological changes in the hormonal stress response, arteries, and immune system that may increase risk for cardiovascular disease. PTS increases risk for cardiovascular disease, particularly for women compared to men. Little research has examined biological mechanisms of cardiovascular disease risk that result from PTS in women, particularly in relation to reproductive hormones that fluctuate as a result of changes in the natural menstrual cycle in younger women. However, studying premenopausal women may provide insight into how certain reproductive hormones and their products could either decrease or exacerbate the stress-related cardiovascular risks associated with PTS. The goal of this project is to identify biological mechanisms that may explain the relationship between PTS and cardiovascular risk in men and women with PTSD by examining biological changes in PTS that affects risk for cardiovascular disease in women. This study will use a recently developed laboratory technique called "metabolomics" that can identify circulating small molecules that affect cell and physiological function. This approach is broad and allows for a comprehensive examination of multiple physiological pathways at the same time that may be missed with traditional, more targeted approaches. A metabolomic analysis examining lipids and reproductive hormones will be performed on stored blood samples that were obtained from premenopausal women and men of similar ages with and without PTS in order to address two specific aims: Aim 1. To examine lipid metabolites that associate with CVD risk and PTS in women (across the menstrual cycle) relative to men. Aim 2. To examine which sex steroids modulate the relationship between CVD risk, PTS and lipid metabolism in women relative to men.					
15. SUBJECT TERMS Sex Differences; Posttraumatic Stress; Cardiovascular Diseases; Metabolomics; Hormones; Lipids					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	12	19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION:

Post-traumatic stress (PTS) has been associated with biological changes in the hormonal stress response, arteries, and immune system that may increase risk for cardiovascular disease. PTS increases risk for cardiovascular disease, particularly for women compared to men. Little research has examined biological mechanisms of cardiovascular disease risk that result from PTS in women, particularly in relation to reproductive hormones that fluctuate as a result of changes in the natural menstrual cycle in younger women. However, studying pre-menopausal women may provide insight into how certain reproductive hormones and their products could either decrease or exacerbate the stress-related cardiovascular risks associated with PTS. The goal of this project is to identify biological mechanisms that may explain the relationship between PTS and cardiovascular risk in men and women with PTSD by examining biological changes in PTS that affects risk for cardiovascular disease in women. This study will use a recently developed laboratory technique called “metabolomics” that can identify circulating small molecules that affect cell and physiological function. This approach is broad and allows for a comprehensive examination of multiple physiological pathways at the same time that may be missed with traditional, more targeted approaches. A metabolomic analysis examining lipids and reproductive hormones will be performed on stored blood samples that were obtained from premenopausal women and men of similar ages with and without PTS in order to address two specific aims: Aim 1. To examine lipid metabolites that associate with CVD risk and PTS in women (across the menstrual cycle) relative to men. Aim 2. To examine which sex steroids modulate the relationship between CVD risk, PTS and lipid metabolism in women relative to men.

2. KEYWORDS:

Sex Differences; Posttraumatic Stress; Cardiovascular Diseases; Metabolomics; Hormones; Lipids

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Study Goals	Timeline (months)	Percentage Complete
1. Study Start Up and Approvals	1-3	100%
2. Coordinate Study Staff for Sample Analysis	1-9	70%
3. Assay Biological Samples	13-17	10%
4. Process Physiological Data	13-17	25%
5. Data Analysis	14-24	0%
6. Finalize Study Requirements and Prepare for Future Funding	22-24	0%

What was accomplished under these goals?

The study started on April 1st 2020. This report describes accomplishments to date. All study start up activities were completed on schedule, including regulatory paperwork and approvals and hiring and coordination of study staff for sample analysis. Receiving laboratories have been contacted, and contracts have been drafted. Procedures for sample shipping and receiving were developed, and a tracking system was created. Biological samples are currently being organized and prepared for shipping. A data analyst has been contacted to process psychophysiological data. Relevant timepoints in psychophysiological data are being labeled before sending the data to the analyst. Our detailed accomplishments to date include:

Major Task 1: Study Start Up and Approvals	Timeline
Subtask 1: Prepare regulatory documents and submit for IRB approval	Completed
Develop IRB application and other regulatory documents	Completed
Submit IRB application to UCSF IRB and obtain full committee review	Completed
Review by SFVAHCS regulatory personnel	Completed
Review by HRPO	Completed
Prepare IRB reports for continuing review approvals	Annually
<i>Milestone Achieved: IRB approval from UCSF, VA, and HRPO</i>	Completed
Major Task 2: Coordinate Study Staff for Sample Analysis	Timeline
Subtask 1: Hiring and Training of Study Staff	
Coordinate with NCIRE to prepare job description and advertisement	Completed
Interview research staff candidates	Completed
Coordinate with SFVAHCS for candidate approval and required trainings	Completed
Training of research staff on study procedures and biospecimen storage, shipping, and receiving	Completed
<i>Milestone Achieved: Research staff hired and trained</i>	Completed
Subtask 2: Coordinate with laboratory personnel for sample shipments	
Contact staff at receiving laboratories (UC Davis)	Completed
Develop procedures manual for sample shipping and receiving	Completed
Develop sample tracking system	Completed
Schedule batched shipments	In progress
<i>Milestone Achieved: Sample shipment protocol established</i>	In progress
Subtask 3: Build database for incoming data	
Establish data extraction protocol and build database	Completed
Establish logistical plan for data quality check	Completed
<i>Milestone Achieved: Database built</i>	Completed
Major Task 3: Assay Biological Samples	Timeline
Subtask 1: Ship stored samples to the receiving laboratory and acquire data	
Package and ship stored samples to UC Davis	In progress
Samples received by UC Davis	In progress

Samples inventoried by UC Davis	In progress
<i>Milestone Achieved: Samples shipped for assay</i>	<i>In progress</i>
Major Task 4: Process Psychophysiological Data	Timeline
Subtask 1: Coordinate with data analyst for psychophysiological data processing	
Contact data analyst and discuss data processing procedure	Completed
Label relevant time points in data for data analyst	In progress
Send psychophysiological data to data analyst	In progress
<i>Milestone Achieved: Psychophysiological data sent for processing</i>	<i>In progress</i>

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

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Plans until next reporting period:

1. Package and ship stored samples to UC Davis to be assayed (Task 3).
2. Send psychophysiological data to data analyst to be processed (Task 4).
3. Acquire assay data (Task 3) and processed psychophysiological data (Task 4). Enter data into database and clean data (Task 5).
4. Finalize data analysis tasks: We are currently working with Dr. Fiehn and the Biostatisticians to conduct chemRich analyses on significant metabolites and conduct correlational analyses with health outcomes (Task 5).
5. Disseminate study findings and finalize study closeout requirements (Task 5).
6. Apply for additional future funding (Task 6).

Major Task 3: Assay Biological Samples	Timeline
Subtask 1: Ship stored samples to the receiving laboratory and acquire data	
Package and ship stored samples to UC Davis	In progress
Samples received by UC Davis	In progress
Samples inventoried by UC Davis	In progress
<i>Milestone Achieved: Samples shipped for assay</i>	<i>In progress</i>
Subtask 2: Assay biological samples	
Perform metabolomics analysis	13-16
Receive data from laboratory	14-16
<i>Milestone Achieved: Samples shipped and data acquired</i>	<i>13-16</i>
<i>Milestone Achieved: Assays complete</i>	<i>17</i>

Major Task 4: Process Psychophysiological Data	Timeline
Subtask 1: Coordinate with data analyst for psychophysiological data processing	
Contact data analyst and discuss data processing procedure	Completed
Label relevant time points in data for data analyst	In progress
Send psychophysiological data to data analyst	In progress
<i>Milestone Achieved: Psychophysiological data sent for processing</i>	<i>In progress</i>
Subtask 2: Process psychophysiological data	
Psychophysiological data cleaned by data analyst	13-16
Psychophysiological data processed by data analyst	14-16
Receive processed data from data analyst	15-17
<i>Milestone Achieved: Data acquired</i>	<i>15-17</i>
<i>Milestone Achieved: Data processing complete</i>	<i>17</i>
Major Task 5: Data Analysis	Timeline
Subtask 1: Enter data and maintain database	
Perform quality checks on incoming data	14-16
Enter all data and maintain database	14-17
Subtask 2: Aim 1. To examine lipid metabolites that associate with CVD risk and PTS in women (across the menstrual cycle) relative to men.	
Clean and process incoming data and prepare for analysis	14-17
Coordinate with Data Management for monitoring data entry and quality	17-19
Work with Biostatistician to conduct analyses	16-20
<i>Milestone Achieved: Aim 1 addressed</i>	<i>20</i>
Subtask 3: Aim 2. To examine which sex steroids modulate the relationship between CVD risk, PTS and lipid metabolism in women.	
Clean and process incoming data and prepare for analysis	14-18
Coordinate with Data Management for monitoring data entry and quality	17-18
Work with Biostatistician to conduct analyses	16-20
<i>Milestone Achieved: Aim 2 addressed</i>	<i>20</i>
<i>Milestone Achieved: Data analysis complete</i>	<i>20</i>
Subtask 4: Share output and findings with co-investigators and with the greater community	
Dissemination of findings (abstracts, presentation, publications, DOD)	16-24
<i>Milestone Achieved: Report results from data analyses</i>	<i>22-24</i>
Major Task 6: Finalize Study Requirements and Prepare for Future Funding	Timeline
Subtask 1: Prepare grant application for DOD or VA Merit Award funding for a clinical trial based on study findings	
<i>Milestone Achieved: Submit grant proposal for future funding</i>	<i>22-24</i>

4. IMPACT:

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

Nothing 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

Due to COVID-19, we experienced laboratory closures at the San Francisco VA Health Care System laboratory and at UC Davis West Coast Metabolomics Center (WCMC). We were therefore unable to organize and ship the blood samples to be assayed for lipids and steroids. We have only recently been granted permission to work on-site at the San Francisco VA Health Care System laboratory, and even then, we are limited to doing so once a week. We expect to ship the biological samples for assays and send the psychophysiological data for processing in the next month. Once we receive data, we will then be able to analyze the data, disseminate our findings, and prepare grant applications for future funding.

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:**Publications, conference papers, and presentations**

Nothing to Report

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**What individuals have worked on the project?**

Name:	Sabra Inslicht
Project Role:	Principal Investigator
Researcher Identifier:	ORCID ID: 0000-0002-2456-1625
Nearest person month worked:	2 person months
Contribution to Project:	Dr. Inslicht has experience conducting clinical trials in Veterans with PTSD and mechanistic studies of human fear conditioning and stress related interactions with complex steroid pathways, reproductive hormones, immune function, metabolic responses, sleep, and health behavior. Dr. Inslicht will assume overall scientific and administrative responsibility for this project, ensuring that research goals are met in a timely manner with scientific integrity. She will design and implement each phase of the research plan and work with the study coordinator to oversee human subjects regulatory documentation and compliance, coordination of personnel involved in this protocol, the coordination of assay completion, as well as the development of a data tracking system to manage participant information, biological samples, and assay data. Over the next reporting period, additional effort will be placed on assisting with sample sorting, for which laboratory access was limited in the prior due to COVID-19 restrictions. Dr. Inslicht will also work with the statistician to conduct data analyses and will prepare manuscripts and disseminate findings.

Name:	Thomas Neylan
Project Role:	Co-Investigator
Researcher Identifier:	ORCID ID: 0000-0002-1572-2626
Nearest person month worked:	1 person month
Contribution to Project:	Dr. Neylan has extensive expertise in the biology of PTSD, sleep, metabolic function, clinical trials, and laboratory-based psychophysiological research. He provides onsite support to Dr. Inslicht on the design, conduction of the proposed project, data analysis and manuscript preparation. As the Director of the Stress and Health Research Program, Dr. Neylan leads a weekly study management meeting in which he addresses scientific, administrative, and data issues.

Name:	Aditi Bhargava
Project Role:	Co-Investigator
Researcher Identifier:	ORCID ID: 0000-0003-1334-0517
Nearest person month worked:	1 person month
Contribution to Project:	Dr. Bhargava is molecular biologist with extensive research experience in the area of metabolomics and neuroendocrinology, including pain, stress, and inflammation. Dr. Bhargava will contribute to study design, execution, data analysis, interpretation of the metabolomics data, and manuscript preparation.

Name:	Joyce Gurdock
Project Role:	Staff Research Associate
Nearest person month worked:	2 person months
Contribution to Project:	Ms. Gurdock assists with study responsibilities including sample organization and preparation, data entry, coding, data cleaning, literature reviews, and editorial support.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Inslicht will increase effort by an addition 0.36 person months to provide more assistance with sample sorting, in light of limited laboratory access due to COVID-19 restrictions.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS:

Collaborative Awards:

N/A

Quad Charts:

See attachment

9. APPENDICES:

Award Chart

PR192475: Sex Differences in Stress-Related Cardiometabolic Risk in PTSD



PI: Sabra Inslicht, NCIRE, CA

Budget: \$306,531

Topic Area: Peer Reviewed Medical Research Program **Mechanism:** Discovery Award

Research Area(s): 0419, 0302

Award Status: 01 March 2020 – 28 February 2022

Study Goals:

We propose to examine biomarkers of cardiovascular health, leveraging upon infrastructure and resources afforded by a previously funded clinical trial, to determine sex differences in biochemical pathways that lead to CVD risk in young, healthy males and females with PTS and controls.

1. Start up study and get approvals
2. Coordinate study staff for sample analysis
3. Assay biological samples
4. Process psychophysiological data
5. Analyze data
6. Finalize study requirements and prepare for future funding

Specific Aims:

1. To examine lipid metabolites that associate with CVD risk and PTS in women (across the menstrual cycle) relative to men
2. To examine which sex steroids modulate the relationship between CVD risk, PTS and lipid metabolism in women relative to men.

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: \$55,269 as of 3/26/2021