THE EFFECTS OF A PRIOR EXERCISE BOUT ON THE ENERGETIC AND CARDIOMETABOLIC RESPONSES TO ACUTE MENTAL STRESS

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Human Movement Science Curriculum in the Department of Allied Health Sciences in the School of Medicine.

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ABSTRACT

Gabriel Hart Zieff: The Effects of a Prior Exercise Bout on the Energetic and Cardiometabolic Responses to Acute Mental Stress
(Under the direction of Lee Stoner)

Background: Mental stress is associated with cardiovascular disease (CVD) risk, but the arterial stiffness and energy expenditure (EE) responses to acute mental stress, and whether prior exercise impacts post-stress cardiometabolic reactivity are not known. The objectives of this dissertation were to assess arterial stiffness and EE responses to acute mental stress and to determine the impact of a prior exercise bout on these responses.

Methods: In addition to a meta-analysis on the effects of acute mental stress on arterial stiffness, this dissertation entailed two randomized cross-over studies. Forty recreationally active young adults (18-30 y) were recruited. For Cross-over 1, 20 participants attended two laboratory visits: i) Trier Social Stress Test (arithmetic + speech), and ii) Control. For Cross-over 2, 20 different participants attended two laboratory visits: i) Exercise + Trier Social Stress Test (psychosocial task), and ii) Exercise + Control. Exercise consisted of 25 minutes of moderate-intensity elliptical. Arterial stiffness and EE were measured by pulse-wave velocity (PWV) and indirect calorimetry, respectively. Measurements took place pre, during (EE only), and post condition. Mixed model linear regression assessed condition x time interactions.

Results: Meta-analysis: Across 17 trials from 9 studies, exposure to acute mental stress caused arterial stiffness to increase (Standardized Mean Difference: 0.45; $p<0.05$). Cross-over 1:

There was a small interaction (B=0.68 m/s, 95%CI: 0.39, 0.97) for PWV [Stress: ↑0.81 m/s, Control: ↑0.15 m/s]. There was also a small interaction (B=0.0010 kcal/kg/min, 95%CI: 0.0004, 0.0015) for EE (Stress: ↑0.0016 kcal/kg/min, Control:↑0.0005 kcal/kg/min). Cross-over 2: There was a small interaction (B=0.47 m/s, 95%CI: 0.21, 0.72) for PWV (Stress: ↑0.43 m/s, Control: ↓-0.05 m/s). For EE, there were small main effects of condition (B=0.0005 kcal/kg/min), 95%CI: 0.0003, 0.0008) and time (B=0.0011 kcal/kg/min, 95%CI: 0.0006, 0.0016). Compared to Cross-over 1, the prior exercise introduced in Cross-over 2 dampened the arterial stiffness and EE responses.

Conclusions: Arterial stiffness and EE may be key players in the relationship between acute mental stress and CVD risk, and exercise may beneficially moderate this relationship. Future research examining the stress-CVD paradigm, including potential protective effects of exercise, will be necessary to inform stress-related CVD prevention and treatment efforts.

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PREFACE

Dissertation Structure

This section outlines the structure of this dissertation, which comprises 8 chapters. The chapters are presented in order of anticipated completion. **Chapter 1** provides a rationale for this dissertation and each of the included studies. **Chapter 2** outlines the candidates training goals. **Chapter 3** is a literature review, which also briefly outlines the significance of the proposed research. **Chapter 4** provides rationale for each study design aspect. **Chapter 5** is a systematic review and meta-analysis. **Chapters 6-7** are the primary experimental research studies. **Chapters 5-7** are each presented in manuscript format and will be submitted for publication. **Chapter 8** summarizes the key findings, discusses the implications of these findings, and makes recommendations for future research.

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LIST OF ABBREVIATIONS

AE	Aerobic Exercise
ANS	Autonomic Nervous System
AS	Arterial Stiffness
CV	Cardiovascular
CVD	Cardiovascular Disease(s)
EE	Energy Expenditure
HRV	Heart Rate Variability
PWA	Pulse Wave Analysis
PWV	Pulse-wave Velocity
RMR	Resting Metabolic Rate
SBP/DBP	Systolic/Diastolic Blood Pressure
TSST	Trier Social Stress Test

CHAPTER 1: INTRODUCTION AND SPECIFIC AIMS

Mental stress is an ubiquitous aspect of the human experience. While some mental stress is adaptive, excessive exposure has been linked to cardiovascular disease (CVD)[1]. A biologically plausible mechanism linking repeated exposure to acute mental stress with CVD is autonomic nervous system (ANS) dysregulation[2, 3]. Another mechanism that is associated with the ANS and CVD risk is energy expenditure (EE)[4–6]. However, EE has not been investigated in the context of acute stress. Importantly, aerobic exercise (AE) positively modulates ANS and EE systems, and may therefore be a cardioprotective effect modifier in the relationship between ANS and EE in the face of stress.

A key knowledge gap that may help explain the link between mental stress and CVD is the influence of stress on EE and metabolism[7], which govern important CVD risk factors such as body composition and glucose regulation[8, 9]. Moreover, EE is tightly regulated by the ANS[10–12], which controls the body's immediate stress response. Despite the plausible link between mental stress and EE, few prior studies have measured the energy expenditure response to acute mental stress in the context of CVD risk [13–16]. Further, beyond regulating EE, the ANS influences hemodynamic factors critical to CVD risk. Thus, another likely contributor to stress-induced CVD risk is ANS dysregulation and ensuing hemodynamic disturbances[3, 17–21]. Importantly, ANS dysregulation is positively modified by aerobic exercise (AE)[11, 22]. While AE, including acute bouts, have been shown to improve mental

affect and perceived stress [23, 24], few studies have examined the effect of a prior AE bout on the post-stress cardiovascular (CV) response[25–27]. Collectively, there is a limited understanding of how repeated exposure to mental stressors leads to CVD, and even less understanding regarding i) the EE associated with acute mental stress and ii) the effects of prior AE on post-stress CV and EE reactivity.

The candidate's long-term goal is to elucidate the mechanisms by which acute mental stressors contribute to CVD risk, thereby facilities the development of lifestyle-based strategies to mitigate CVD risk. Since a strong foundational knowledge of the current literature must be established in order to successfully execute and ultimately contextualize findings from the experimental studies of this dissertation (described below), **Aim 1** was to conduct a systematic review and meta-analysis on the effects of acute mental stress on arterial stiffness (AS) in disease-free adults was conducted prior to the experimental studies.

Considering the nascent understanding of the effect of acute mental stress on EE and cardiovascular reactivity, **Aim 2 (Experimental Study 1)** assessed the energy expenditure and arterial stiffness response to an acute mental stressor. To address this aim, indirect calorimetry was used to assess EE[28]. Indirect calorimetry is a reliable technique that is sensitive to acute changes in EE, and enabled simultaneous EE measurement during and following the administration of the mental stressor. Arterial stiffness was assessed using pulse-wave velocity, a well-established subclinical, non-invasive measure of arterial stiffness which is an important marker of vascular aging and early CVD risk[29, 30]. Since AE may positively modify the psychophysiological stress response, **Aim 3 (Experimental Study 2)** determined the impact of a prior AE bout on EE and AS during and following an acute stressor.

Aim 1: Systematic Review and Meta-Analysis assessed the state of the literature surrounding acute mental stress and arterial stiffness. Studies were included if (i) arterial stiffness was measured non-invasively using pulse wave velocity (PWV) or pulse transit time (PTT), (ii) arterial stiffness was measured pre- and post- administration of an acute mental stressor, (iii) participants were adults (≥18 years) free of vascular-acting medication and overt CVD or CVD risk at the time of testing, and (iv) the mental stressor was purely psychological (rather than purely physiological or both psychological and physiological) in nature

Aim 2: **Experimental Study 1 determined the EE and AS response to an acute mental stressor**. **Population.** Twenty recreationally active adults (18-30 y) were recruited. This age range was selected to minimize age-related biological variance. **Protocol.** Each participant attended two 105 min laboratory visits in a randomized order consisting of: i) Trier Social Stress Test (TSST; mental arithmetic + psychosocial stressors), and ii) Control (no-stress).Prior to, during (EE only), and throughout the 60-min period following the TSST, EE and AS were assessed. EE was assessed via indirect calorimetry with a ventilated canopy and metabolic cart. AS was measured by brachial-femoral pulse-wave velocity (bfPWV). **Hypothesis.** The stressor TSST will increase EE and AS.

Aim 2: Experimental Study 2 determined the effects of a prior AE bout on AS and EE following exposure to an acute mental stressor. A separate group 20 recreationally active adults (18-30 y) attended two 135 min visits consisting of AE+TSST and ii) AE only (no stress). The TSST is a validated laboratory stressor which has been shown to elicit a robust physiological response[31, 32]. The dual-study design allowed us to address the following distinct aims and eliminate potential confounding due to TSST habituation (e.g. multiple TSSTs not required with

current study/dissertation design)Participants engaged in 25 min of moderate-intensity (50% heart-rate reserve) AE prior to the TSST. This duration and intensity of AE was chosen as it was likely to induce a robust AS and EE response and is in line with national and international physical activity guidelines. On the other hand, the AE was likely not at an intensity high enough to prompt mental distress in this population. The outcomes were EE and AS, measured before, during (EE only), and throughout the 60 min period following the TSST. **Hypothesis.** The stressor increase EE and AS. Compared to Aim 1, the AS and EE will be dampened.

Significance. Findings will i) contribute to a greater understanding of how repeated exposure to acute mental stress leads to CVD risk over years and decades, ii) determine whether a prior AE bout may combat negative CV responses associated with an acute mental stressor, and iii) contribute to a paradigm shift in human physiology, such that mental-stress-related EE is considered alongside the more canonical resting, physical activity, and digestion-based components of EE. **Innovation.** The methodology contains novel aspects, including assessment of bfPWV and EE during and following an acute mental stressor. **Key Strengths.** The candidate gathered a multi-disciplinary team with expertise in CVD risk and EE assessment. This expertise, coupled with the strong study design, provides a promising opportunity to better understand mental stress and CVD risk, which in turn sets the stage for lifestyle-based CVD prevention efforts centered on modulating cardiometabolic reactivity to mental stress.

CHAPTER 2: CANDIDATE TRAINING GOALS

Overview

My ultimate goal is to establish my own lab at a top-tier research institution studying the interplay among lifestyle factors, stress, and health. Specifically, I am interested in understanding how repeated acute exposures to mental stress impact cardiometabolic disease risk. I am also interested in developing and implementing lifestyle interventions for the prevention and treatment of chronic stress and stress-related diseases. This series of studies were designed to provide the training need to develop leadership and managerial skills, develop robust data management skills, execute a comprehensive meta-analysis, and obtain expertise in acute stress studies related to modifiable lifestyle factors and cardiometabolic function, all of which will help me attain my ultimate goal. (See **Table 2.1**):

Table 2.1. Training goals.

#	Training Goal	Why Important	Study
1	Leadership and managerial skills	Lead/organize team of students and researchers as a faculty member	1-3
2	Data management skills	Tools necessary for overseeing multiple projects with a variety of study designs/exposures/outcomes	1-3
3	Perform Meta-Analysis	Comprehensively assess state of the literature	1
4	Acute stress/CM study development/implementation	Career trajectory is geared towards exploring inter-relationships among stress physiology and cardiometabolic health	2-3

Abbreviations: *CM, cardiometabolic*

Goal 1: Leadership and Managerial Skills

I developed substantial skills pertaining to leadership including from a mentorship perspective as well as a general managerial perspective. In terms of the former, I aim to have a regular team of undergraduate research assistants – including those obtaining and not obtaining EXSS 395 credit - assist with data collection throughout the completion of this dissertation. I developed mentorship skills by leading weekly team and individual meetings and by helping guide those taking research for credit to develop and answer their own research questions from the data that we are collecting. From a managerial perspective, this dissertation required simultaneous administration of numerous devices and pieces of equipment to collect data. I developed managerial skills by implementing a team scheduling system so that I have two assistants for each participant visit, and I also trained certain individuals on specific pieces of equipment depending on their skill level, availability, and area of interest. These leadership and managerial skills provided a strong foundation for me during my post-graduate career as I seek to direct a dynamic laboratory of undergraduate, graduate, and post-doctoral researchers in a top research institution.

Goal 2: Data Management Skills

I developed a variety of data management skills, which is critical for organizing and maintain a diverse set of data across multiple studies with varying exposures and outcome variables. These skills were facilitated by the incorporation of laboratory templates related to data folder infrastructure, the creation of individual and summary data spreadsheets (for data reduction), which ultimately enabled data spreadsheets designed for input into R and Jamovi statistical software for linear mixed model analysis. I also utilized redundancy throughout my

data storage system so that all data is backed up across multiple locations. Honing these skills have prepared me to manage larger scale projects (e.g. community-based participatory research, community interventions, population-level large data sets, etc.) during my post-graduate career.

Goal 3: Perform Meta-Analysis

I have assisted with a number of meta-analyses and systematic reviews investigating lifestyle factors (e.g. exercise, sedentary behavior, sedentary behavior interruptions) on physiological outcomes (e.g. endocrine, vascular). However, performing a meta-analysis as part of this dissertation gave me experience leading this type of analysis including mentoring an undergraduate assistant throughout the entire meta-analysis process – from searching and critiquing the literature to writing, submitting, and publishing the final manuscript. Meta-analysis findings are harmonized consolidations of existing literature on a topic and are considered some of the highest levels of evidence used to inform public health and policies. Gaining this experience in administering and mentoring an assistant throughout the meta-analysis process gave me a foundation to perform and teach meta-analyses related to lifestyle factors and health throughout my career.

Goal 4: Execute Psychophysiological Research Pertaining to Mental Stress and CVD Risk

Performing this dissertation allowed me to continue my expertise in acute physiological studies pertaining to the effects of mental stress on cardiometabolic function. I plan on centering my career on the effects of stress and lifestyle factors on cardiometabolic health. Thus, further experience in this area – especially related to acute stress administration – are critical for my future success as an independent investigator focused on stress-related

psychobiology. Additionally, the incorporation of a modifiable lifestyle factor (acute aerobic exercise) in combination with the stressor is a study design/research focus which I aim to continue to explore. For example, I plan on investigating the interrelationships among stress and other potentially modifiable lifestyle factors (e.g. sedentary behavior, dietary factors, sleep, mindfulness, etc.) on cardiometabolic health. As such, the experimental studies outlined in this dissertation gave me a strong set of tools and experiences to build off during my post-graduate career investigating stress, lifestyle, and health.

CHAPTER 3: REVIEW OF LITERATURE

This section will define important concepts and provide a topical overview of key background information relevant to this dissertation. This information is key to understanding the physiology, scientific approach, and public health perspective that have shaped this dissertation.

Definitions

Cardiovascular Disease

Cardiovascular Disease (CVD) is a broad umbrella term for numerous cardiovascular pathologies. Some pathologies such as certain arrhythmias or valvular disorders are congenital in nature; however, in the context of this dissertation, CVD focused primarily on those conditions which arise from atherosclerotic and arteriosclerotic processes, and are thus largely modifiable. These types of cardiovascular disease include, but are not limited to coronary artery disease, myocardial infarction (heart attack), cerebrovascular ischemia or hemorrhage (stroke), peripheral arterial disease, hypertension, and congestive heart failure. Not only is CVD an important public health concern to investigate due to it being the number one cause of morbidity and mortality in the United States and globally[33, 34], but also because its modifiable nature presents a golden opportunity to positively impact public health.

Arterial Stiffness

Arterial stiffness (AS) is considered the gold-standard non-invasive biomarker of vascular aging[35]. A healthy arterial system gradually stiffens from the aorta towards the periphery[36,

37]. AS impairs this gradient and is associated with myocardial burden and CVD[35–39]. AS is an early marker of CVD risk, making it a relevant biomarker in relatively healthy populations without overt CVD[35, 40, 41]. Further, AS is modifiable and predictive of future CVD independent of established risk factors[42, 43]. The gold-standard technique for measuring AS is pulse-wave velocity (PWV), which is the speed of the forward traveling pressure wave in a given arterial segment (higher=worse). PWV-derived AS is a modifiable, sensitive index of early CVD risk and is a relevant outcome for investigating mental stress-related CVD risk in disease-free adults.

Energy Expenditure

Total energy expenditure (EE) is usually expressed as the number of daily kilocalories used for all metabolic processes and physical movement. Importantly, in addition to basal (or resting) metabolic rate (BMR [or RMR]; the metabolic cost of simply existing and maintaining normal organ/cellular functions), EE also encompasses the thermic effect of food (energetic cost of digestion) and the thermic effect of activity (energetic cost of physical movement). Energy expenditure is primarily determined by body mass and body composition, with larger individuals and those with greater lean mass expending a greater amount of energy. Energy expenditure is related to the autonomic nervous system (ANS) and CVD risk. Additionally, EE may be related to CVD risk within the context of the constained energy theory, which postulates that stress-related EE – when not tempered by physical activity-related EE, may contribute to excess inflammatory actions. Collectively, EE is an understudied, potentially critical variable to investigate in the context of mental stress and CVD risk.

Mental Stress

In general, stress can be described as a real or perceived threat to homeostasis. In the case of mental stress, this stress can best be thought of as perceived (e.g. it is not the result of a stressor such as encountering a large predator that might kill you or cause you direct physical harm). Importantly, mental stress is not the same as a mental stressor. Rather, mental stress is a function of the perception of, and response to a given mental stressor. Example mental stressors that are commonplace in modern society include being stuck in traffic, financial insecurity, racial (or other) discrimination, experiencing social challenges in the workplace, or taking care of an elderly or ill family member. Thus, mental stress can be thought of as the internalized perception and response to these external stimuli. Given its prevalence in society today and association with modifiable CVD risk, mental stress is an important variable to investigate.

Trier Social Stress Test

The Trier Social Stress Test (TSST) is an ecologically valid laboratory stressor used to provoke psychophysiological stress responses for research purposes[32, 44]. Modified versions of the TSST have been employed including with varied test durations, administering the test to single and multiple participants, use of a virtual-reality interface, and varied math and speech tasks specific to the population of interest (e.g. children)[32].

Acute, Moderate-Intensity, Whole-Body Aerobic Exercise

Acute, moderate-intensity whole-body aerobic exercise (AE) can be defined as a single bout of continuous movement of large muscle groups in both the upper and lower-limbs over a sustained duration such that aerobic cellular respiration (e.g. aerobic glycolysis, beta oxidation)

is required as the primary fuel source for energy production to sustain the activity. Moderate-intensity AE requires moderate, but not maximal exertion and effort. There are many ways to define or characterize moderate-intensity. Objectively, intensity can be measured using physiological variables such as heart rate, oxygen consumption (VO_2), or blood lactate, and can be reported in absolute or relative terms[45]. Subjectively, intensity level can be determined using a Rate of Perceived Exertion scale[45, 46].

Stress: A Brief Overview and Historical Perspective

This section will provide a brief topical overview of stress as it relates to organisms' relationships with their internal and external environment, as well as historical context regarding some of the pioneering advances in our scientific understanding of how stress interacts with human physiology. This is important in order to view and understand stress through both an evolutionary and physiological lens.

Stress is an ubiquitous term, but can be described as "a real or perceived state of threatened homeostasis[47]." All organisms experience stress, and while it most commonly has a strong negative connotation, stress is normal and often adaptive, including in humans[48]**.** For example, AE places a stress on the cardiovascular system, which leads to positive adaptations such as reduced heart rate (HR) and improved oxygen utilization[49]. Similarly, in the case of mental stress, moderate exposure to mental, cognitive, or psycho-social challenges (i.e., academic exams, having social anxiety at a public event) may improve performance when future challenges arise[50].

Exposure to stressors and the stress response has been defined as the "allostatic load," and while "normal" or moderate exposure to stressors promote beneficial adaptations, a load

that is too great, whether mental or physical in nature, is thought to induce negative physiological states, among them elevated CVD risk[1, 50]. In this schema, stressors may be categorized as either a eustress (positive) or distress (negative)[51]. The physiological understanding of this paradigm can be traced back to the work of Hans Seyle in the early-mid 20th century and his proposed "General Adaptation Syndrome." Seyle's "General Adaptation Syndrome" expanded upon (and ultimately deviated from) Walter Canon's earlier interpretation of stress as simply a "fight or flight" response, and ushered in a more nuanced understanding of stress physiology in which excessive stress exposure decreases the ability to respond to and recover from stress, and ultimately causes a cascade of unfavorable neuroendocrine and autonomic responses leading to potentially long-term disease states [52]. Not surprisingly, a host of factors determine an organism's psychophysiological response to stress including whether it manifests as maladaptive or adaptive. While many factors are involved, at the most basic level, these determinants of the stress response include stress perception, appraisal and coping.

Chronic Mental Stress is Common, Disrupts Systems Physiology, and Increases CVD Risk

This section will discuss the ubiquity of stress in contemporary Western society and its relationship with CVD. This is important because this information illustrates the relevance of this topic to public health and underscores the significance of mental stress as a modifiable and important CVD risk factor.

Elevated Mental Stress is Highly Prevalent in Today's Society

Importantly, the pace, pressure, and socio-political landscape of contemporary Western society is conducive for high levels of stress. Thus, it is not surprising that the majority of the

United States population reports high levels of stress, and that the prevalence of high levels of stress is increasing nationwide. For example, in 2015, 24% of Americans reported “extreme” levels of stress, up from 18% in 2014[53]. The same study also showed that over one third of Americans reported that their stress levels had increased from the previous year[53]. Examples of daily mental stress that have been associated with negative health outcomes include work-related stress[54], marital stress[55], racial discrimination[56], and caring for a sick spouse[57]. It would also be remiss not to acknowledge the enormous toll that the COVID-19 pandemic has had on stress-related mental and physical health. Scientific and public health communities are only beginning to understand the extent to which pandemic-related mental stress has had and will continue to have on psychological and physiological function[58, 59].

Chronic Mental Stress Increases Cardiovascular Disease Risk

One particularly concerning physiological repercussion of chronic mental stress given its prevalence and insidious etiology is increased CVD risk [60, 61]. However, despite strong longitudinal associations between chronic life stress and CVD risk[62–65], the precise mechanisms underpinning the stress-CVD relationship are not fully understood. Generally, the disruptions in autonomic and neuroendocrine function as a result of chronically elevated levels of stress are thought to contribute to CVD risk by causing hemodynamic and vascular disruptions, metabolic dysfunction, and inflammation[4, 60, 61, 66, 67]. However, the majority of studies that have investigated how repeated exposures to mental stressors may affect CVD risk have predominantly focused on a limited number of outcome variables including heart rate (HR) and blood pressure (BP). Further complicating matters, excess mental stress may impact CVD risk indirectly by promoting negative lifestyle behaviors including increased sedentary

behavior[68], poor sleep quality[4], overeating[69, 70], and substance use[71]. Indirect mediators aside, there is currently an incomplete understanding of the psychophysiological chain of events that explain the link between elevated exposure to mental stress and CVD risk. Thus, there is a need to better understand the mechanisms linking repetitive exposure to acute mental stress with CVD risk, as well as techniques that can reduce stress, regulate the stress pathways, and prevent the development of stress-related CVD risk.

Cardiovascular Disease Mechanisms are Likely Multi-Factorial

Threats to homeostasis – or stressors – provoke a range of physiological responses[72]. Thus, it is not surprising that the CVD mechanisms associated with mental stress are multi-factorial[1, 64]. For example, autonomic disturbances may lead to unfavorable changes in heart rate and blood pressure, while direct vascular insults may also occur due to altered local vascular functioning which disrupt hemodynamics, shear stress, endothelial function, and contribute to AS and maladaptive wave morphologies, ultimately increasing systemic resistance and myocardial burden[3, 16, 20, 73, 74]. Additionally, since the stress response is largely centered around making energy readily available to cope with the stressor, metabolic function is also likely implicated in stress-related CVD risk including via factors such as the regulation of balance between anabolic and catabolic actions, glucose and insulin dynamics, body composition, and energy expenditure[14, 15, 51, 75]. The following section will delve more deeply into how a number of complex, and inter-related systems may contribute to mental stress-induced CVD risk.

Mental Stress Disrupts Systems Physiology

Acute and repeated (or constant, unwavering) mental stress causes direct and indirect insults to multiple physiological systems that impact cardiovascular (CV) health, and may contribute to mental stress-related CVD risk. This section will discuss what is known and not known regarding how acute exposure to mental stress may contribute to CVD risk. This information is important as it provides an overview of the current understanding of the pathophysiological processes linking mental stress to CVD, which is necessary to fully grasp and contextualize the key knowledge gaps that this dissertation fills.

In terms of stress-related health impacts, excessive mental stress is most commonly associated with negative mental health outcomes such as anxiety and depression. However, as alluded to in Seyle's General Adaptation Syndrome, acute and chronic stress also impact a range of physiological systems including autonomic and neuroendocrine systems. Interestingly, chronic physical stress (e.g. sport-related overtraining) and chronic mental stress (e.g. care-giving) induce largely similar effects such as increased negative affect[5, 76], fatigue[77, 78], and weakened immune function[79, 80], suggesting activation of similar stress pathways. Indeed, regardless of whether physical or mental in nature, stress stimulates the sympathetic branch (SNS) of the autonomic nervous system (ANS), and the hypothalamic-pituitary-adrenal (HPA) axis to assist in the response to, and recovery from stress[5]. With chronic exposure to stress, dysregulation of these pathways occurs, which is thought to influence a number of physiological and psychological conditions[4, 64, 75, 81, 82]. In terms of negative physiological effects, chronic stress is associated with immune dysfunction[4, 82], inflammation[64],

decreased telomere length[83, 84], and tumor growth[85], among others[4, 66]. Further, elevated mental stress is related to worse outcomes in individuals with chronic illnesses[55].

Autonomic Dysfunction

The body's initial response to acute stress is through the SNS[86]. The SNS stimulates the secretion of the catecholamines epinephrine and norepinephrine from the adrenal medulla and sympathetic nerves[1]. In conjunction with the HPA axis, the SNS-mediated rise in catecholamines induces the "fight or flight" response by increasing heart rate, cardiac contractility, and vasoconstriction[1]. Unremittent exposure to stress decreases the capacity of the parasympathetic nervous system to moderate sympathetic activity both at rest and in response to stress[87]. This causes an imbalance of the SNS and PNS, which in turn is associated with inflammation (e.g. via increasing pro-inflammatory cytokines such as Interleukin-6), one of the driving factors behind atherosclerosis. In addition to traditional measures such as HR and BP, the effects of mental stress on the autonomic nervous system have most notably been investigated using heart rate variability (HRV). HRV is a measure of sympatho-vagal balance and has been associated with all-cause CVD and morbidity[1, 67]. Additionally, increases in HR, decreases in HRV, and elevated levels of pro-inflammatory cytokines have been reported at rest and following acute stress in populations experiencing chronic stress[1, 67]. While beyond the scope of this review of literature, it is also worth noting that autonomic dysfunction can also mediate CVD directly wherein elevations in myocardial burden and systemic vascular resistance increase the likelihood of an acute plaque rupture or cardiac event, though this direct impact may be more relevant in individuals with pre-existing and/or advanced CVD[1].

Direct Vascular Assaults

Hemodynamic Disturbances and Morphology of the Pressure Wave

ANS dysfunction and AS are each associated with hemodynamic disturbances and unfavorable changes to the pressure wave that are important to CVD risk. For example, decreased shear stress (a eustress resulting from laminar flow of red blood cells against the endothelium) promotes a pro-atherosclerotic environment by decreasing endothelial function (see below). In terms of unfavorable changes to the pressure wave, increased velocity and amplitude of the reflected pressure wave increase myocardial burden[36][88]. Thus, other likely contributors to stress-induced CVD risk are hemodynamic disturbances and maladaptive morphological changes to the pressure wave [3, 17–21].

Endothelial Dysfunction

Stress may also promote CVD risk by causing endothelial dysfunction, the precursor to atherosclerosis and subsequent CVD risk[89–91]. For example, acute mental stress has been shown to impair endothelial function for up to four hours[91]. Further, multiple studies have reported associations between chronic stress (e.g. caregiving stress) and endothelial dysfunction[60, 89, 90]. Mental Stress may lead to endothelial dysfunction by decreasing shear stress and subsequent bioavailability of nitric oxide, a critical vasodilator, antioxidant and regulator of platelet and monocyte adhesion in the endothelium[73, 90]. Reduced formation and/or increased degradation of nitric oxide promotes free-radicals and accumulation of platelets and monocytes on the endothelium, which contribute to atherosclerosis and CVD risk[92, 93].

Arterial Stiffness

Both acute and chronic stress negatively impact arterial stiffness[20, 89, 90, 94–96]. Vlachopolous et al. have also shown in several well-designed randomized controlled cross-over trials that among healthy adults, an acute laboratory stressor induces arterial stiffening as measured by PWV. In one creative study, Vlachopolous et al. not only demonstrated that exposure to a horror film increased PWV (increasing stiffness), but also that exposure to a comedy film significantly *decreased* PWV. However, AS is a relatively novel biomarker of vascular structure and function, particularly in the context of mental stress, and further work is needed to understand the effects of mental stress on arterial stiffness, which in turn may improve our understanding of stress-mediated CVD risk.

Metabolism and Energy Expenditure

It is well known that one of the primary adaptive responses to stressors of any kind is to mobilize energy stores in order to cope with the given stressor. For example, the two primary drivers of the acute stress response are the SNS and the HPA axis. SNS-mediated catecholamines stimulate glycogenolysis and promote rapid increases in blood glucose. Meanwhile, the end product of the HPA axis, cortisol, stimulates catabolic actions such as lipolysis, while also promoting an immunosuppressive effect as a means to triage energy away from non-essential functions[6, 82]. Several preliminary studies have attempted to investigate metabolic responses to an acute mental stressor with data indicating that mental stress does indeed seem to increase EE acutely [13–16]. However, stress-related impacts on metabolism have been understudied in the context of CVD. Variables related to metabolic function such as EE, including TEE and RMR, are associated with key cardiometabolic and CVD risk factors[8, 9].

For example, EE has been independently associated with BP, insulin resistance, body fatness, hyperglycemia, obesity, metabolic syndrome, type II diabetes, as well as all-cause mortality [8, 9, 97–99]. It should be noted, however, that some studies also indicate that changes in body fat may substantially mediate associations between EE and CVD risk[100, 101]. Indeed, whether tissue is composed of fat or muscle dictates that tissue's metabolic activity, and thus EE. The EE associated with a given tissue impacts cellular and hormonal signaling and orchestrates the interplay between anabolic and catabolic actions[102]. While both tissues are metabolically active, muscle is associated with improved glycemic control as well as greater metabolic activity and fuel use compared to fat[28, 103–105]. In addition to EE being related to body composition, it is also related to the distribution of that composition, which is highly relevant to CVD risk[106]. For example, fat distribution is more strongly associated with CVD risk than total fat, especially when adipose accumulates viscerally[107, 108]. In addition to the more traditional potential mediators of EE and CVD, increased attention has recently focused on the role of the gut-brain axis (e.g. connection between gut microbiome and neuroendocrine regulation) in the interplay between EE and cardiometabolic dysregulation[109]. In sum, since EE is associated with key cardiometabolic and CVD risk factors, EE may be a missing link of sorts in our current understanding of how repeated exposure to acute mental stressors, over time, may contribute to CVD risk.

Critical Knowledge Gap #1: Energy Expenditure and Metabolic Rate are Associated with CVD Risk, but the Effects of Acute Mental Stress on Energy Expenditure are Unclear

This section will outline the critical knowledge gap that the first experimental study sought to fill, as well as describe the importance of adding this new knowledge.

Interactions Among Mental Stress, EE, and CVD Risk are Unknown

It is well established, and also quite intuitive, that stressors are associated with a range of physiological events which seek to make energy readily available[48]. Evolutionarily, it was advantageous for your typical sub-Saharan hunter-gatherer, upon encountering the neighborhood lion, to experience an immediate mobilization of energy stores, rise in blood glucose, and heightened alertness in order to improve chances of survival and ultimately produce offspring. Indeed, SNS-mediated catecholamines and HPA axis-mediated cortisol help the body respond to stress by increasing energy availability through gluconeogenesis, glycogenolysis, and lipolysis, and assists in suppression of non-essential functions such as growth and reproduction[51, 110]. While much of our knowledge on this topic has been garnered through the observation of changes in blood and salivary hormone levels in response to stressors, few studies have directly assessed EE in response to an acute stressor. The studies that have assessed EE in response to mental stress have shown increases in EE ranging from 7-28% in both clinical and healthy populations [13–16]. However, these studies were published between 2000-2007 and are therefore outdated in several respects. For example, one critical limitation of the existing literature is the use of inappropriate methods for sensitively measuring EE[14–16]. Additionally, these studies were either limited by small sample sizes, failed to measure and/or report post-stress EE, were not specifically designed to assess the energetic expenditure associated with mental stress, and/or did not simultaneously assess sensitive markers of early CVD risk[13–16]. Collectively, few studies have precisely measured the energy expenditure associated with acute mental stress. The influence of stress on EE and metabolism may provide further insight into our understanding of how repeated exposures to

mental stressors contribute to long-term CVD risk[7]. More broadly, this understanding may support a more sophisticated understanding of total energy expenditure beyond the traditional thinking that EE is simply an additive sum of RMR and thermic effects of activity and food.

Critical Knowledge Gap #2: Aerobic Exercise Positively Modifies Stress Reactivity, but Effects of a Prior Exercise Bout on Post-Stress Arterial Stiffness and Energy Expenditure are Not Known

This section will outline the critical knowledge gap that the second experimental study sought to fill, as well as describe the importance of adding this new knowledge.

Aerobic Exercise Can Positively Modify Psychophysiological Reactivity to Mental Stress

There is substantial evidence demonstrating beneficial effects of AE on both psychological and physiological reactivity to stress including in animal models as well as in both healthy and clinical populations[11, 111]. Psychologically, acute and chronic AE have been shown to reduce symptoms of stress, anxiety, and depression (including to the same extent as standard pharmacological treatment [i.e. selective serotonin reuptake inhibitor])[112–114], while promoting relaxation and improved mood and cognitive performance, [22, 112, 115]. Physiologically, stress-related benefits of AE have been demonstrated, including improved resting and post-acute stress CV and ANS function as measured by BP, HR, HRV, and AS[116–120]. Specifically, the vasculature may be better able to cope with the stressors as a function of improved ANS (central, systemic) and endothelial (local) functioning[116–120]. Such acute cardio-protection in the face of a stressor, may, over time, partially account for the decreased risk of CVD that is observed among individuals (independent of age) with higher levels of cardiorespiratory fitness[121–123]. Though less consistently reported than ANS and vascular measures, neuroendocrine factors related to mental stress have also been shown to be

affected as a result of AE. For example, greater aerobic fitness has been associated with decreased HPA reactivity in response to a lab-stressor, as well as improvements in resting levels of brain-derived neurotropic factor (BDNF) and neurogenesis, which are associated with inflammation, and in turn may be related to CVD risk[124, 125]. A full description of neuro-protective effects of AE that may subsequently confer downstream cardioprotective effects is beyond the scope of this review. However, for further insight, the curious reader is directed to reviews by Heijnen et al, Chen et al, and Tarumi et al[22, 114, 129]. The idea that AE contributes to dampened stress reactivity is also in line with the constrained energy theory, which posits that elevations in physical activity-related EE reduce non-essential metabolic processes including SNS, HPA, and inflammatory actions[126–128].

How a Prior Aerobic Exercise Bout Impacts Post-Stress Arterial Stiffness and Energy Expenditure are Not Known

While AE, including acute bouts, have been shown to reduce perceived stress[23, 24], few studies have examined the effect of a prior AE bout on the post-stress CV [25–27] and EE response. In terms of the CV response, research has most commonly shown that AE can promote post-stress reductions in traditional CV measures such as HR and BP[130–132]. However, few studies have assessed how AE may modulate more novel, sensitive markers of early CVD risk in response to mental stress[25, 133]. Among a sample of young men, Kume et al. showed that 10 minutes of light-intensity AE at 35% heart rate reserve in young men eliminated post-stress increases in AS across multiple arterial segments, though in this study AE was performed after, not before the stressor was induced[25]. Allan et al. also reported findings that among adults with metabolic syndrome, 40 minutes of leg cycling at 80% ventilatory threshold (likely moderate-intensity) mitigated stress-induced endothelial

dysfunction as measured by brachial flow-mediated dilation[133]. These studies, though few, demonstrate adaptive vascular and endothelial effects of acute AE in the face of mental stress. However, few studies which we are aware of have sensitively examined EE responses to mental stress, let alone a combined assessment of how prior AE impacts both the EE and CV responses to mental stress. Filling this knowledge gap improves the mechanistic understanding of how CV and EE systems interact in response to AE, including with and without exposure to mental stress. More importantly, findings have the potential to inform lifestyle strategies and public health recommendations for mitigating stress-related CVD risk (e.g. utilize AE prior to instances in which individuals anticipate exposure to excessive stress, such as a stressful work meeting or care-giving period).

Literature Review Summary

Why are These Studies Needed?

Mental stress is an ubiquitous aspect of the human experience, and contributes to CVD risk. The prevalence of both high levels of mental stress and CVD risk in contemporary society merits further investigation into the mechanisms linking acute mental stress to CVD risk, as well as potential lifestyle-based strategies to offset stress-related CVD risk.

What is Known

While some mental stress is adaptive, acute stress negatively impacts CV parameters, and chronic stress is associated with CVD risk [1].

What is Not Known

EE has not been investigated in the context of acute stress. Further, it is unclear how AE may modulate AS and EE in response to stress.

Critical Need

There is a need to better understand the mechanisms underlying the impact of acute mental stressors on CVD risk, and in turn develop evidence-based strategies to offset stress-related CVD risk. Based on the review of literature, two separate randomized cross-over studies help to fill these knowledge gaps while maximizing internal validity, ensuring project feasibility, and limiting participant burden. Study 1 employed precise measurement of AS and EE parameters during and following exposure to acute mental stress. Study 2 assessed these parameters in response to a pre-stress AE bout.

Key Considerations for Design and Implementation of Research

- Internal validity
- Addresses key knowledge gaps that will guide future psychophysiological and translational research
- Researcher and participant burden
- Feasibility
- Specific committee expertise

What this Study Adds

Findings will help i) contribute to a greater understanding of how repeated exposure to acute mental stress leads to CVD risk over years and decades, ii) determine whether a prior AE bout may combat negative CV responses associated with an acute mental stressor, and iii) contribute to a paradigm shift in human EE physiology, such that mental-stress-related EE is considered alongside the more canonical resting, physical activity, and digestion-based categories of EE.

CHAPTER 4: RATIONALE FOR APPROACH

Major Methodological Considerations

The aims for each study are provided in **Table 4.1**.

Table 4.1. Study aims

Study	Aims(s)
1 (Review 1)	To conduct a systematic review and meta-analysis in order to consolidate and synthesize the literature pertaining to the acute effect of mental stress on AS
2 (Experimental Study 1)	To determine EE and AS response to an acute mental stressor
3 (Experimental Study 2)	To determine how an acute prior moderate-intensity AE bout impacts post-stress CV and EE response

Abbreviations: *AE, Aerobic Exercise; AS, Arterial Stiffness; CV, Cardiovascular; CVD, Cardiovascular Disease; EE, Energy Expenditure; TSST, Trier Social Stress Test*

The major considerations for each experimental study are provided in **Table 4.2**.

Table 4.2. Major considerations for each study.

Study	Population	Study Design	Primary Outcome	Secondary Outcome(s)	Experimental Timeline

2 (Experimental Study 1)	Recreationally active young adults (18-30 y)	Randomized X-Over	AS, EE	HRV, PWA	• 2 visits (Experimental [TSST] and Con [No TSST])
					• 105 min/visit
					• Primary outcomes measurement timing:
					  • EE: Continuous Pre/During/60min post TSST/Con [EE]
					  • AS: Pre/During/Post TSST/Con and every 15 min after TSST/Control for 60 min (AS)

3 (Experimental Study 2)	Recreationally active young adults (18-30 y)	Randomized X-Over	AS, EE	HRV, PWA	• 2 visits (Experimental [AE+TSST] and Con [AE Only])
					• 135 min/visit
					• Primary outcomes measurement timing:
					  • EE: Continuous Pre/During/60min-post TSST/Control
					  • AS: Pre/Post AE and Pre/During/Post TSST/Control and every 15 min after TSST/Control for 60 min (AS)

Abbreviations: *AE, Aerobic Exercise; AS, Arterial Stiffness; EE, Energy Expenditure; HRV, Heart Rate Variability; PWA, Pulse Wave Analysis; TSST, Trier Social Stress Test; y, years*

Measurement Considerations

This section will describe the primary constructs that were evaluated in the current project, the variety of methodological options that were considered in this approach, and the rationale for choosing the adopted approach. The specific constructs that were discussed include arterial stiffness (AS), energy expenditure (EE), autonomic nervous system (ANS) function, and perceived stress. **Table 4.3** shows major measurement considerations for the two experimental studies.

Table 4.3. Major measurement considerations for experimental studies

Outcome	Construct	Options	Key Considerations	Selection
Primary	AS	• Tonometry • Oscillometry • Ultrasound	• Validity and reliability • Time • Participant burden • Researcher burden • Validity and reliability • Ability to measure simultaneously with EE • Ability to test multiple AS measurements simultaneously • Ability to time-align with PWA measurements	Oscillometry

Primary	EE	• Doubly Labeled water
		• Indirect Calorimetry
		  ○ Portable metabolic analyzer (e.g. OxyCon)
		  ○ Metabolic cart (Facemask with or without mouthpiece)
		  ○ Metabolic Cart – RMR – using ventilation canopy
		• Direct Calorimetry
		• Estimations from Body Comp
		  ○ DEXA
		  ○ Bioimpedance Analysis
		• Calculations (age, sex, height, weight, PA status)
			• Validity and reliability
			• Ability to administer TSST (e.g., ability to communicate verbally with participant)
			• Sensitivity to acute perturbation
			• Ability to test AS simultaneously
			• Time
			• Participant burden
			• Researcher burden
			• Validity
			• Ecological Validity
				• Indirect calorimetry
				  ○ Metabolic Cart – RMR – using ventilation canopy

Secondary	ANS	• HR • HRV • GSR • Blood catecholamines	• Validity and reliability • Ability to measure HR, HRV, GSR, and respiration rate simultaneously and on same device • Participant Burden • Researcher Burden	HR, HRV, GSR (Same device; Equivital, ADInstruments)

Secondary	PWA: Central BP and Waveform Dynamics	• Oscillometry
		  ○ XCEL
		  ○ Vicorder
		  ○ BP+
		  ○ Mobil-o-graph
		  ○ OSCAR
			• Validity and reliability
			• Time
			• Participant burden
			• Researcher burden
			• Ability to measure simultaneously with AS and EE
			• Ability to test multiple PWA measurements simultaneously
			• Ability to time-align with AS measurements
				Vicorder, BP+

Secondary	Stress level (state)	• Likert Scale • Visual Analog Scale • Questionnaire • Objective markers (salivary/blood cortisol)	• Time • Participant burden • Stress-related confounding (e.g. blood draw may elicit stress) • Self-report bias	VAS

Abbreviations*: ANS, Autonomic Nervous System; AS, Arterial Stiffness; BP, Blood Pressure; DEXA, Dual X-Ray Absorptiometry; EE, Energy Expenditure; GSR; Galvanic Skin Response; HR, Heart Rate; HRV, Heart Rate Variability; PWA, Pulse Wave Analysis; RMR, Resting Metabolic Rate; TSST, Trier Social Stress Test; VAS, Visual Analog Scale.*

Measure 1: Arterial Stiffness (Primary Outcome)

Rationale for Measuring Arterial Stiffness

A healthy arterial system gradually stiffens from the aorta towards the periphery, which prevents harmful pulsatile forces to the microcirculation[36, 37]. With arterial stiffening, this gradient is compromised, and potentially reversed in extreme cases. Additionally, impairment of the stiffness gradient quickens and augments reflected waveforms, which in turn increases systolic pressure and myocardial burden. Adding further insult, the more rapid return of the reflected waveform is associated with reduced diastolic pressure which limits optimal coronary perfusion [36, 37]. The multi-faceted, yet subclinical vascular consequences of aortic stiffness underscores its utility as an early marker of cardiovascular disease (CVD) risk (e.g. may precede risk factors such as obesity, hypertension). As such, AS is a relevant biomarker of CVD risk in

otherwise healthy adults[35, 40, 41]. Further, AS is modifiable and predictive of future CVD independent of established risk factors[42, 43]. With respect to mental stress, acute and longitudinal studies support a negative effect of mental stress on AS[20, 25, 94–96]. The principle investigator has reported that mental stress acutely increased AS by 0.28 m/s as measured by brachial-femoral pulse wave velocity (bfPWV)[134]. Mechanistically, acute stress-induced sympathetic activation may decrease ANS function, increase systemic vascular resistance, and impair endothelial function, all of which may contribute to increases in AS[92, 125–128]. Over time, AS leads to deleterious arterial remodeling[35], which helps to explain the link between chronic mental stress, AS, and CVD[139–141]. AS is a sensitive index of early CVD risk and is a relevant outcome for investigating stress-related CVD risk in otherwise healthy adults.

Methodological Options for Measuring Arterial Stiffness and Rationale for Chosen Approach

AS is most commonly assessed using PWV, which can be measured using a variety of non-invasive methods. Two-point AS measures assess the stiffness of a certain arterial segment, which may include central and/or peripheral arterial segments. Two-point measures of AS can be assessed using applanation tonometry, oscillometry, combined tonometry + oscillometry, as well as combining electrocardiography with photoplethysmography and doppler ultrasound[142, 143]. While PWV devices using tonometry are considered the gold-standard (e.g. carotid-femoral PWV [cfPWV] as assessed by combined oscillometric + tonometric SphygmaCor XCEL is most common clinical and research practice) [127, 128], these approaches involve placing a tonometer firmly over the carotid artery, which may introduce a confounding source of mental stress. Single-point measurements of AS are also possible using ultrasound-

based analyses of vessel diameter and blood flow, though these assess local, rather than segmental AS[144, 145]. Ultimately, oscillometric brachial-femoral PWV (bfPWV) was chosen for the current project. Oscillometric bfPWV offers precise measurements that can be performed quickly, thereby minimizing researcher and participant burden. It also enabled simultaneous assessment of central blood pressure via pulse-wave analysis (PWA). Most importantly, bfPWV enabled a sensitive assessment of AS while the participant was under the ventilated canopy for EE measurement.

Principles and Key Considerations for PWV Measurements of Arterial Stiffness

The Vicorder (SMT Med) and BP+ (USCOM) was used to measure bfPWV and aortic PWV respectively. In terms of the Vicorder, bfPWV (m/s) was calculated by dividing the arterial path length (D) by the pulse transit time (TT) between the proximal (brachial) and distal (femoral) arterial site. Specifically, D was measured as the distance between the suprasternal notch (SSN) and the umbilicus. To measure TT, the cuffs inflate to a sub-diastolic (~50 mmHg) pressure when it detects an acceptably strong signal. The device then uses a proprietary algorithm to calculate the time between the foot of the proximal pressure waveform to the foot of the distal pressure waveform. Each measurement cycle lasts 60 s. This method has excellent between-day reliability (ICC: 0.98), and has been validated against the non-invasive gold-standard cfPWV as well as magnetic-resonance based AS measures[146–148]. Further, our laboratory has shown that bfPWV is sensitive to acute laboratory and lifestyle perturbations. bfPWV is non-invasive, induces little participant discomfort, and precise measurements can be performed quickly.

In terms of the BP+, aortic PWV was calculated by dividing the arterial path length (2 x D from SSN to umbilicus) by the reflected wave transit time (peak to peak). This transit time is

equal to t3-t1 where t3 is the peak of the reflected wave and t1 is the peak of the incident wave. To derive this transit time, the device uses a supra-systolic pressure and proprietary algorithm to decompose the blood pressure waveforms. Each measurement cycle lasts 60 s. This method has excellent within-day reliability (Intraclass Correlation Coefficient [ICC]: 0.90-0.94) and has been validated against invasive and non-invasive AS measurements[149–151].

Several key considerations must be taken into account in terms of best practice and interpretation of PWV. First, for PWV assessments in general, a clinically meaningful change in AS is typically considered 1m/s[152], and both the Vicorder and BP+ have the sensitivity to detect changes of this magnitude. Second, visual inspection of the waveforms is needed for quality control purposes (e.g. ensuring proper identification of pressure wave feet and peaks). Third, multiple pre-testing and testing factors can influence the PWV measurement including body posture, time of day, fasted state, caffeine, recent vigorous exercise, and medications that impact cardiovascular function. These factors were considered in this project, and are further outlined in the pre-assessment guidelines described later in this chapter. Finally, the principal investigator was well-versed in both the Vicorder and BP+, and the oscillometric nature of the device requires little technical training.

Measure 2: Energy Expenditure (Primary Outcome)

Rationale for Measuring Energy Expenditure

Resting metabolic rate (RMR) is a measure of EE that represents the amount of energy in kilocalories (kcal) that is expended at rest, and is typically expressed in kcal/day. While measurement of EE during a mental stressor is not truly a resting measurement per se, the RMR test is the appropriate test to consider in the context of this dissertation (e.g. the research

questions are focused on the energetic response to mental stress, not how mental stress impacts *total* energy expenditure). RMR is controlled by central regulators including the hypothalamus, brainstem, and ANS. Additionally, RMR is associated with CVD risk factors including body composition and glucose regulation[99, 100, 153]. Since EE is a primary outcome of this dissertation, and based on the adopted study design for achieving the study aims, RMR is a requisite measurement.

Methodological Options for Measuring AS and Rationale for Chosen Approach

Several techniques exist for measuring or estimating RMR. Direct and indirect calorimetry assess energy expenditure using amounts of heat captured and gas exchanged (oxygen consumed, carbon dioxide expelled), respectively[153, 154]. Another technique more suitable for free-living conditions is doubly-labeled water, which assesses changes in isotope levels (deuterium, oxygen-18) to estimate carbon dioxide and EE[126, 127]. However, doubly-labeled water is expensive, time-intensive, and requires urine samples across multiple days. Multi-compartment body composition assessments can also be used to estimate EE based on total and relative amounts of fat and lean mass. Population-specific equations that take into account variables including demographics, anthropometrics, and physical activity status can also be used to estimate EE. With the exception of direct and indirect calorimetry, these techniques are not well-suited to assess EE with high-temporal resolution. For example, doubly-labeled water assesses total energy expenditure over a certain multi-day period based on urine samples, but would not have the capacity to continuously detect acute changes in EE across a distinct laboratory perturbation such as a mental stressor or exercise bout. On the other hand, direct and indirect calorimetry can assess acute changes in EE continuously. With respect to

calorimetry, whereas indirect calorimetry requires the use of a mask, mouthpiece, or ventilated canopy to trap and analyze expired air, direct calorimetry can be conducted in a metabolic chamber (specialized room) that captures expelled body heat. Thus, using a direct calorimetry method (e.g. metabolic chamber) would have been ideal from an external (ecological validity) standpoint. For instance, mental stress could be used in a relatively "real-life" scenario using a metabolic chamber, whereas indirect calorimetry requires the participant to be subjected to unnatural research equipment such as a mask or canopy. Nevertheless, indirect calorimetry measurement of RMR was chosen for the current dissertation as the principal investigator did not have access to a metabolic chamber. Moreover, RMR from indirect calorimetry may be able to more sensitively detect acute and subtle changes in EE compared to direct calorimetry.

Principles and Key Considerations for Energy Expenditure Measurement

There are standard protocols for indirect calorimetry measurements of RMR. A ParvoMed metabolic cart was calibrated prior to each test. An RMR-specific gas cylinder, dilution pump, and software (ParvoMed) was used. Data points were obtained every minute, but the first 10 minutes of collected data were excluded from data analysis because 10 minutes are needed to achieve steady-state metabolism, and allow breathing and dilution rate to normalize[153]. Steady state was defined as 10 minutes during which the oxygen consumption, minute ventilation, and respiratory exchange ratio do not vary by more than 10%. Participants who do not reach a steady state were not included in the analysis. RMR measurements, including those obtained in our lab (Applied Physiology Lab, UNC), demonstrate excellent test-retest reliability (ICC: 0.94), with a standard error of measurement of 125.6 kcal/day, and a mean detectable difference of 244.3 kcal/day[153].

Several key considerations must be taken into account in terms of best practice and interpretation of RMR. First, a clinically meaningful change in RMR is difficult to ascertain as it may vary considerably based on body mass, body composition, age, sex, and race[155]. However, 3500 kcal can be used as a general indicator of practical significance as this corresponds to approximately one pound of fat[156]. Related to the variation in EE that is associated with body mass and composition, bioelectric impedance was also used in the current project as it may be appropriate to statistically account for these variables[103]. Second, for quality control purposes, dilution rate must be adjusted during the first 5 min of the test, and potentially throughout the test (e.g. particularly during the TSST) so that the fraction of expired carbon dioxide is between 0.9 and 1.2%. Third, multiple pre-testing and testing factors can influence the RMR measurement including body posture, body movement, speaking, time of day, fasted state, caffeine, recent vigorous exercise, and medications that impact metabolic function and arousal state. These factors were considered in this project, and are further outlined in the pre-assessment guidelines described later in this chapter. Finally, RMR is a relatively new technique for the principal investigator. However, pilot testing included training from the Applied Physiology Lab Director, Dr. Abby Smith-Ryan. Further training took place under the supervision of committee-member Dr. Herman Pontzer. The principal investigator also conducted a small reliability study under resting and TSST conditions to ensure technical proficiency and sensitivity of the measurement to the TSST.

Measure 3: Heart Rate Variability (Secondary Outcome)

Rationale for Measuring Heart Rate Variability

Measurement of heart rate variability (HRV) reflects the cardiovascular (CV) ANS, and is an independent predictor of CV events[157]. A recent study of 77 adults found that greater reductions in HRV (worse outcome) during a psychological stressor were associated with shorter relative buccal telomere length (↑cellular aging)[158]. Moreover, HRV is sensitive to acute laboratory perturbations and has been shown to be negatively impacted by both acute stress and stress-related chronic states such as depression[3, 159, 160] Since the ANS is one of the primary orchestrators of the physiological stress response and is implicated in both AS and EE, HRV is an appropriate method to assess ANS function in this dissertation.

Methodological Options for Measuring Heart Rate Variability and Rationale for Chosen Approach

HRV is measured by analyzing the variation in R-R intervals and is thus most commonly measured using a standard electrocardiogram (ECG). Typically, a 3- or 10-lead ECG involves placing electrodes on the participant's chest and abdomen area in order to capture continuous R wave peaks from the ECG QRS complexes. For the current project, an Equivital (ADInstruments) belt was used to capture R-R intervals. This method has built-in electrodes, and thus does not require placement and removal of individual electrodes on the participant's body. Moreover, the Equivital system can simultaneously capture respiration rate, which may influence HRV, as well as galvanic skin response, another useful marker of ANS, and in particular SNS, reactivity. Based on the time-period of testing (described later in chapter), the relatively healthy sample being recruited, and the expected autonomic perturbation, the specific time- and frequency-domain indices that were used for HRV analysis in this dissertation included the

standard deviation of R-R intervals, the root mean square of the standard deviation between consecutive R-R intervals, high frequency power, low frequency power, and the high-frequency:low frequency ratio.

Principles and Key Considerations for Heart Rate Variability Measurement

HRV is an umbrella term for many indices that characterize the variability or complexity in the R-R interval. The vacillations of a healthy heart are complex, which allow the CV system to quickly adjust to sudden environmental and homeostatic challenges. The most widely used parameters include time-domain, frequency-domain, and non-linear metrics. Time-domain indices reflect the amount of variability during monitoring periods that range from 2 min to 24 hours. Frequency-domain values determine the absolute and relative amount of signal energy within component frequency bands and are thought to represent SNS or PNS more heavily depending on the specific measure and context (e.g. RMSSD, or the root mean square of subsequent R-R interval differences is thought to be primarily vagally driven) differences although not all researchers are in agreement[161, 162]. Non-linear measurements quantify the unpredictability and complexity of a series of R-R intervals.

Collectively, ECG-based HRV is a reliable (ICC: 0.68-0.88 depending on specific metric) and valid technique[159, 163]. However, HRV is a complex and sensitive measure and data must be interpreted in the context of recording period length, age, and sex. For example, 24 hour, short-term, and ultra-short-term normative values are not interchangeable. The multitude of possible recording periods and metrics make it difficult to discern a change in HRV that may be clinically significant, particularly in relatively healthy individuals. Nevertheless, various indices (mostly from 24-hour recording periods) have been shown to have differing

capacities to predict various CVDs and CV events[164]. Not surprisingly, multiple pre-testing and testing factors can influence the measurement including body posture, time of day, fasted state, caffeine, recent vigorous exercise, and medications that impact ANS function. These factors were considered in this project and are further outlined in the pre-assessment guidelines described later in this chapter. Lastly, the principal investigator is well-versed in ECG and HRV analysis; no extra training is needed for the execution of this project. A full explanation of all methodological considerations and potential clinical implications of HRV are beyond the scope of this review. However, the interested reader is directed to several useful reviews[164, 165].

Measure 4: Pulse Wave Analysis (Secondary Outcome)

Rationale for Measuring Pulse Wave Analysis

Pulse-wave analysis comprises a number of important vascular measurements that reflect central blood pressure and pressure wave interactions. The committee chair and others have reported oscillometric-based cSBP (ICC: 0.89) and arterial wave reflection (systolic augmentation index, SAI; ICC: 0.79-0.94) to be highly reliable[166–168]. The prognostic value of cSBP has been recognized by expert consensus[169, 170] and sAI has also been shown to explain variation in CVD risk beyond traditional risk predictors[171]. The committee chair has also shown that SAI decreases by 16.2% in response to acute aerobic exercise in adults[172].

Methodological Options for Measuring Pulse Wave Analysis and Rationale for Chosen Approach

Several products have been used for clinical and research purposes, each with slightly different technologies and proprietary algorithms. The Vicorder and BP+ were chosen as tools to assess PWA primarily due to the fact that they were also used for measurement of the

primary AS outcome, PWV. This approach enabled multiple and simultaneous assessments of PWV and PWA (e.g. time aligned readings of two PWV measurements [one at each limb], two PWA measurements [one at each limb], which is an attractive advantage when assessing the effects of an acute perturbation such as a laboratory-based mental stressor.

Principles and Key Considerations for Pulse Wave Analysis Measurement

PWA is measured oscillometrically in order to decompose pressure waveforms at peripheral sites (typically at brachial artery). The decomposed waves are analyzed using proprietary algorithms that quantify the time and magnitude of forward and reflected waveforms. Ultimately, these central (aortic) pressure waveforms are used to estimate cSBP and arterial wave reflection[173, 174]. The cSBP reflects overall myocardial stress and sAI estimates the proportion of cSBP being driven by arterial reflection from peripheral factors, including AS. As far as clinical implications, a meta-analysis[175] of 11 longitudinal studies (n = 5,648, mean follow-up 45 months) reported that for a 10% increase in SAI, the risk of future CV events and all-cause mortality increased by 32% and 38%, respectively[164]. Like PWV, multiple pre-testing and testing factors can influence PWA measurement including body posture, time of day, fasted state, caffeine, recent vigorous exercise, and medications that impact CV function. These factors were considered in this project, and are further outlined in the pre-assessment guidelines described later in this chapter. Finally, the principal investigator is well-versed in both the Vicorder and BP+, and the oscillometric nature of the device requires little technical training.

Methodological and Rigor Considerations

This section will explain factors that will be considered to ensure methodological and scientific rigor.

- **Consideration of relevant biological variables**. Both mental stress reactivity and CVD risk trajectories differ by sex, and racial/ethnic minorities may have greater baseline and post-stress CV reactivity than whites[176–178]. The current study was not be powered to examine sex and race differences, however separate analyses were conducted to stratify by race and sex.
- **Scientific rigor.** Considerations including experimental design, external validity, quality control, and methodology are described below.
- **Study design.** Rationale for the design of each aim is described and rationalized above.
- **External validity.** The goal is to ensure a representative sample of recreationally active adults. Findings from this proposal provided important insights to guide further investigation of stress-related CVD risk in otherwise healthy adult populations.
- **Quality control.** Quality control includes standard operating procedures, staff training, analysis supervision, data quality monitoring. The PI held weekly meetings with the research staff to review participant recruitment and retention, adverse events, study administration, and quality of data collection.
- **Methodology.** Extensive rationale are provided above. All measures are reliable and valid. The Principal Investigator provided pre-study training to research assistants and reviewed all collected data. If an adequate level of quality was not achieved (e.g., poor quality pressure waveforms), the data were not included in the analysis and the analyst was re-trained.

Study Design Considerations

Table 4.4 outlines potential study design options and the rationale for the chosen design.

Table 4.4. Potential study design options.

Aim (Study)	Consideration	Choices	Selection	Explanation
1, 2	Study Design	• Observational • Interventional • Multi-group RCT • Randomized Cross-Over	• Randomized Cross-Over	• Minimizes between-subject variability • Only necessitates each participant take TSST 1x, eliminating potential for bias due to TSST habituation

Abbreviations: *RCT, Randomized Controlled Trial; TSST, Trier Social Stress Test*

Considerations Related to the Laboratory Stressor and AE Eustress

Laboratory Stressor: Trier Social Stress Test

Trier Social Stress Test (TSST; mental arithmetic + psychosocial stressors). The TSST is a validated, short battery of mental stressors, which has been shown to reliably elicit a robust physiological response[31, 32]. The TSST reliably provokes a stress response by incorporating social evaluation and unpredictability, most commonly via a public speaking task followed by a mental arithmetic task[32, 44]. Since it is both i) well-established in the literature[31, 32, 44], and ii) effective at eliciting a reliable and robust psychophysiological response[31, 32, 44], the TSST is an appropriate technique to use in research investigating the relationship between acute mental stress and cardiometabolic factors related to CVD risk. The same research assistant administered the TSST for all participants, and verbal communication necessary to administer the test was scripted to ensure between-subject consistency.

Laboratory Eustress: Moderate-Intensity, Whole-Body Aerobic Exercise

Participants engaged in twenty-five minutes of moderate-intensity elliptical AE during one of the two visits for the experimental Study 2. This duration and intensity of AE elicited a robust cardiometabolic response and is in line with national and international physical activity

guidelines[45, 179]. On the other hand, the AE intensity was likely not at an intensity high enough to prompt mental distress in recreationally active adults. The elliptical was chosen as a means to promote a whole-body aerobic stimulus and shear-stress. Exercise intensity was monitored by rate of perceived exertion (RPE) and by HR using the Karvonen Method of Heart Rate Reserve (HRR) [45]. Specifically, the following equation determined the target HR: [(Age-predicted HR_{max} – HR_{rest})(%Intensity)] + HR_{rest}. Age-predicted HR_{max} was calculated as 220-age[45]. Resting heart rate was determined by a chest-worn HR monitor (Polar) following 10 minutes of supine rest at the beginning of the first visit for **Study 2**.

Pre-Assessment Guidelines

To ensure participants reported for each experimental visit under standardized conditions a number of factors were considered. These factors are outlined in **Table 4.5**.

Table 4.5. Pre-assessment guidelines for experimental study visits.

Consideration	Explanation	Control Procedure
PA	Recent vigorous exercise alters ANS and EE	No Vigorous PA for 24 hrs prior
Sleep	Sleep impacts AS and metabolism	Ensure normal sleep for given participant; no differences in duration/quality between visits (Use Karolinska Sleep Diary)
Food/Caffeine	Influences AS and EE	12 hr fast (besides water).
Medications	Influences AS and EE	Abstain from CV and metabolic drugs (e.g. beta blockers, diuretics)
Mode of Transport	Walking or cycling to lab visit will influence AS and EE	Offer parking pass/Help participant navigate public transport
Quiet rest period prior to measurements	Influences AS and EE	20 min supine rest in quiet area without distractions prior to baseline testing

Abbreviations: *ANS, Autonomic Nervous System; CV, Cardiovascular; EE, Energy Expenditure; Hrs, Hours; PA, Physical Activity; TSST, Trier Social Stress Test; VAS, Visual Analog Scale*

Internal Validity

Table 4.6 outlines considerations for ensuring internal validity.

Table 4.6. Considerations for ensuring internal validity for experimental studies.

Consideration	Explanation	Control Procedure
Posture during measurement	Posture impacts EE and AS measurements	Semi-reclined position at same angle will be used consistently to balance measurement validity and ecological validity
Equipment Calibration	Calibration of gases and flow rates necessary for valid EE measurement; PWA devices must be calibrated for valid BP readings	Metabolic cart for EE will be calibrated prior each visit; Manometers will be used to calibrate pressures for PWA devices every month
Stress Level	Stress-related states will impact primary outcomes and confound TSST. Want to ensure "normal" stress level for given participant	Use VAS prior to supine rest period. Note, this VAS will also be used after TSST/Control periods to assess perceived effect of TSST/Control period relative to baseline.
TSST Consistency and Efficacy	Variation in TSST introduces confounding; failure to produce a reliable stress response introduces confounding	Non PI administrator of stressor, piloting to ensure perceived and physiological stress response, Same administrator for all participants
Consistent AE	Between- and within-subject variation in AE intensity introduces confounding to primary outcomes	Use of RPE and HR (using Karvonen HRR method) to monitor exercise intensity

Abbreviations: *AE, Aerobic Exercise, EE, Energy Expenditure; HR, Heart Rate; PI, Principal Investigator; PWA, Pulse Wave Analysis; RPE, Rate of Perceived Exertion, TSST; Trier Social Stress Test; VAS, Visual Analog Scale*

Population/Sampling

Findings are generalizable to a large segment of disease-free, recreationally active young age adults aged 18-30 years. This age range minimized age-related biological variance (e.g. AS naturally increases with age). This population was recruited from the local Chapel Hill area. The sample size (n=20) and dual-visit cross-over design for each experimental study enabled detection of statistically significant group x time interactions, ensure feasibility, and

prevent unnecessary participant and researcher burden. Inclusion and exclusion criteria are described in **Tables 4.7** and **4.8** below.

Table 4.7. Eligibility criteria.

Criteria	Method	Rational
Age 18-30 y	Screening interview	Minimizes age-related biological variance, while still capturing large segment of the adult population
Recreationally active: 500–1000 MET min/wk of reactional PA or exercise/week	Screening interview; IPAQ	Generalizable to large portion of young adults. Ensures participants are not psychologically averse to physical activity.

Abbreviations*: IPAQ, International Physical Activity Questionnaire; MET, Metabolic equivalent (=3.5 ml/kg/min of O_2 consumption); Min, Minutes; PA, Physical Activity; Wk, week; Y, years*

Table 4.8. Exclusion criteria

Aims(s) Criteria	Method	Rational
Cardiometabolic disease/medication use	Screening interview/MHQ	Likely to have an influence on primary and secondary outcomes
Mental Illness/medication use	Screening Interview/MHQ	Mental illness likely to be substantially mediated via current and/or history of excessive mental stress

Abbreviations*: MHQ, Medical History Questionnaire*

Sex as a Biological Factor

The Principal Investigator attempted to recruit equal sex distribution in each experimental study (Experiment 1: 11 females/ 9 males; Experiment 2: 12 females/ 8 males). While not powered to address sex disparities, sex was not a significant covariate in the primary analysis models for changes in arterial stiffness and energy expenditure.

Ethnicity/Race

The Principal Investigator attempted to recruit an ethnically/racially diverse population in each experimental study (Experiment 1: n=7 [35%] non-white [5 Asian, 2 African-American]; Experiment 2: n=9 [45%] non-white [4 Asian, 4 African-American, 1 Hispanic]). However, the current studies were not powered to address ethnicity/race disparities.

External Validity / Generalizability

Results were not generalizable to all young adults. Nonetheless, findings guided future investigations of acute mental stress and CVD risk. Based on the scope and resources of the current proposal, the current approach is a promising opportunity to spur future inquiry into mental stress-related CVD risk as well as lifestyle-based strategies to mitigate this risk.

Statistical Considerations

Based on the cross-over study design of the experimental studies, linear mixed model regression with random effects were used. For each study, factors were specified for condition and time. Specifically, condition (2 conditions), time (pre, post, 15 min, 30 min, 45 min, 60 min = six time points), and condition X time interaction effects were tested. The mixed model allowed for participants to be nested in condition and time factors. As this is a cross-over trial, each participant served as their own control, removing between-subject variance. Additionally, to evaluate the influence of concomitant changes in mean arterial pressure on AS and of %body fat on EE, additional models adjusted for these variables.

Potential Challenges & Alternative Strategies

The most probable challenge was the potential for missing data. Two missing data scenarios were possible: participant dropouts, and poor or incomplete data collection. However, missing data was allowable within the mixed model analysis. Another potential challenge was trying to ensure that simply resting under the ventilated canopy for >1 hour was not unduly stressful in and of itself. However, this was countered by device familiarization on a separate day prior to the actual experimental visits.

Unmet Recruitment Targets

A key challenge may be recruitment of non-white individuals. The principle investigator worked with North Carolina Translational and Clinical Sciences Institute (NCTraCS) and community outreach groups to ensure inclusive recruitment and study inclusion.

Carry-Over Effects

Attempting to answer the research questions with a single experimental study would have necessitated >1 TSST administrations for each participant, which would likely be subject to a carry-over or habituation effect (e.g. the TSST may be impart less stress with each exposure to the participant). An advantage of the dual-study study is that it only requires that each participant take the TSST one time, thereby eliminating the potential for bias due to a TSST learning effect.

Timeline

Table 4.9 Outlines the expected timeline of this dissertation.

Table 4.9. Project timeline & milestones.

Activities	Start Date	End Date
Pilot Testing	10/25/21	11/22/21
Equipment/Standard Operating Procedures current	10/25/21	11/15/21
Protocol/Institutional Review Board	10/25/21	11/15/21
Staff training	11/1/21	11/22/21
Study forms/ database	11/1/21	11/22/21
Set-up filling system (OneDrive)	10/25/21	11/1/21
Recruitment	11/22/21	12/20/22
Data collection	11/29/21	12/31/22
Aim 1 analysis	4/11/22	2/28/23
Aim 2 analysis	4/11/22	2/28/23
Hand Document to Committee	3/10/23	
Defend	3/20/23	
Respond to defense changes	4/1/23	
Submit thesis to graduate school	4/10/23	
Authorship order agreement	5/1/23	
Submit Manuscripts	6/1/23	8/1/23
Milestones		
50% recruitment	6/22/22	
50% Data collection	8/16/22	

CHAPTER 5: STUDY (REVIEW) 1: EFFECTS OF ACUTE MENTAL STRESS ON ARTERIAL STIFFNESS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Preamble

It was important that this meta-analysis was conducted first to consolidate the available literature on the effects of acute mental stress on arterial stiffness, which is one of the primary outcomes in the subsequent experimental studies. Arterial stiffness is a biomarker of arterial structure and function, and has significant prognostic value.

Overview

Chronic mental stress is associated with cardiovascular disease (CVD) risk. This association has been found primarily in longitudinal/observational studies that do not reflect the direct mechanism(s) linking stress and CVD. Moreover, a majority of the acute studies relating stress to CVD risk have used traditional indices such as blood pressure and heart rate. Arterial stiffness is a sensitive, sub-clinical, composite measure of arterial structure and function that may help better explain the mechanism associating stress and CVD risk.

Objectives: The objective of this meta-analysis was to synthesize the existing literature regarding the effect of acute mental stress on arterial stiffness.

Data Sources: Electronic databases (PubMed) from inception to February 2023. The reference lists of eligible studies and relevant reviews were also checked.

Study Selection: Inclusion criteria: Studies were included if (i) arterial stiffness was measured non-invasively using pulse wave velocity (PWV) or pulse transit time (PTT), (ii) arterial

stiffness was measured pre- and post- administration of an acute mental stressor, (iii) participants were adults (≥18 years) free of vascular-acting medication and overt CVD or CVD risk at the time of testing, and (iv) the mental stressor was purely psychological (rather than purely physiological or both psychological and physiological) in nature.

Appraisal and Synthesis Methods: Initially, 11,689 studies were identified. After evaluation of study characteristics, quality, and validity, data from 9 studies (17 trials) involving 254 participants were extracted for meta-analysis. Standardized mean difference (SMD) and 95% confidence intervals (95% CI) were calculated using random-effects meta-analysis modelling. SMD was used to determine the magnitude of effect.

Results: The overall effect size of mental stress on arterial stiffness from all studies was 0.45 ($p<0.005$). Additionally, there was insubstantial heterogeneity between studies (Cochran's $Q(16)=7.02$ ($p=0.97$)).

Conclusions: Exposure to acute mental stress causes arterial stiffness to transiently increase, potentially leading to cardiovascular disease risk over time.

Introduction

Cardiovascular disease (CVD) risk is elevated with repeated exposure to psychological stress [1]. Chronic psychological stress is a general term that encompasses myriad, common sources of severe life stress including, but not limited to caregiving, having a history of adverse childhood events, community violence, and discrimination. These chronic sources of life stress are associated with elevated cardiovascular morbidity and mortality[66]. For example, large epidemiological studies have highlighted the relationships between marital stress, adverse childhood events, and stress-related psycho-social factors with atherosclerosis, peripheral

artery disease, as well as incident myocardial infarction cerebrovascular events[1, 65, 180]. However, the physiological mechanisms responsible for the relationship between chronic stress and CVD risk is not well understood. A better understanding of the physiological chain of events that explain the relationships between chronic life stress and CVD risk is necessary to optimally inform prevention and treatment of stress-related CVD risk.

Psychological stress is a complex variable which likely interacts with a number of other factors to influence CVD risk, such as stress-related effects on important health behaviors known to impact cardiovascular health such as diet and physical activity[181]. Nevertheless, previous studies and reviews have identified a several CVD risk factors which reliably increase in response to acute stress exposure[31, 32, 182]. For example, elevations in blood pressure (BP), heart rate, and sympathetic nervous system activity following exposure to acute laboratory stressors (e.g. public speaking, mental arithmetic, etc.) are well documented in the literature[31, 32, 182]. These acute effects of psychological stress on CVD risk factors suggest that direct effects on physiological functioning contribute to the relationship between chronic stress and increased CVD risk[183]. However, the majority of studies investigating the effects of acute stress on CVD risk have focused on the traditional cardiovascular measures mentioned previously, including BP and heart rate[31, 32, 182]. This narrow focus on traditional cardiovascular markers has contributed to the limited understanding of the physiological mechanisms underlying the relationship between chronic psychological stress and CVD risk.

A small, but growing body of literature has investigated the effects of acute mental stress on arterial stiffness, a construct which reflects vascular structure and function. Arterial stiffness is influenced by both central (e.g. autonomic) and local (e.g. endothelial) factors, and

provides superior prognostic capacity of CVD risk compared to traditional factors such as heart rate and BP [142, 184]. Moreover, arterial stiffness is considered a sensitive marker of vascular health in young and otherwise healthy populations prior to traditional clinical manifestations of CVD risk[35, 185]. The comprehensive reflection of vascular structure and function of arterial stiffness, coupled with its prognostic utility in young and generally healthy populations make this novel biomarker an attractive candidate for exploring the mechanisms underlying the relationship between repeated or prolonged exposure to acute mental stress and CVD risk.

Objective

As far as we are aware, no prior studies have systematically consolidated the literature pertaining to the effects of acute psychological stress on arterial stiffness. Therefore, the objective of this review was to systematically review, critique, and consolidate the existing body of literature that has assessed the effects of acute psychological laboratory stressors on arterial stiffness, as measured by pulse-wave velocity (PWV) or pulse-transit time (PTT), in disease-free adults.

Methods

This meta-analysis was carried out in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines[186].

Data Sources and Searches

Electronic databases (PubMed, SportDiscus, Google Scholar) were searched by two authors independently (GZ, NS) utilizing the keywords:((psychological stress[Title/Abstract]) OR (mental stress [Title/Abstract]) OR (acute stress [Title/Abstract]) AND (arterial stiffness [Title/Abstract]) OR (pulse wave velocity [Title/Abstract]) OR (aortic stiffness [Title/Abstract])).

The reference lists of all identified trials and relevant reviews or editorials were also examined. The search was limited to English language studies published between inception and February 2023.

Article Selection

For the purpose of this meta-analysis the term 'article' is used synonymously with 'study', and 'trial' is the unit included in the meta-analysis. A given article may have resulted in more than one eligible 'trial' if the article included more than one intervention group. Initially, article titles and abstracts were screened for relevance. The full-text of potentially eligible articles were obtained to review eligibility for inclusion. The following criteria were used to select trials for inclusion in the review: (i) arterial stiffness was estimated non-invasively using PWV or PTT, (ii) arterial stiffness was measured pre- and post- administration of an acute mental stressor, (iii) participants were adults ($\geq$18 years) free of vascular-acting medication and overt CVD or CVD risk at the time of testing, and (iv) the mental stressor was purely psychological (rather than purely physiological or both psychological and physiological) in nature.

Data Extraction and Quality Assessment

Data extracted for each eligible trial included bibliographic information (author, publication year), baseline participant characteristics, details of intervention(s), and results of reported outcomes. Study quality and bias were assessed using a risk of bias assessment tool (Risk of Bias 2 software for Crossover Trials), which produced a score from 1-3 with 1 being low risk of bias, 2 being moderate risk of bias, and 3 being high risk of bias. The rating system

considered factors including randomization, period and carryover effects, outcome data, outcome measurement techniques, and selection of reported results.

Data Synthesis

For each outcome of interest, the pre- and post-intervention values (mean and standard deviation) were entered into a spreadsheet. When mean differences and associated standard deviations were not published, they were requested from the corresponding study author(s). For one study, where we were unable to reach the author, image analysis software (ImageJ) was used to extract data from a figure provided in the article. [2] The post-measurement data point used for analysis was the measurement taken immediately following cessation of the stressor (e.g. Time 0 minutes post-stress). For studies that did not measure arterial stiffness immediately following the stressor, a mid-stress measurement was used as the "post" time-point. When there was not a measure taken during or immediately after the stress exposure, the first post-stress measure was used for analysis. Primary study outcomes were PWV and PTT. Aggregation and calculation of final results was conducted by one author (GZ).

Data Analysis

Data were analyzed using the 'metafor' package (R version 4.2.0). Outcome measures were expressed as standardized mean differences (SMD). SMD was used rather than weighted mean difference in order to accommodate the joint analysis of both PWV and PTT measurements. Since a higher (faster) PWV corresponds with a lower PTT, estimates for PTT were inversed within the random-effects model to maintain consistency in terms of the directionality of the effect. The SMD was used to determine the magnitude of effect where <0.2, 0.2, 0.5 and 0.8 were defined as trivial, small, moderate and large, respectively[187]. A

random-effects model was fitted to the data and a corresponding forest plot was created. The amount of heterogeneity (i.e., τ^2), was estimated using the DerSimonian-Laird estimator (DerSimonian & Laird, 1986). In addition to the estimate of τ^2 , the Q-test for heterogeneity (Cochran, 1954) and the I^2 statistic (Higgins & Thompson, 2002) are reported. In case any amount of heterogeneity is detected (i.e., $\tau^2 > 0$, regardless of the results of the Q-test), a prediction interval for the true outcomes is also provided (Riley et al., 2011). Studentized residuals and Cook's distances are used to examine whether trials may be outliers and/or influential in the context of the model (Viechtbauer & Cheung, 2010). The rank correlation test (Begg & Mazumdar, 1994) and the regression test (Sterne & Egger, 2005), using the standard error of the observed outcomes as predictor, were used to check for funnel plot asymmetry. Two authors (GZ and NS) conducted the data analysis.

Results

Literature Search and Trial Selection

A total of 11,689 potentially eligible articles were identified. Of these, 33 articles were identified for full-text screening, of which 24 were excluded, leaving 17 trials from 9 studies for inclusion[20, 94, 95, 188–192, 218] (**Figure 5.1**).

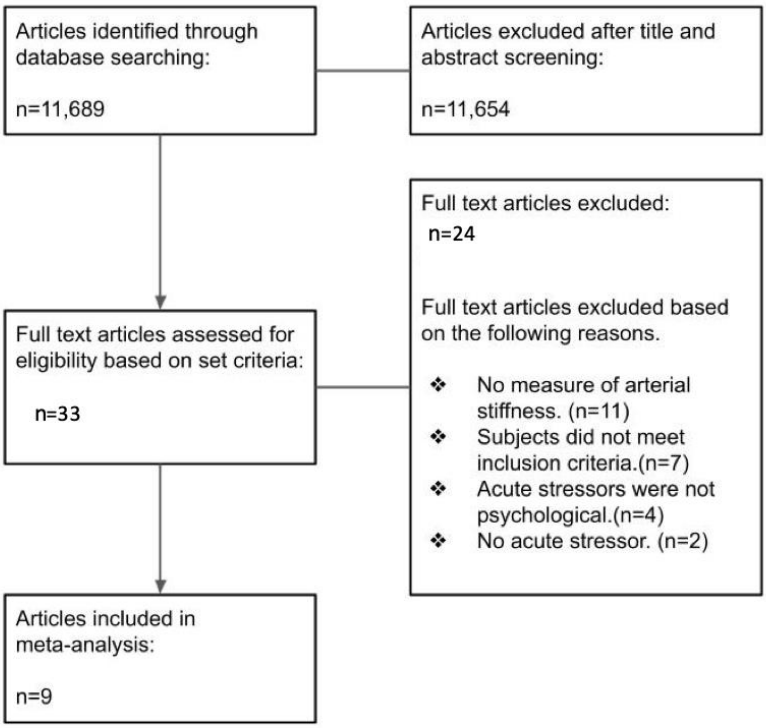

Figure 5.1. Flow-chart of study selection.

Description of the Included Trials

Trial Setting and Participants

Included study characteristics are summarized in **Table 5.1**. The studies were carried out in the United States (n=1)[95], Greece (n=2)[20, 94], Japan (n=2)[188, 218], Italy (n=1)[190], Ireland (n=1)[191], Canada (n=1)[192] and New Zealand (n=1)[189]. The number of participants in each trial ranged from 15 to 85. One trial [95] included only female participants and three trials [25, 192, 218] included only male participants. The mean age of the participants ranged from 20.1 years to 30.1 years. There were a total of 254 participants. One study, Dutch et al[189], did not specify biological sex, and they also did not report average and standard

deviation of the age of participants. Besides Dutch et al[189], the analysis included 218 participants (25.42 [3.43] years, 61% being female).

Interventions

A brief description of the interventions is given in **Table 5.1**. All studies utilized a laboratory stressor, psychological or combined psychological & physiological stressors.

Table 5.1. Study characteristics.

Reference	N	Biological Sex	Mean Age (years) [SD]	Mental Stressor	Duration of Stressor (minutes)	Methodological Assessment(s)	Non-stress Comparison Group? (Y/N)	Covaried for BP? (Y/N)	Quality (1-3)
Szabo, A.[192]	15	15 Males	30.1 [6.7]	-Mental Arithmetic	1	PTT	N	N	1.5
Vlachopoulos et al. 2009[20]	18	10 Females 8 Males	26.9 [2.6]	-Distressing Film	30	PWV c-f	Y	Y	1.0
Vlachopoulos et al. 2006[94]	19	10 Females 9 Males	28.5 [0.6]	-Mental Arithmetic	3	PWV c-f	Y	Y	1.0
Logan et al.[95]	85	85 Females	28.78 [9.84]	-TSST (speech task + mental arithmetic)	10	PWV c-f	Y	Y	2.5
Kume et al. 2020[188]	17	17 Males	20.1 [0.7]	-Mental Arithmetic	5	haPWV hbPWV baPWV	Y	N	1.0
Kume et al. 2022[218]	15	15 Males	21.7 [0.3]	-Mental Arithmetic	5	haPWV hbPWV baPWV	N	N	1.0
Dutch et al.[189]	36	Not Specified	Not specified. Range: 18-28 years	-Geometrical problems -Reaction Times -Video Game	Not specified. ~ <5min	PTT	N	N	2.5
Gentilin et al.[190]	26	13 Females 13 Males	24.25 [2.85]	-Mental Arithmetic	10	PWV h-f	N	N	1.5
Jatoi et. al[191]	23	14 Females 9 Males	23 [3.0]	-Mental Arithmetic	3	PWV c-f	N	Y	2.0

Median quality score for included trials: 1.56 (Range 1-3: 3-high risk of bias, 1-low risk of bias)
Abbreviations: *BP, blood pressure; cfPWV, carotid-femoral pulse-wave velocity; haPWV, heart-ankle pulse-wave velocity; hbPWV, heart-brachial pulse-wave velocity; baPWV, brachial-ankle pulse-wave velocity; hfPWV, heart-femoral pulse-wave velocity; PTT, pulse-transit time; TSST, Trier Social Stress Test.*

Methodological Quality Assessment

Quality scores were determined using Risk of Bias 2 (Beta Version 7) for cross-over trials. This software provided qualitative ratings for each study that were then quantified on a scale of

1-3. The qualitative ratings provided were Low Concern, Some Concern, and High Concern. Low concern was given a score of 1, some concern was given a score of 2, and high concern was given a score of 3. Thus, the studies with the least bias had a score of 1. The criteria that were used by the Risk of Bias tool to determine quality scores considered: i) randomization process, ii) bias arising from period and carryover effects, iii) deviations from intended interventions, iv) missing outcome data, v) outcome appropriateness, and vi) appropriateness of analysis strategy. Because the software was designed for cross-over trials, it assumed that studies indeed utilized a cross-over design and a control comparison group. However, several studies did not employ a cross-over design and/or a control comparison group. For these studies, the researchers added 1 point from the software-derived quality score. When the ratings of software and researchers were different from each other, the ratings were averaged together. The methodological assessment of included trials is summarized in **Table 5.1**. The quality of the included studies ranged from 1–3, with a median quality score of 1.56 such that the risk of bias was low. Six of the nine studies were randomized cross over trials. Three studies[95, 189, 190] were not crossover studies. Dutch et al[189] and Logan et al[95] used different groups for different conditions, while Gentilin et al.[190] only used a pre-post design. Five of the 9 studies did not use a non-stress control condition or control group as a comparison[189–192, 218]. Of these studies, besides Gentilin et al[190] which used the pre-post design, multiple stressors were compared against each other in the different conditions[189, 191, 192].

Synthesis of the Results

Arterial Stiffness

A total of k = 17 trials were included in the analysis. The observed SMD ranged from 0.01 to 0.78, with 100% of the estimates being positive (**Figure 5.2**) . The estimated average outcome based on the random-effects model was $\mu = 0.45$ (95% CI: 0.30 - 0.60, $p < 0.0001$). According to the Q-test, there was no significant amount of heterogeneity in the true outcomes ($Q(16) = 7.02$, $p = 0.97$, $\tau^2 = 0.00$, $I^2 = 0.00\%$). Visual inspection of the funnel plot (**Figure 5.2**) indicated no evidence of publication bias, and the sensitivity analysis indicated that none of the trials unduly influenced the outcome. While no trials unduly influenced the outcome, one trial (13) had a relatively large weight compared to the rest of the studies. Subsequent analysis whilst removing this trial (13) showed that the SMD decreased slightly to 0.42, but still remained statistically significant.

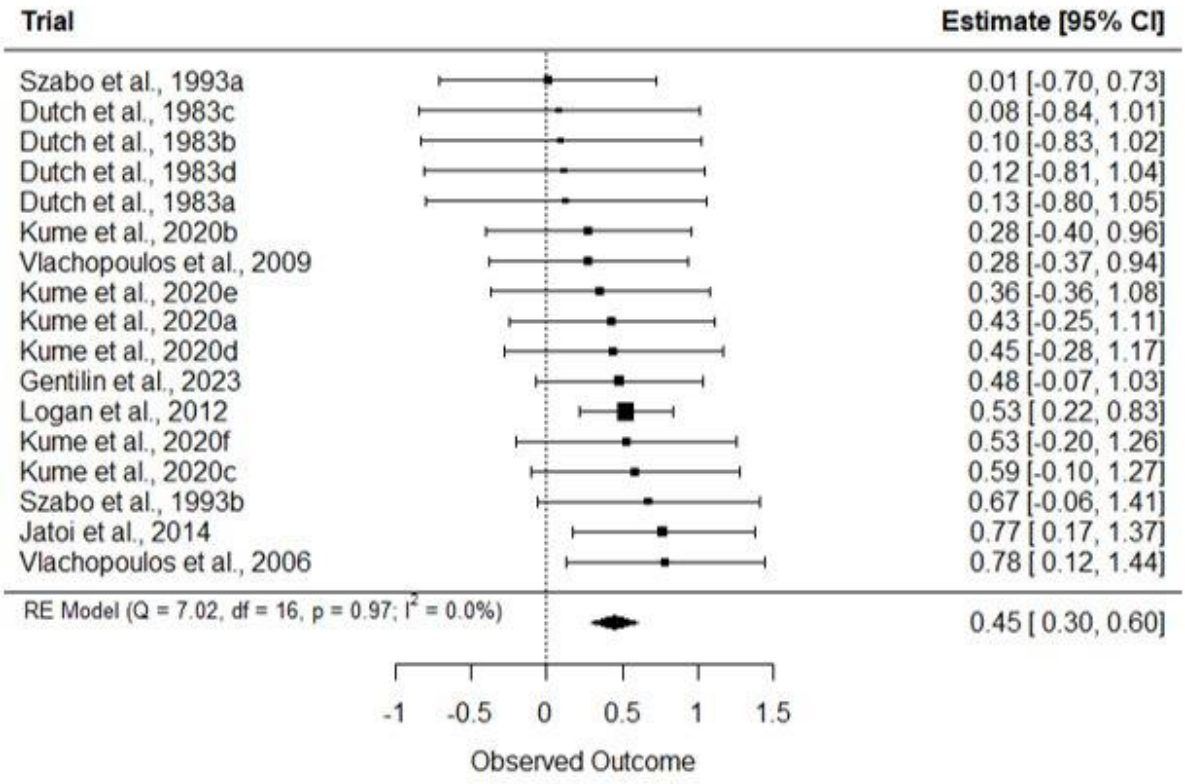

Figure 5.2. Forest plot showing the observed outcomes and the estimate of the random-effects model. Abbreviations*: 95% CI, 95% confidence interval.*

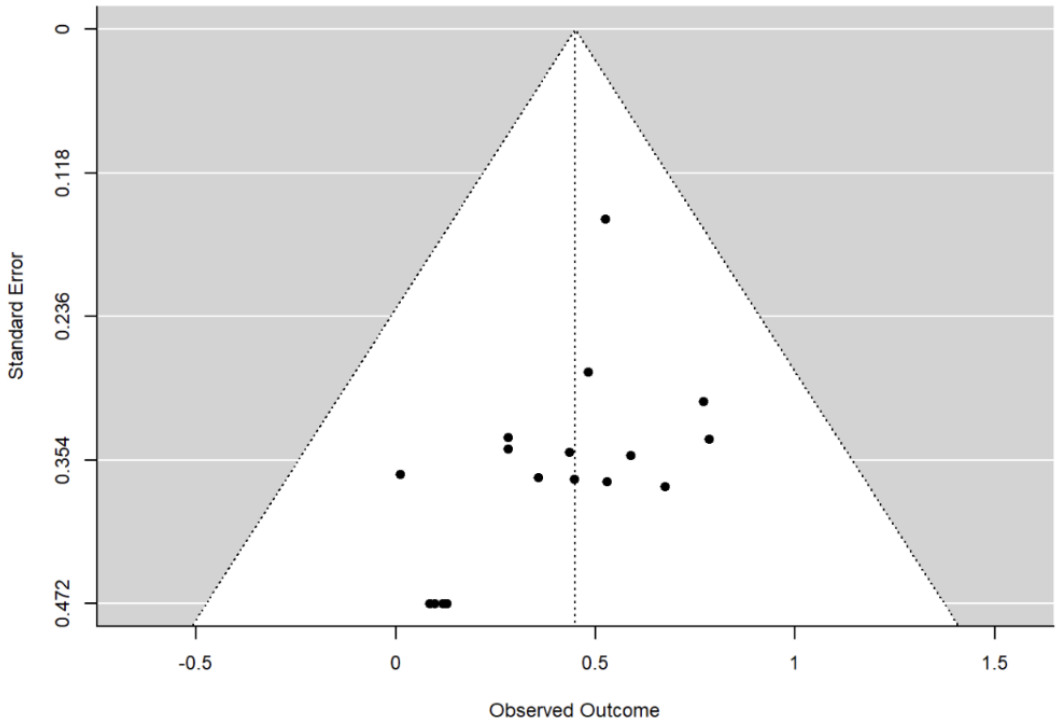

Figure 5.3. Funnel plot for studies assessing the acute effects of mental stress on arterial stiffness.

Discussion

The aim of this meta-analysis was to consolidate the existing literature relating to the effect of acute mental stress exposure on arterial stiffness, as measured by PWV or PTT. The main findings were that acute exposure to mental stress resulted in significant small-moderate increase (SMD = 0.45) in arterial stiffness.

Limitations

Several limitations should be borne in mind when considering the findings of this meta-analysis. First, the sample size of included trials was generally small; further trials are required to more clearly delineate the interactions between acute mental stress and arterial stiffness. With the small number of studies heterogeneity was introduced due to i) a number of different arterial segments used for PTT and PWV calculations, and ii) multiple types of mental stressors.

Another limitation is that over half of the included studies did not covary for BP which is important since BP is a known confounder of arterial stiffness estimates[145]. Third, the majority of trials were mixed-sex and did not report results stratified by sex. It remains unclear whether arterial stiffness responses to acute mental stress differ between sexes. Lastly, only four of the nine studies compared their results against a neutral or non-stress control group or condition. These factors partially contributed to the median risk of bias score being > 1.

Comparison with other Studies

Findings from the current meta-analysis were that acute exposure to mental stress resulted in significant small-moderate increase (SMD = 0.45) in arterial stiffness as assessed by PWV and PTT. In response to acute stress exposre, on average, studies presenting PWV data showed an increase of 0.3 $\pm$ 0.25 m/s, and studies presenting PTT data showed an average decrease of 0.003 $\pm$ 0.005 seconds.

No prior reviews that we are aware of have assessed the arterial stiffening response to acute mental stress. Physiologically, acute mental stress may elevate arterial stiffness via both central (autonomic and local (endothelial) systems [91, 193]. Indeed, previous work has also shown that exposure to acute mental stress stimulates increases in circulating catecholamines[193] and reductions in flow-mediated dilation[91]. In terms of endothelial function, prior studies – including by our group[137] – have demonstrated that PWV is partially driven by endothelial function, and that endothelial function is impaired following acute stress exposure[91]. In terms of autonomic function, increases in arterial stiffness in response to acute mental stress are likely due to elevations in sympathetic, and tempering of parasympathetic, branches of the autonomic nervous system[3, 6]. For example, our prior work has

demonstrated that PWV measurements are influenced by autonomic-mediated changes in BP[145, 194]. Thus, the fact that nearly half of the selected studies did not control (or did not mention controlling) for blood pressure does limit the inferences that can be made from the current results. To best understand the contributions from different physiological systems in terms of their role in stress-related CVD pathophysiology, it is necessary for future studies to adjust arterial stiffness measures – particularly PWV - for simultaneous changes in BP. Controlling for BP enables a better reflection of pressure-independent, alterations in intrinsic stiffness properties. Collectively, it is likely that acute stress-mediated arterial stiffening is partially driven by both central (autonomic) and local (endothelial) mechanisms.

Implications

The pace, pressure, and socio-political landscape of contemporary Western society is conducive for high levels of mental stress. Thus, it is not surprising that the majority of the United States population reports high levels of stress, and that the prevalence of high levels of stress is increasing nationwide. For example, in 2015, 24% of Americans reported “extreme” levels of stress, up from 18% in 2014[53]. The same study also showed that over one third of Americans reported that their stress levels had increased from the previous year[53]. Moreover, It would be remiss not to acknowledge the enormous toll that the COVID-19 pandemic has had on stress-related mental and physical health. It is arguably now more important than ever before to investigate how acute repeated or prolonged exposure to mental stress may contribute to CVD risk over time. Scientific and public health communities are only beginning to understand the extent to which pandemic-related mental stress has had and will continue to have on psychological and physiological function[58, 59]. Further efforts are

warranted to better understand the relationship between acute stress exposure and arterial stiffness, particularly amongst studies which more consistently i) assess arterial segments with clear prognostic capacity, and ii) also account for changes in BP. It is also important to acknowledge that while exposure to stress cannot be completely eliminated (though efforts can be made to reduce exposure), cardiovascular and arterial stiffness reactivity to acute mental stress may be able to be modified through healthy lifestyle factors such as physical activity, exercise, and mindfulness meditation[11, 125, 195]. Better understanding the mechanisms as well as interactions with other lifestyle factors (e.g. exercise, physical activity, sleep, sedentary behavior) which influence the relationship between acute stress exposure and CVD risk is needed to most optimally inform lifestyle-based strategies for off-set stress-related CVD risk.

Conclusions

Chronic psychological stress is a known contributor to elevated cardiovascular disease (CVD) risk, and severe levels of mental stress are commonplace in today's society[1]. Exposure to acute mental stress causes arterial stiffness to increase, potentially leading to CVD risk over time. While the current findings do seem to demonstrate stress-induced arterial stiffness elevations, the magnitude and consistency of such elevations are not fully clear – particularly given the heterogeneity in stress protocols and arterial pathlengths, as well as the lack of controlling for blood pressure in much of the current literature. Future studies are warranted to explore how other lifestyle behaviors (e.g. exercise, mindfulness, diet, sleep) and lifestyle-associated factors (e.g. psychological resilience, cardiorespiratory fitness) of cardiovascular function may also influence the effects of repeated or prolonged exposure to acute stress on CVD risk.

CHAPTER 6: STUDY 2 (EXPERIMENT 1): EFFECTS OF ACUTE MENTAL STRESS ON ARTERIAL STIFFNESS AND ENERGY EXPENDITURE

Preamble

It was important that this experimental study was conducted first to assess the impacts of acute stress on arterial stiffness and energy expenditure without a prior aerobic exercise bout. This was critical so that findings could be compared to the subsequent study involving pre-stress exercise in a similar population.

Overview

Background: Chronic mental stress has been linked to cardiovascular disease risk [1]. A biologically plausible mechanism linking repeated exposure to acute mental stress with CVD is autonomic nervous system-driven arterial stiffening. Another mechanism which is associated with the ANS, and which also impacts CVD risk is energy expenditure [4–6]. However, Energy Expenditure has been seldomly investigated in the context of acute stress. Therefore, the purpose of this randomized cross-over trial with a time-matched control was to assess energetic and cardiovascular reactivity during and following an acute mental stressor.

Methods: A randomized cross-over design was utilized. Twenty [21.0 (2.5) years, 55% female, 23.6 (4.0) kg/m^2)] recreationally active adults attended two visits in a randomized order: i) Trier Social Stress Test, and ii) Control (no-stress). Energy expenditure and arterial stiffness were assessed before and after exposure to stress and control conditions. Data was analyzed using linear mixed models and area under the curve.

Results: There was a small condition x time interaction effect (B=0.68 m/s, 95%CI: 0.39, 0.97, d=0.30) for PWV [Stress: ↑0.81 m/s (13%), Control: [↑0.15 m/s (2%)] and a small interaction effect (B=0.0010, 95%CI: 0.0004, 0.0015, d=0.31) for energy expenditure [Stress: ↑0.0016 kcal/kg/min (10%), Control:↑0.0005 kcal/kg/min (3%)].

Conclusion: Compared to a neutral time-matched control condition, exposure to acute mental stress caused significant arterial stiffness and energetic responses, which may be important markers in stress-mediated CVD pathophysiology.

Introduction

Mental stress is a ubiquitous aspect of the human experience. While some degree of mental stress is adaptive, excessive exposure has been linked to poor health [1]. One particularly concerning physiological repercussion of chronic mental stress is increased CVD risk [60, 61]. Generally, the disruptions in autonomic and neuroendocrine function as a result of chronically elevated levels of stress are thought to contribute to CVD risk by causing hemodynamic and vascular disruptions, metabolic dysfunction, and inflammation[4, 60, 61, 66, 67]. However, despite strong longitudinal associations between chronic life stress and CVD risk[62–65], the precise factors underpinning the stress-CVD relationship are not fully understood. Further complicating matters, excess mental stress may impact CVD risk indirectly by interacting with other lifestyle factors including 24-hour activity behaviors (physical activity, sedentary behavior, sleep)[4, 68], dietary patterns[69, 70], and substance use[71]. Indirect mediators aside, there is currently an incomplete understanding of the psychophysiological chain of events that explain the link between elevated exposure to mental stress and CVD risk.

Hampering a more comprehensive understanding of the role of stress on CVD risk, the majority of studies that have investigated how repeated exposures to mental stressors may affect CVD risk have mostly focused on a limited number of outcome variables including heart rate (HR) and blood pressure (BP)[1]. Arterial stiffness is a continuous and sensitive biomarker of vascular structure and function[36]. Arterial stiffness is influenced by both central (autonomic) and local (endothelial) factors, and provides superior prognostic capacity of CVD risk compared to HR and BP[142, 196]. Arterial stiffness is sensitive to acute stress[20, 94] and is considered an important marker of vascular health in young and otherwise healthy populations prior to traditional clinical manifestations of CVD risk[184]. In addition to vascular effects, the effect of stress on CVD risk may also be influenced by metabolic function including energy expenditure (EE)[4–6]. EE and metabolism govern important CVD risk factors such as body composition and glucose regulation[7–9]. Moreover, EE may be implicated in stress-related CVD risk by influencing the degree to which stress-induced energy expenditure is allocated towards maladaptive processes such as inflammation (e.g. constrained energy theory)[128, 197]. However, EE has been seldom investigated during and following acute stress, let alone concurrently with arterial stiffness in the context of CVD risk.

A better understanding of the relationships between mental stress and CVD risk is necessary to optimally inform prevention and treatment of stress-related CVD risk. Filling this knowledge gap can aid in informing health strategies to reduce stress, regulate biological stress pathways, and prevent the development of stress-related CVD risk. Therefore, the purpose of this study was to assess the arterial stiffness and EE response to an acute, psychosocial laboratory stressor.

Methods

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines. Ethical approval was obtained from the University of North Carolina at Chapel Hill Institutional Review Board. All participants provided written informed consent prior to participating in the study.

Participants

Twenty-three young (18-30 years), healthy, recreationally active young adults were recruited from the host institution, a large state university. A recreationally active sample was recruited in order to minimize the influences of physical fitness on the primary outcomes. Recreationally active was defined as engaging in structured moderate-to-vigorous exercise for at least 30-minutes two times per week. A healthy population sample was recruited to mitigate the risk of age or disease-related influences on the primary outcomes. Participants were excluded if they were pregnant, were taking medications known to affect cardiovascular function, or reported smoking. Since mental stress was the primary exposure variable, participants were also excluded if they had a diagnosed mental health condition or were currently taking medication for a mental health condition.

Experimental Design and Procedures

This was a randomized cross-over trial in which cardiometabolic measures were assessed before and after exposure to an acute mental stressor and a time-control condition. The order of the experimental and control visits was randomized using an online number randomizer (www.randomizer.org). Participants first attended a familiarization visit in which they were familiarized with the equipment and devices including the metabolic canopy. They

also completed the Perceived Stress Scale[198] during the familiarization visit to characterize their general stress levels over the past month. Following the familiarization visit, all measurements were collected on experimental and control visits separated by 2-7 days. For both visits, participants arrived the laboratory having abstained from i) consuming food and drink other than water for 8 hours prior, ii) vigorous physical activity for 24 hours prior, and iii) alcohol consumption 24 hours prior to the visit.

Upon arrival at the laboratory for each visit, hydration status was tested using a refractometer (specific gravity $\leq$ 1.0025[199]) (10440, American Optical Corp, Keene, NH), body mass and lean mass were assessed using bioelectrical impedance (InBody USA, Cerritos, CA). If participants did not meet the hydration cut point, they were given 240 grams (1 cup) of water to drink. Participants then underwent a venipuncture in the antecubital vein of the arm of their choosing. A vacutainer and needle were used to collect 10 ml of blood, which was centrifuged for serum extraction, and placed in a -80° freezer for future analysis of inflammatory cytokines. Next, participants were then fitted with a wireless physiological monitoring vest for assessment of heart rate variability (HRV), respiration rate, and galvanic skin response. Participants were then positioned in a semi-reclining 30° position and were fitted with oscillometeric cuffs for pulse-wave velocity (PWV) and pulse wave analysis (PWA) measurements. Next, for indirect calorimetry EE measurement, a ventilated canopy was placed over the participant's head and tucked around the upper torso to minimize air penetration. Following 30 minutes of quiet rest, baseline PWV and PWA measurements were taken simultaneously.

After baseline measurements, an 11-minute laboratory stressor – the Trier Social Stress Test (TSST), or a neutral condition, was administered. PWV and PWA measurements were

repeated immediately (Time 0), and 5, 10, 15, 30, 45, and 60 minutes following the TSST/control condition. A 1-10 Likert Scale was administered immediately before and after the TSST/control conditions to assess present-moment perceived stress[200]. For PWV and PWA, semi-automated measurements were made in triplicate at each timepoint, with the average of the closest two measures used for analysis. Indirect calorimetry and wireless physiological measurement vest data were collected continuously from the beginning of the rest period until 60 minutes following the TSST. The entire testing period was conducted with the participant in the semi-reclined 30° position so as to maximize internal validity of the physiological measurements, while also ensuring ample external validity of the TSST, which includes social interactions and is normally completed in a seated. A schematic outlining the timeline of the study procedures is shown in in **Figure 6.1**.

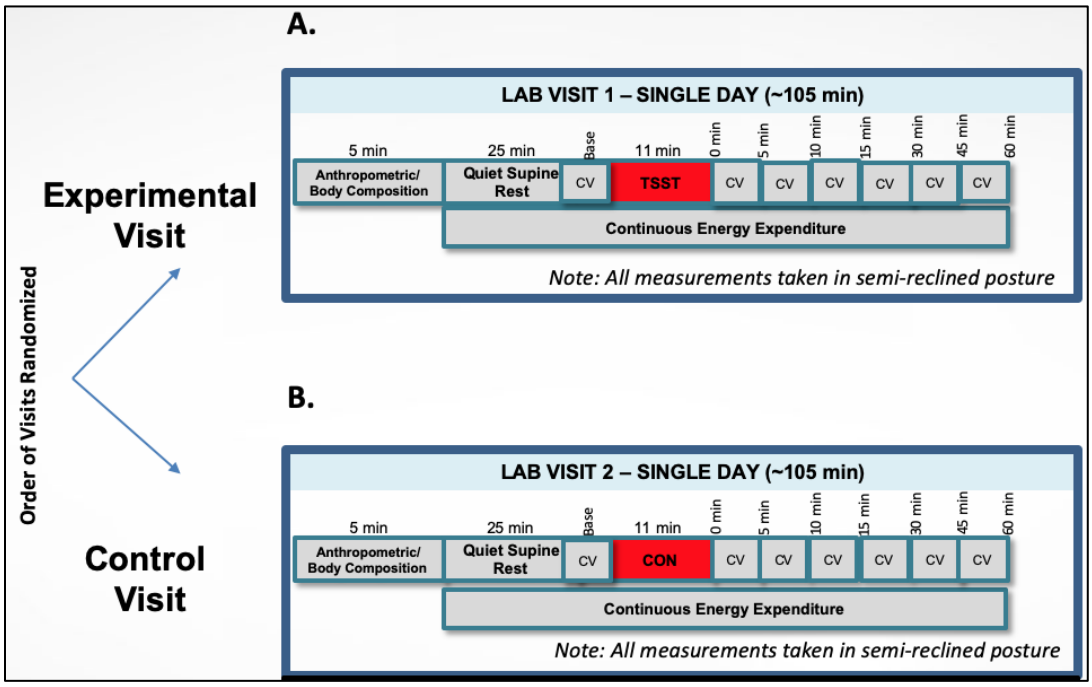

Figure 6.1. Experimental study diagram. A. Experimental and B. Control Visits. Abbreviations*: Con, Control condition; CV, Cardiovascular Measurements (i.e. pulse-wave velocity, pulse wave analysis); Min, Minute; TSST, Trier Social Stress Test.*

Primary Outcomes

Pulse-Wave Velocity – Arterial Stiffness

For assessment of arterial stiffness, the Vicorder (SMT Medical, Wuerzburg, Germany) was used to measure brachial-femoral PWV (bfPWV). Per device guidelines, cuffs were placed around the right arm over the brachial artery and around the right thigh over the femoral artery as high up on the leg as possible. PWV (m/s) was calculated by dividing arterial path length by the pulse transit time. The distance used was the distance between the suprasternal notch and the umbilicus. To measure pulse transit time, the two cuffs were simultaneously inflated to a low (~50 mmHg) pressure, and a proprietary algorithm calculated the time between the foot of the proximal and distal pressure waveforms. This method has excellent between-day reliability (ICC: 0.98), and has been validated against the non-invasive gold-standard cfPWV as well as magnetic-resonance based AS measures[146–148].

Indirect Calorimetry – Energy Expenditure

Indirect calorimetry using the dilution method (TrueOne 2400 Canopy System, ParvoMedics, Inc., Sandy, UT) was used to assess energy expenditure (kcal/kg/min) and was calibrated prior to each use. Data points were obtained continuously (breath-by-breath analysis) and was smoothed by averaging continuous 15-second segments of data. The first 5 minutes of data was excluded from data analysis because $\geq$ 5 minutes are needed to achieve steady-state metabolism, and to allow breathing and dilution rate to normalize[153]. Dilution rate is normally adjusted during the first five minutes of the test so that the fraction of expired carbon dioxide is between 1.0 and 1.2%. However, following consultation with the manufacturers, we adjusted the dilution so that the fraction of expired carbon dioxide during

the first five minutes was targeted at 0.9. This protocol modification was necessary to account for the increased expelling of carbon dioxide which would be expected with the participants exposure to the lab stressor. The baseline period used for mixed model analysis was the 10-minute period (within the first 30 minutes of indirect calorimetry measurement) with the least variance in the fraction of expired carbon dioxide, oxygen consumption, minute ventilation, and respiratory exchange ratio. EE was also assessed using the trapezoidal method for area under the curve (AUC). With this analysis, the first 10 minutes of data were removed, and AUC was calculated continuously until 60 minutes following the stressor/control condition. Energy expenditure measurements, including those obtained in our lab, demonstrate excellent test-retest reliability (ICC: 0.94), with a standard error of measurement of 125.6 kcal/day, and a mean detectable difference of 244.3 kcal/day[153].

Secondary Outcomes and Control Measures

Pulse-Wave Analysis – Central Hemodynamics

Central BP (cSBP, cDBP), peripheral BP (SBP, DBP), systolic wave reflection (systolic augmentation index) (sAI), and pulse rate were measured using the BP+ device (USCOM BP+, Sydney, Australia). Measurements were taken on the left upper arm following standard manufacturer guidelines.[201] Each measurement cycle was approximately 40 seconds, consisting of a brachial blood pressure recording then a 10-second suprasystolic recording. A corresponding aortic pressure waveform was generated using a validated transfer function which estimated cSBP. Arterial wave reflection was calculated from the suprasystolic waveform using the formula: SAI = (P3-P0)/(P1-P0), where P0 is the pressure the pulse onset, P1 is the highest pressure at the incident wave, and P3 is the highest pressure of the reflected wave.

Oscillometric-based between-day reliability for cSBP is high (intraclass correlation coefficient: 0.90), and both cSBP (r=0.94) and sAI (r=0.71) have also been validated against gold-standard tonometry[202, 203].

Equivital – Autonomic Measures

Autonomic data consisted of heart rate variability (HRV), respiration rate, and galvanic skin response (GSR; assessment of sympathetic skin conductivity), which were assessed using the Equivital wireless physiological monitoring system (Equivital Inc, New York, NY) and analyzed in ADInstruments Labchart Labchart software (ADInstruments, Colorado Springs, CO). Data was measured continuously. Ten 5-minute segments of the data were averaged and subsequently used for analysis at the following timepoints: i) baseline (prior to start of experimental/control condition), ii) speech task (part 1 of condition), iii) arithmetic task (part 2 of condition), iv) immediately (0-5 minutes) after condition, v) 5-10 minutes after condition, vi) 10-15 minutes after condition, vii) 15-20 minutes after condition, viii) 25-30 minutes after condition, ix) 40-45 minutes after condition, and x) 55-60 minutes after condition. For measurements viii-x, data selections for analysis were chosen such that they occurred at times when the PWV and PWA devices were not inflating in order to reduce the autonomic data to be impacted by the inflations. Data was collected at the following sample rates: electrocardiogram (256 HZ), respiration rate (25.6 HZ), GSR (8 HZ). For HRV settings, the R-R interval threshold was set to 500 to 1600 ms and ectopic beats were excluded. Measures of HRV included standard deviation of R-R intervals (SDRR), the root-mean-square of successive differences of R-R intervals (RMSSD) and the low-frequency (LF) power, high-frequency (HF) power, and frequency power ratio (LF/HF). Based on visual inspection of Q-Q plots, the frequency-domain variables

(LF, HF, LF/HF) had non-uniformity of error. Thus, we applied a natural logarithm for analyses of the frequency-domain data.

Actigraph - Accelerometry

Prior to participating in the laboratory experiments, participants wore an accelerometer (Actigraph GT3X+, Pensacola, FL, USA) to characterize their typical physical activity levels. The accelerometers were worn on their non-dominant wrist during an 8-day free living period. Using NHANES protocols[204], Hildebrand (in)activity cutoff points[205, 206], and the GGIR package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria) participants' daily average minutes spent in vigorous, moderate, light and (in)-activity were calculated. Epoch length was set at 5 seconds, non-wear detection resolution at 15 mins, and the non-wear detection window at 60 minutes.

Experimental Condition: Trier Social Stress Test

The TSST, a short battery of mental stressors (speech task + mental arithmetic test) which has been shown to elicit a robust CV response [27, 28], was administered on the experimental visit. The TSST is a reliable and valid acute stressor that can induce a robust physiological response[32]. A modified version of the TSST was used in which the normally 3-minute preparatory period (prior to speech task starting) was shortened to 2 minutes due to the long duration of the entire test period as well as the added stress of having a metabolic canopy covering their body during exposure to the TSST. Additionally, participants typically use the 3-minute preparatory period to write down thoughts on a piece of paper to prepare for the speech task[32]. However, since participants were administered the TSST during the same time as the EE measurement, they were not able to write notes since their arms and hands were

sealed under the ventilated canopy. Following the 2-minute preparatory period, participants were instructed to think of their ideal job and explain to the TSST administrator (someone who unfamiliar to participants) why they were "the best candidate for their ideal job" in five minutes. Next, participants were given a surprise 5-minute mental arithmetic task and told that their responses would be monitored and compared to the norm. At the beginning of each minute of the 5-minute arithmetic task, participants were told to subtract the number 7 or 13 (alternated at each minute) from a new 4-digit number. Participants were also asked to answer at the pace of a metronome, which was surreptitiously increased for each minute of the test (min 1: 12 bpm; min 2: 14 bpm; min 3: 16 bpm; min 4: 18 bpm; min 5: 20 bpm). Other key aspects of the TSST included i) use of a video-camera that was not recording, but made to look like it was recording, ii) shining a bright light towards the subject, and iii) having the TSST administrator and 1-2 assistants wear white lab coats during TSST administration. Additional details of the TSST are reported in a comprehensive 2017 review by Allen et al.[32].

Control Condition: Neutral Task

For the control visit, a neutral task similar in structure to the TSST was administered. The protocol was time-matched with the TSST and included similar components consisting of a 2-minute preparatory period, a 5-minute speech period, and a 5-minute arithmetic period. However, participants were told that their responses would not be critiqued or compared to other scores. For the speech period, participants were instructed to "talk about a boring or neutral activity such as sitting in a dull lecture, waiting for the bus, or sitting in a waiting room." For the math period, participants were instructed to count over and over again from 1-10 at a slow, calm pace.

Sample Size

Sample size calculations were made using GPower 3.1.9. Prior studies have reported increases in PWV ranging from 0.29-0.57 $\pm$ 0.2-1.06 m/s[20, 94]. Based on the moderate effect seen in these studies, we expected to observe a similar moderate effect in the current study. With a moderate effect size (d=0.5) , an alpha level set at 0.05, the power set at 0.8, 8 measurement time points and a moderate (0.5) correlation among repeated measures, the sample size was computed to be 16[187]. We inflated the sample to 23 participants to account for attrition and the chance of poor or incomplete data.

Statistical Analysis

Statistical analyses were performed using Jamovi (2022, Version 2.3.21.0) for linear mixed models and the pkr package for R (R Foundation for Statistical Computing, Vienna, Austria) for area under the curve analyses (AUC). The significance level was set *a priori* for all statistical procedures at alpha = 0.05, however interactions between group and time were considered significant at the alpha < 0.1 level because of the increased power needed to detect interactions. Raw data are presented as mean (SD) and mixed model data are presented as mean (95% CI). Effect sizes(ES) were calculated as Cohen's *d* where $\leq$ 0.2, 0.2-0.3, 0.4-0.8, and $\geq$ 0.8 were defined as trivial, small, moderate, and large, respectively[187]. The corresponding author (GZ) had full access to the data in the study and was responsible for the integrity of the data set and the data analysis. Based on the cross-over study design of the experimental studies, linear mixed models with random effects were used to test the effects of the TSST and control conditions on PWV and EE. Effects were specified as random for time (slope) and subject (intercept), and fixed for condition. The models were adjusted for baseline values as

specified by Kenward and Roger[207]. EE data was also tested using the trapezoidal method for AUC. Paired samples T-tests with MD and 95% CI were used to test differences in AUC between conditions. PWV and EE were also corrected for mean arterial pressure and lean muscle mass due to the pressure-dependency of arterial stiffness [145, 208] and the influence of body composition on metabolic rate[153], respectively.

Results

Participants

Twenty-three participants were recruited, of which 20 [21.0 (2.5) years, 55% female, 23.6 (4.0) kg/m^2)] completed the study. Participants identified as white (n=13) and non-white (n=7; 5 Asian, 2 Black). The three participants that dropped out attributed their attrition to exam stress and discomfort with the blood draw (n=1) and to discomfort associated with the indirect calorimetry device (n=2). The CONSORT diagram is presented in **Supplement Figure 6S.1.**

Baseline Data

Table 6.1 displays demographics and participant characteristics.

Table 6.1. Participant characteristics.

	Mean/n	SD/%
Biological Sex (M/F)	9 M/11F	45% M/55% F
Age (years)	21.0	2.5
Height (cm)	167.1	8.4
Weight (kg)	65.7	12.3
BMI (kg/m^2)	23.5	4.0
% Body Fat	24.3	7.8
Perceived Stress (PSS Score)	14.2	4.7
Activity Status		
MVPA (min/day)	77.5	30.6
VPA (min/day)	1.7	2.6
MPA (min/day)	75.8	29.1
LPA (min/day)	175.1	79.0
Sedentary (hrs/day)	11.9	1.4

Abbreviations: *cm, Centimeters; F, Female; hrs, hours; kg, kilograms; LPA, light physical activity, M, Male; m, meters; MPA, moderate physical activity; MVPA, moderate-vigorous physical activity; PSS, Perceived Stress Scale, VPA, vigorous physical activity*

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Control Measures

Acute Changes in Perceived Stress

Data from the 1-10 Likert Scale (administered immediately before and immediately after the experimental and control conditions) indicated that the experimental condition (mean difference: 3.4 ($p<0.001$), but not the control condition (mean difference: 0.3; $p=0.19$), resulted in a significant increase in perceived stress.

Cardiovascular Outcomes

Primary Cardiovascular Outcome: Arterial Stiffness

Arterial stiffness PWV measures adjusted for mean arterial pressure are presented in **Table 6.2** and **Figure 6.2.** There was a small interaction effect (B=0.68 m/s, 95%CI: 0.39, 0.97,

d=0.30) of condition and time. Compared to the control condition [↑0.15 m/s (2%)], the experimental condition induced a greater increase in PWV [↑0.81 m/s (13%)] across time.

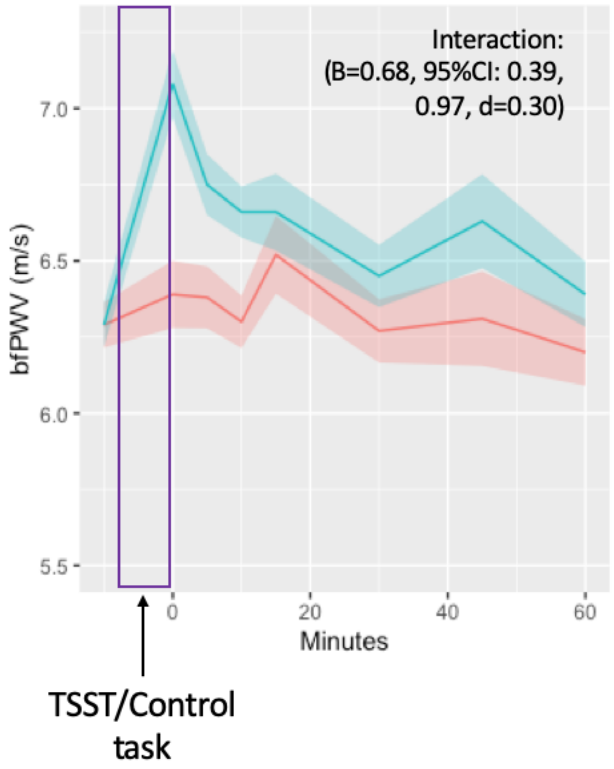

Figure 6.2. Pulse-wave velocity before and after condition exposure. Control (red line) and experimental (green line) condition responses are shown. Plotted data are the estimated marginal means from the linear mixed models with the shaded region representing the standard error. Eight measurement timepoints were at -10 minutes (pre-condition), and at 0, 5, 10, 15, 30, 45, and 60 minutes post-condition. Abbreviations**:** *bfPWV, brachial-femoral pulse-wave velocity; m/s, meters per second; TSST, Trier Social Stress Test.*

Secondary Cardiovascular Outcomes: Blood Pressure and Hemodynamics

BP and central hemodynamic measures are presented in **Table 6.2.** There were significant interaction effects (ranging from trivial to moderate in terms of effect sizes) of condition and time for SBP (B=9.34 mmHg, 95%CI: 6.09, 12.60, d=0.36), DBP (B=3.09 mmHg, 95%CI: 0.41, 5.78, d=0.15), cSBP (B=6.89 mmHg, 95%CI: 3.68, 10.10, d=0.27), and cDBP (B=4.97 mmHg, 95%CI: 2.17, 7.77, d=0.23), with the greatest change between conditions occurring from

pre- to immediately post-condition exposure. There was a moderate main effect of condition for sPR (B=3.35 bpm, 95%CI: 2.37, 4.34, d=0.43). No significant main or interaction effects were observed for sAI ($p>0.05$).

Table 6.2. Cardiovascular results. Pre and immediately post-condition data, as well as results from the linear mixed model are shown.

		bfPWV m/s	SBP mmHg	DBP mmHg	cSBP mmHg	cDBP mmHg	sAI %	sPR bpm
Means								
CON	PRE	6.29	107.00	64.00	98.20	65.20	45.80	60.30
	POST	6.44	108.00	62.90	100.00	66.00	48.60	62.00
EXP	PRE	6.28	105.00	65.50	96.90	63.20	45.30	62.60
	POST	7.09	116.00	67.30	106.00	68.90	45.40	68.40
Standard Deviations								
CON	PRE	0.90	7.98	5.98	7.07	5.98	13.70	7.74
	POST	0.85	9.81	7.01	9.00	7.29	15.20	7.29
EXP	PRE	1.04	8.90	5.27	7.30	6.09	17.20	9.82
	POST	1.37	8.80	6.33	7.46	6.94	15.00	11.20
Condition Effect								
	β	0.28	1.93	0.91	1.28	0.98	-1.16	3.35
	P	<0.001	<0.001	0.009	0.002	0.007	0.218	<0.001
	ES	0.48	0.30	0.17	0.20	0.18	-0.08	0.43
	LCI	0.21	1.11	0.24	0.47	0.27	-3.00	2.37
	UCI	0.36	2.75	1.59	2.08	1.68	0.68	4.34
Time Effect								
	β	0.44	6.10	2.87	5.44	3.23	1.54	3.64
	P	<0.001	<0.001	0.001	<0.001	<0.001	0.14	0.049
	ES	0.26	0.44	0.23	0.39	0.23	0.05	0.23
	LCI	0.23	4.35	1.32	3.66	1.48	-2.16	1.60
	UCI	0.66	7.86	4.43	7.21	4.97	5.23	5.67
Interaction Effect								
	β	0.68	9.34	3.09	6.89	4.97	-2.90	4.17
	P	<0.001	<0.001	<0.001	<0.001	<0.001	0.753	0.249
	ES	0.30	0.36	0.15	0.27	0.23	-0.05	0.13
	LCI	0.39	6.09	0.41	3.68	2.17	-10.21	0.24
	UCI	0.97	12.60	5.78	10.10	7.77	4.42	8.11

Abbreviations*: B, beta coefficient; Con, control; cDBP, central diastolic blood pressure; cSBP, central systolic blood pressure; DBP, diastolic blood pressure; ES, effect size; EXP, experimental; LCI, lower confidence interval; p, p-value; bfPWV, pulse-wave velocity; sAI, systolic augmentation index, SBP, systolic blood pressure; sPR, systolic pulse rate; UCI, upper confidence interval.*

Energy Outcomes

Primary Energy Outcome: Energy Expenditure

Energy expenditure measures adjusted for lean muscle mass are presented in **Table 6.3.** With the linear mixed models, there was a small condition x time interaction (B=0.0010 kcal/kg/min, 95%CI: 0.0004, 0.0015, d=0.31) with the greatest change in EE between conditions occurring from pre-condition to during-condition. Specifically, the experimental condition caused a greater increase in EE [↑0.0016 kcal/kg/min (10%)] compared to the control condition [↑0.0005 kcal/kg/min (3%)].

With the AUC analysis, the EE in the experimental condition was not significantly different than the control condition (mean difference: 0.06 kcal/kg, 95%CI: -0.13, 0.02, p=0.12, d=0.29). Post-Hoc analyses showed that when the AUC analyses was limited only to the baseline period and the first 35 minutes (rather than full 60 min post-condition period), the difference in EE responses between the experimental and control conditions (experimental had higher EE response than control) approached significance (mean difference: 0.04 kcal/kg, 95%CI: -0.08, 0.001, p=0.05). EE AUC responses averaged across participants for the experimental and control conditions are displayed in **Figure 6.3**.

Secondary Energy-Related Measures

Secondary energy-related measures are shown in **Table 6.3**. For relative VO_2, METS, and RQ, there were time x condition interactions with the greatest changes in measurements occurring from pre-condition to during-condition. Effect sizes were trivial for relative VO_2 (B=0.23 ml/kg/min, 95%CI: 0.02. 0.43, d=0.17) and METS (B=0.06, 95%CI: 0.003, 0.120, d=0.17), and moderate for RQ (B=0.06, 95%CI: 0.04, 0.08, d=0.44). Ancillary energy-related measures

(fractional content of carbon dioxide, fractional content of oxygen; resting energy expenditure; carbon dioxide production, minute ventilation, oxygen consumption) are presented in **Supplement Table 6S.1.**

Table 6.3. Energy results. Pre and during stress data are presented, as well as the analysis from the linear mixed models.

		EE kcal/kg/min	VO_2/kg ml/kg/min	METS	RQ
Means					
CON	PRE	0.0150	3.15	0.90	0.77
	DURING	0.0155	3.26	0.93	0.80
EXP	PRE	0.0153	3.21	0.92	0.77
	DURING	0.0169	3.54	1.01	0.86
Standard Deviations					
CON	PRE	0.0022	0.47	0.13	0.08
	DURING	0.0025	0.52	0.15	0.07
EXP	PRE	0.0025	0.52	0.15	0.07
	DURING	0.0031	0.64	0.18	0.07
Condition Effect					
	β	0.00	0.15	0.04	0.00
	P	<0.001	<0.001	0.034	0.96
	ES	0.03	0.37	0.37	0.00
	LCI	0.00	0.08	0.02	-0.01
	UCI	0.00	0.21	0.06	0.01
Time Effect					
	β	0.00	0.22	0.06	0.06
	P	<0.001	0.002	0.002	<0.001
	ES	0.42	0.31	0.31	0.44
	LCI	0.00	0.11	0.03	0.04
	UCI	0.00	0.33	0.09	0.08
Interaction Effect					
	β	0.00	0.23	0.06	0.06
	P	<.001	0.034	0.034	<0.001
	ES	0.31	0.17	0.17	0.24
	LCI	0.00	0.02	0.00	0.02
	UCI	0.00	0.43	0.12	0.09

Abbreviations: *B, beta coefficient; Con, control; EE, energy expenditure; ES, effect size; EXP, experimental; LCI, lower confidence interval; METS, metabolic equivalents; RQ, respiratory quotient; p, p-value; UCI, upper confidence interval; VO_2/kg, relative oxygen consumption*.

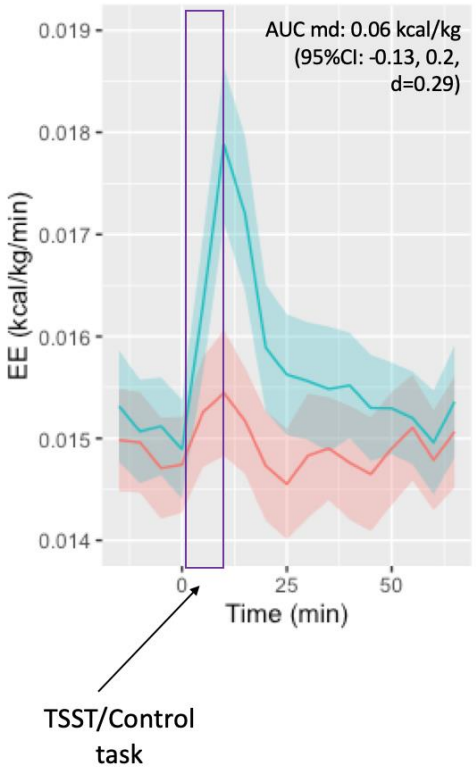

Figure 6.3. The area under the curve for the energy expenditure response. Data is averaged across participants for the control (red line) and experimental (green line) condition. Abbreviations**:** *95% CI, 95% confidence interval; AUC, area under the curve; EE, energy expenditure; kcal, kilocalories; kg, kilograms; md, mean difference; min, minute; TSST, Trier Social Stress Test.*

Heart Rate Variability, Galvanic Skin Response, and Respiration Rate

There were time x condition interaction effects for heart rate (B=12.98 bpm, 95%CI:8.78, 17.19, d=0.34), GSR (B=0.65 uS, 95%CI: 0.10, 1.20, d=0.13), and respiration rate (B=-0.51 BrPM, 95%CI: -1.97, 0.95, d=-0.04). There were main effects of both condition and time for RMSSD, SDRR, LF, and HF (all $p<0.05$). For LF/HF, there was a main effect of time ($p<0.05$). Full analyses from the mixed models for HRV, GSR, and respiration rate are reported in **Supplement Table 6S.2.**

Discussion

Chronic mental stress is a contributor to CVD risk[1]. However, the mechanistic underpinnings of this relationship are poorly understood. This study investigated the energetic and arterial stiffness responses to an acute mental stressor. Findings suggest that, compared to a neutral control condition, acute mental stress causes immediate increases in arterial stiffness and energy expenditure – responses that may be implicated in the relationship between repeated exposures to mental stress and CVD risk.

Limitations and Strengths

Limitations and strengths are provided to help contextualize findings. First, a relatively homogenous sample of young, healthy adults was recruited, which limits generalizability to other populations (e.g. different ages and health/disease statuses). However, it is first imperative to study the biological responses to mental stress in a sample which limits the potential for confounding of age and disease processes that strongly influence cardiovascular and metabolic physiology. Second, the current study was not powered to investigate effects of biological sex. Future research is warranted to investigate potential sex differences in the vascular and energetic response to acute mental stress. Third, the TSST was not administered by the same individuals for each experimental condition. However, the same two individuals were the lead administrators of the TSST and they administered the stressor using the same script. Major strengths of the study were the development and implementation of a time-matched neutral, control condition, as well as the use of sensitive measurements of cardiovascular and metabolic constructs that may be implicated in stress-related CVD risk.

Comparison to Literature

Arterial Stiffness

Exposure to acute mental stress increased bfPWV by 0.81 m/s (95%CI: 0.03, 1.59, d=0.67) in the time immediately following the TTST. These findings are in line with several other studies suggesting acute mental stress negatively impacts vascular parameters including arterial stiffness[20, 89, 90, 94–96]. In several controlled studies, Vlachopolous et al. reported that among healthy adults, an acute laboratory stressor increased the gold-standard carotid-femoral PWV (cfPWV) by 0.29-0.57 m/s. We assessed PWV using bfPWV. The greater increase in the current study compared to those by Vlachopolous et al. may be partially attributed to the bfPWV pathway, which captures both the proximal aorta and muscular arteries (subclavian, brachial). The proximal aorta appears to be more impacted by advancing age and disease status than the arteries in the cfPWV pathway, and thus may be more sensitive to acute perturbations or early changes in CVD-related pathophysiology[20]. In addition to the advantages of the inclusion of both elastic and muscular artery components of its path-length, bfPWV also does not necessitate applanation of the carotid artery. Carotid applanation is fairly intrusive and may promote discomfort and distress, which could confound measurements – particularly in studies seeking to investigate the physiological responses to acute mental stress. On a more practical level, assessment of bfPWV also allowed for the simultaneous indirect calorimetry measurement of EE under the ventilated canopy, which would not have been possible if carotid applanation was needed (e.g. with cfPWV measurement). Since seated/reclining positions generally result in higher measurements of cardiovascular parameters compared to supine positions due to the added challenge that gravity poses to the system (e.g. in terms of

maintaining venous return)[144, 194], another possible reason for the greater PWV values in the current study was the semi-recumbent posture of the participants. However, compared to a supine position, the semi-recumbent posture enabled a more ecologically valid mental stressor to be administered.

Physiologically, acute mental stress may elevate arterial stiffness via both central (autonomic and local (endothelial) systems [91, 193]. In terms of endothelial function, prior studies – including by our group[137] – have demonstrated that PWV is partially driven by endothelial function, and that endothelial function is impaired following acute stress exposure[91]. In terms of autonomic function, increases in arterial stiffness in response to acute mental stress are likely due to elevations in sympathetic, and tempering of parasympathetic, branches of the autonomic nervous system[3, 6]. Indeed, the observed time x condition interactions in autonomic variables GSR, HR, and respiration rate do suggest that autonomic modulation may have contributed to the increases in arterial stiffness Additionally, our prior work has demonstrated that PWV is influenced by autonomic-mediated changes in BP[145, 194]. In the current study we adjusted for simultaneous changes in BP to better reflect pressure-independent, alterations in intrinsic stiffness properties. As such, our data support the notion that acute stress-related arterial stiffening is likely driven by both central (autonomic) and local (endothelial) mechanisms. Our study further extends the literature supporting multi-system reactivity to acute stress by demonstrating stress-related changes in autonomic (HRV, GSR, respiration rate) and metabolic function including EE, which will be discussed in the following section.

Energy Expenditure

The current study demonstrated significant increases in EE with acute mental stress exposure compared to a neutral control condition. Few studies have directly assessed EE in response to an acute stressor. The limited studies that have assessed EE in response to mental stress have shown increases in EE ranging from 7-28% in both clinical and healthy populations [13–16]. These data are in line with findings from the current study which showed that acute mental stress caused a 10.5% increase in EE. The considerably greater EE in several of these studies compared to the current study may be partially attributable to differing methodologies. For example, Seematter et al. used a venous hyperinsulinemic clamp to investigate gluco-regulatory function in response to stress which exposed participants to an additional stressor/challenge [15]. Others used laboratory stressors that were longer in duration (e.g. 15-30 minutes rather than 11 minutes), utilized a predominantly cognitive (e.g. color Stroop task) rather than psychosocial stressor [14–16], and/or used an all-male [16] or small (e.g. $n \leq 10$) [13, 14] sample. The existing studies were published in the early 2000s and the majority [13–15] also failed to report EE results that accounted for body mass or composition which may have inflated EE changes since these variables strongly impact metabolic rate[153]. In addition to accounting for body composition, the current study extends the current literature by demonstrating stress-related increases in EE compared to a neutral control task. This is especially important because, independent of whether perceived stress is elevated, EE would be expected to increase simply by the act of engaging in a social or cognitive task due to increases in neural (e.g. executive) demand and muscular (e.g. speaking) activity.

Physiologically, EE has long been known to be related to a number of metabolic markers that are strongly related to CVD risk including glucose and lipid regulation[99][209]. More recently, the constrained energy theory has postulated that stress-related EE may contribute to CVD risk by increasing inflammation[127, 210]. More specifically, the theory postulates that while total daily EE may be somewhat hard-wired and genetically pre-determined, allocations of energy may be partitioned/dispersed differently depending on lifestyle factors such as physical activity and exercise[126]. Collectively, we extend the literature by complementing more traditional markers of post-stress cardiovascular function (e.g. PWV, PWA) with assessments of stress-induced EE to gain a more comprehensive understanding of the effects of acute stress on global cardiovascular and metabolic function. While the current study did not directly test each of the biomarkers and causal pathways in **Figure 6.4**, findings do highlight that the variables shown in this figure may be involved in the relationship between repeated exposures to acute mental stress and CVD risk over time. Specifically, **Figure 6.4** illustrates the putative biological framework linking acute stress-related increases in arterial stiffness and energy expenditure to CVD risk.

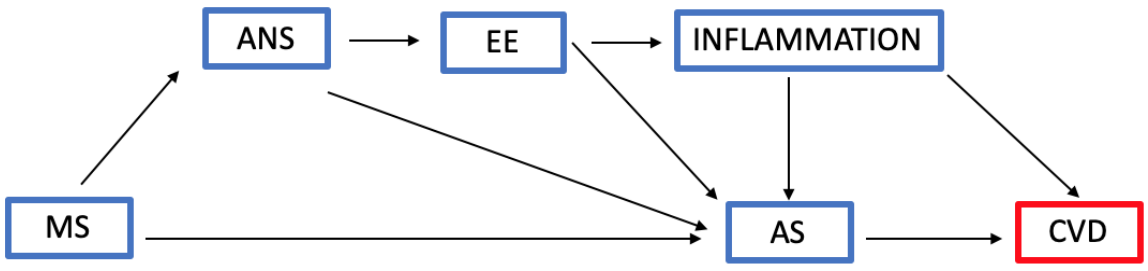

Figure 6.4. Conceptual framework using a directed acyclic graph (DAG) for plausible pathway linking repeated/prolonged acute mental stress exposure and CVD risk over time.
Abbreviations: *ANS, autonomic nervous system; AS, arterial stiffness; CVD, cardiovascular disease; EE, energy expenditure; MS, mental stress (acute).*

Implications

Mental stress is an unavoidable aspect of the human experience. However, compared to lifestyle factors such as physical activity and exercise, mental stress is understudied in the context of modifiable CVD risk. A better understanding of how repeated or prolonged exposure to acute stress leads to CVD risk is necessary to pave the way for optimal stress-related CVD prevention and intervention strategies. Our findings indicate that the cardiovascular and metabolic systems work in tandem to respond to acute mental stress. The significant arterial stiffening and energetic responses observed in the current study suggest that these biomarkers may be important stress-CVD mediators, and should be explored further in larger, less homogenous samples. From a CVD risk reduction standpoint, stress-related CVD risk could arguably be reduced through three primary strategies: i) reduce stress exposure, ii) improve psychological and/or biological resilience in the face of a given stressor, and/or iii) - as alluded to in the brief discussion of the constrained energy theory above – elevate physical activity levels to re-allocate energy resources away from stress-reactivity and subsequent inflammation which may contribute to CVD risk[197, 211]. While this study does not directly address the constrained energy theory, it does set the stage for future inquiries into the potential moderating effects of physical activity and exercise on multi-system cardiometabolic stress reactivity and associated CVD risk.

Conclusion

Compared to a neutral control condition, exposure to acute mental stress caused significant small effect size increases in arterial stiffness and energy expenditure, which may be important markers in stress-mediated CVD pathophysiology. Better understanding the

pathophysiological chain of events implicated in the stress-CVD paradigm will be necessary to inform stress-related CVD prevention and treatment efforts. Future work is warranted to further explore the mechanisms, and interactions with other key lifestyle factors that may be involved in the relationship between repeated or prolonged mental stress exposure and elevated CVD risk.

Experiment 1 Supplement

Table 6S.1. Ancillary energy data. Pre and during stress data are presented, as well as the analysis from the linear mixed models.

		VO_2 ml/min	VCO_2 ml/min	VE L/min	FEO_2 %	FECO2 %	REE kcal/D
Means							
CON	PRE	207.00	159.00	23.20	19.90	0.87	1415.00
	DURING	214.00	171.00	22.70	19.80	0.95	1473.00
EXP	PRE	206.00	158.00	23.50	19.90	0.87	1410.00
	DURING	227.00	194.00	22.60	19.70	1.11	1583.00
Standard Deviations							
CON	PRE	34.50	24.20	2.92	0.12	0.05	226.00
	DURING	35.50	27.20	2.74	0.13	0.07	235.00
EXP	PRE	39.80	27.60	3.07	0.09	0.05	264.00
	DURING	43.90	38.80	3.02	0.17	0.17	303.00
Condition Effect							
	β	4.32	3.69	0.12	-0.04	0.04	30.07
	P	0.039	0.051	0.594	<0.001	<0.001	0.034
	ES	0.16	0.16	0.04	-0.32	0.38	0.17
	LCI	1.72	0.01	-0.33	-0.05	0.02	2.49
	UCI	12.03	7.38	0.57	-0.02	0.05	57.70
Time Effect							
	β	13.75	23.87	-0.71	-0.12	0.16	115.02
	P	0.001	<0.001	0.045	<0.001	<0.001	<0.001
	ES	0.30	0.47	-0.15	-0.52	0.55	0.36
	LCI	6.70	16.09	-1.47	-0.16	0.12	65.74
	UCI	20.79	31.66	0.05	-0.09	0.21	164.30
Interaction Effect							
	β	14.11	23.90	-0.36	-0.11	0.14	117.08
	P	0.055	<0.001	0.994	<0.00	<0.001	0.009
	ES	0.16	0.16	-0.04	-0.29	0.45	0.20
	LCI	0.45	11.51	-1.87	-0.17	0.09	24.71
	UCI	27.76	36.29	1.15	-0.05	0.19	209.40

Abbreviations: *B, beta coefficient; Con, control; ES, effect size; EXP, experimental; $FECO_2$, fractional content of carbon dioxide; FEO_2, fractional content of oxygen; LCI, lower confidence interval; p, p-value, REE, resting energy expenditure; UCI, upper confidence interval; VCO_2, carbon dioxide production, VE, minute ventilation; VO_2, oxygen consumption.*

Table 6S.2. Mixed model analysis for autonomic nervous system data from the Equivital Wireless Physiological Monitoring System. Frequency-domain measures underwent log transformation due to heteroscedasticity.

	RMSSD	SDRR	LF Log (Hz)	HF Log (Hz)	LF/HF Log (Hz)	GSR (uS)	Resp Rate (BrPM)	HR (bpm)
Condition Effect								
β	-8.22	-6.60	-0.13	-0.16	0.03	0.57	-0.07	6.05
P	<0.001	<0.001	<0.001	<0.001	0.227	<0.001	0.68	<0.001
ES	-0.30	-0.24	-0.31	-0.37	0.07	0.51	-0.02	0.71
LCI	-11.26	-9.61	-0.17	-0.20	-0.02	0.44	-0.40	5.10
UCI	-5.17	-3.58	-0.08	-0.11	0.07	0.69	0.26	7.01
Time Effect								
β	-28.42	-20.58	-0.19	-0.44	0.25	2.18	-1.07	16.50
P	0.002	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001
ES	-0.27	-0.18	-0.12	-0.30	0.23	0.28	-0.09	0.50
LCI	-40.24	-33.42	-0.36	-0.61	0.13	1.30	-2.44	12.80
UCI	-16.59	-7.74	-0.02	-0.28	0.38	3.06	0.31	20.19
Interaction Effect								
β	-8.39	-10.59	-0.22	-0.19	-0.03	0.65	-0.51	12.98
P	0.44	0.12	0.15	0.196	0.056	<0.001	<0.001	<0.001
ES	-0.07	-0.09	-0.12	-0.10	-0.02	0.13	-0.04	0.34
LCI	-21.75	-23.83	-0.42	-0.39	-0.22	0.10	-1.97	8.78
UCI	4.97	2.66	-0.02	0.02	0.17	1.20	0.95	17.19

Abbreviations*: B, beta coefficient; BPM, beats per minute; BrPM, breaths per minute; ES, effect size; GSR, galvanic skin response; HF Log, logarithm transformed high-frequency band; HR, heart rate; LCI, lower confidence interval; LF Log, logarithm transformed low-frequency band; LF/HF Log, logarithm transformed ratio of low: high frequency bands; UCI, upper confidence interval; p, p-value*; RMSSD, root mean square of successive differences between R-R intervals; SDRR, standard deviation of R-R intervals; uS, micro-Siemens.

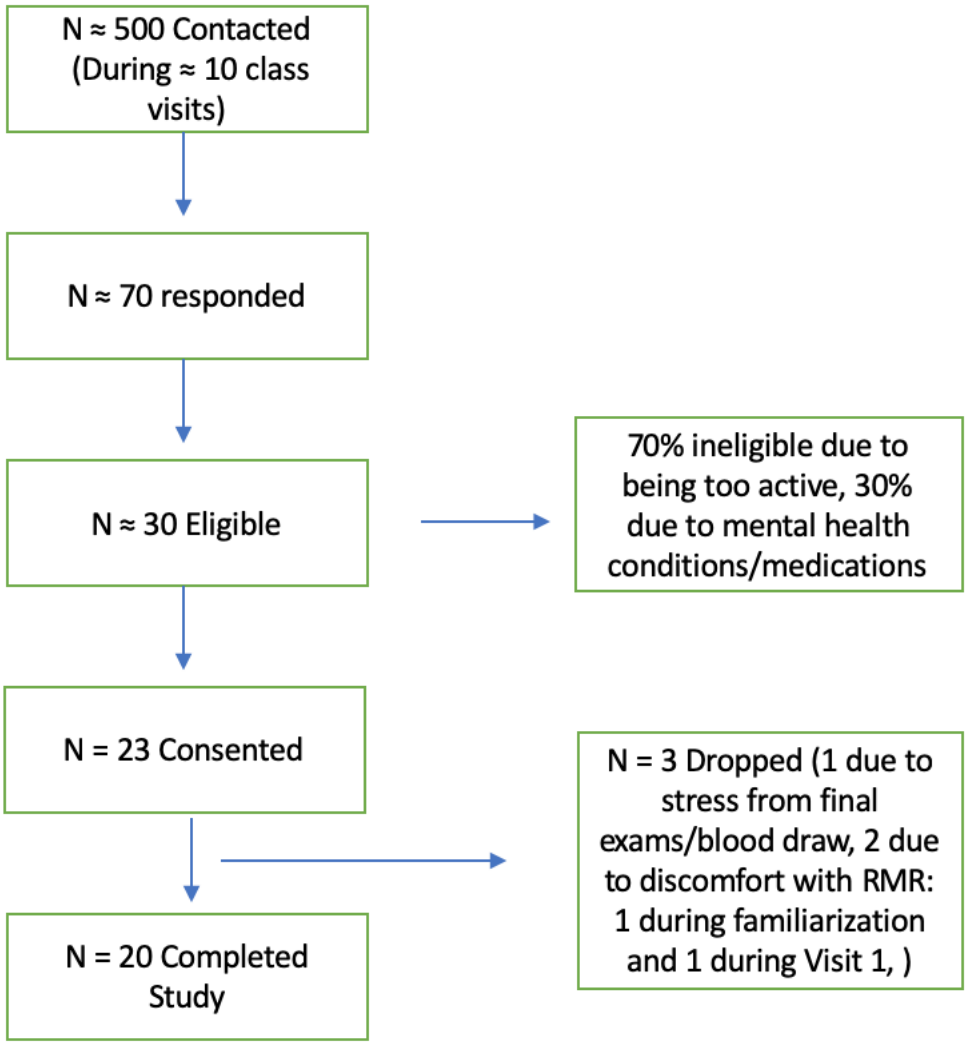

Figure 6S.1. Participant flow-chart. Consolidated Standards of Reporting Trials (CONSORT) diagram shows participant recruitment, attrition, and study completion. Abbreviations**:** *RMR, resting metabolic rate.*

CHAPTER 7: STUDY 3 (EXPERIMENT 2): EFFECT OF PRIOR AEROBIC EXERCISE ON THE ENERGETIC AND ARTERIAL STIFFNESS RESONSES TO ACUTE MENTAL STRESS

Preamble

It was important that this experimental study was conducted following the initial experiment to assess the impacts of a prior aerobic exercise bout on the arterial stiffness and energy expenditure responses to acute mental stress. It was important that this study was conducted separately (e.g. rather than having one study with the same participants completing four conditions: no exercise & stress; no exercise & no stress; exercise & stress; exercise & no stress) to avoid the possibility of a habituation effect with the stress exposure. With the current dissertation and study structure, all participants were only exposed one time to the acute stress condition, whilst still being able to capture a highly similar sample population.

Overview

Background: Aerobic exercise positively modulates autonomic, cardiovascular, and metabolic systems, and may therefore protect against the negative cardiovascular effects mental stress. The purpose of this randomized cross-over trial with a time-matched control was to determine the impact of a prior exercise bout on the energy expenditure and arterial stiffness response to acute mental stress.

Methods: A randomized crossover design was utilized. Twenty healthy young adults [20.0 (1.90) years, 60% female, 24.2 (4.5 kg/m^2)] attended two visits in a randomized order: 25 minutes of moderate aerobic exercise followed by i) an acute stressor or by a ii) a neutral

control task. Energy expenditure and arterial stiffness were assessed before and for the 60 minutes after stress and control conditions. Data was analyzed using linear mixed models.

Results: For pulse-wave velocity, there was a small effect size condition x time interaction (B=0.47 m/s, d=0.23, 95%CI: 0.21, 0.72) with the acute stress condition causing a greater increase in PWV [↑0.43 m/s (7%)] compared to the control condition [↓0.05 m/s (-1%)]. For EE, there were small main effects of condition (B= 0.0005 kcal/kg/min, 95%CI: 0.0003, 0.0008, d=0.33) and time (B=0.0011 kcal/kg/min, 95%CI: 0.0006, 0.0016, d=0.31).

Conclusion: Exposure to post-exercise acute mental stress induced increases in, but still likely dampened arterial stiffness. Energy expenditure did not significantly increase after post-exercise mental stress exposure. Prior aerobic exercise may be a feasible lifestyle-based strategy to off-set stress-related cardiometabolic reactivity and subsequent CVD risk.

Introduction

Mental stress is a ubiquitous aspect of the human experience. The majority of the United States population reports high levels of stress, and the prevalence of high levels of stress is increasing nationwide[53]. While some mental stress is adaptive, excessive exposure has been linked to elevated cardiovascular disease (CVD) risk[1]. Two biologically plausible mechanisms linking repeated exposure to acute mental stress with CVD risk are arterial stiffness and energy expenditure (EE).[2–6]. Arterial stiffness and EE are both strongly governed by the autonomic nervous system and are sensitive markers of cardiovascular and metabolic function including in young, healthy populations[95, 142, 219]. They may also interact with other lifestyle factors to influence the stress-CVD risk relationship[197]. For example, cardiovascular and metabolic systems are positively influenced by modifiable lifestyle factors

including aerobic exercise (AE) [212, 213]. Therefore, AE may help protect against the negative effects of acute exposure to mental stress on cardiovascular disease (CVD) risk by priming the autonomic nervous system and vasculature to dampen stress-related arterial stiffness and energetic responses.

There is substantial evidence demonstrating beneficial effects of AE on both psychological and physiological reactivity to stress [11, 111]. Psychologically, AE has been shown to improve mood and reduce symptoms of stress, anxiety, and depression (including to the same extent as standard pharmacological treatment such as selective serotonin reuptake inhibitors[22, 112–115]. Physiologically, stress-related benefits of AE have been demonstrated, including improved resting and post-acute stress autonomic and vascular function as measured by blood pressure (BP), heart rate (HR), and heart rate variability (HRV)[116–120]. However, fewer studies have assessed the effects of acute exercise on cardiometabolic stress reactivity[25, 214], and no studies that we know of have assessed how prior exercise impacts the effects of stress on arterial stiffness and EE simultaneously. The examination of EE may be a particularly informative marker to examine in the context of acute stress and CVD risk. For example, the constrained energy theory posits that physical activity may re-allocate energy resources away from stress-reactivity and subsequent inflammation which may contribute to CVD risk[197, 211]. Thus, the assessment of arterial stiffness, and especially EE, during post-exercise stress exposure has the potential to both i) better our understanding of biological stress pathways and CVD risk, and ii) inform simple behavioral strategies for stress-related CVD risk prevention.

The purpose of this study was to investigate how mental stress impacts arterial stiffness and EE responses to acute mental stress following a prior aerobic exercise bout. Filling this knowledge gap will improve the mechanistic understanding of how cardiovascular and EE systems respond to post-exercise acute stress, while also helping to inform behavioral strategies for prevention and treatment of stress-related CVD risk. Therefore, this study assessed how a prior bout of moderate-intensity aerobic exercise impacts the arterial stiffness and EE responses to a short psychosocial laboratory stressor in comparison to a neutral non-stress control task.

Methods

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines. Ethical approval was obtained from the University of North Carolina at Chapel Hill Institutional Review Board. All participants provided written informed consent prior to participating in the study.

Participants

Twenty-four young (18-30 years), healthy recreationally active young adults were recruited from the host institution, a large state university. A recreationally active sample was recruited in order to minimize the influences of physical fitness on the primary outcomes, as well as to ensure that the moderate-intensity AE exposure would induce a significant physiological response. Recreationally active was defined as engaging in structured moderate-to-vigorous exercise for at least 30-minutes two times per week. A healthy population sample was recruited to mitigate the risk of age or disease-related influences on the primary outcomes. Participants were excluded if they were pregnant, were taking medications known to affect

cardiovascular function, or reported cigarette smoking. Since mental stress was the primary exposure variable, participants were also excluded if they had a diagnosed mental health condition or were currently taking medication for a mental health condition.

Experimental Design and Procedures

This was a randomized cross-over trial in which, following an aerobic exercise bout, cardiometabolic measures were assessed before and after exposure to an acute mental stressor. Participants first attended a familiarization visit in which they were familiarized with the equipment and devices including the metabolic canopy. They also completed the Perceived Stress Scale[198] during the familiarization visit to characterize their general stress levels over the past month. Following the familiarization visit, all measurements were collected on experimental and control visits separated by 2-7 days. The order of the experimental and control visits was randomized (www.randomizer.org).

On experimental and control visits, participants arrived to the laboratory having abstained from i) consuming food and drink other than water for 8 hours prior, ii) vigorous physical activity for 24 hours prior, and iii) alcohol consumption 24 hours prior to the visit. After ensuring adequate hydration using a refractometer (specific gravity $\leq$ 1.0025[199]) (10440, American Optical Corp, Keene, NH) body weight and lean mass were assessed at each laboratory visit using bioelectrical impedance (InBody USA, Cerritos, CA). If participants did not meet the hydration cut point, they were given 240 grams (1 cup) of water to drink. Participants then underwent a venipuncture in the antecubital vein of the arm of their choosing. A vacutainer and needle were used to collect 10 ml of blood, which was centrifuged for serum extraction, and placed in a -80° freezer for future analysis of inflammatory cytokines. Next,

participant in a semi-reclining 30° posture for 10 minutes of quiet rest. After 10 minutes of quiet rest, Baseline 1 pulse-wave velocity (PWV) and pulse-wave analysis (PWA) measurements were taken. Next, the participants completed 25 minutes of moderate-intensity AE performed on an elliptical machine.

After the aerobic exercise, participants were provided with a mass-dependent (3.63 grams of water per kilogram of body mass) amount of water to drink and were given an opportunity to use the restroom. This quantity of water (≈ 1 cup) was chosen as it was enough to aid in post-exercise rehydration and quench thirst, while also being unlikely to prompt a need to urinate during the subsequent hour of testing. Next, participants were fitted with a wireless physiological monitoring vest for assessment of heart rate variability (HRV), respiration, and galvanic skin response. They then returned to the semi-reclining 30° posture and were fitted with oscillometric cuffs for pulse-wave velocity (PWV) and pulse wave analysis (PWA) measurements. After five minutes of rest, Baseline 2 PWV and PWA measurements were taken. Next, for indirect calorimetry measurement, a metabolic canopy was placed over the participants' head and tucked around the upper torso to minimize air penetration. Following 20 minutes of indirect calorimetry measurement (10 of these minutes were used for baseline EE analysis), Baseline 3 PWV and PWA measurements were taken simultaneously. Next, a 10-minute laboratory stressor – the Trier Social Stress Test was administered. PWV and PWA measurements were repeated immediately (Time 0), and 5, 10, 15, 30, 45, and 60 min after the TSST. For the PWV and PWA, semi-automated PWV and PWA measurements were made in triplicate at each timepoint, with the average of the closest two measures used for analysis. Indirect calorimetry and wireless physiological monitoring vest data were collected

continuously from the beginning of the rest period until 60 minutes following the TSST. The entire testing period was conducted with the participant in the semi-reclined 30° position so as to maximize internal validity of the physiological measurements (validated in a supine position), while also ensuring ample external validity of the TSST, which includes social interactions with test administrators and is typically completed in a seated position (e.g. similar to an academic exam or professional interview context). A schematic outlining the timeline of the study procedures is shown in **Figure 7.1**.

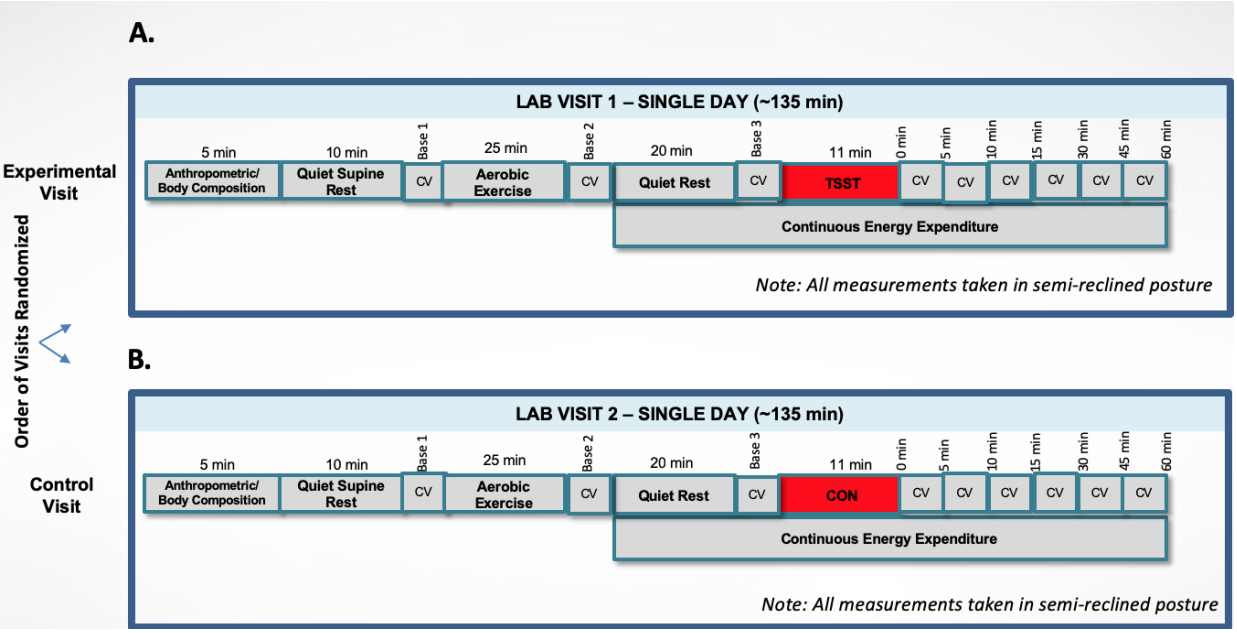

Figure 7.1. Experimental study diagram. A. Experimental and B. Control Visits. Abbreviations*: Con, Control condition; CV, Cardiovascular Measurements (i.e. pulse-wave velocity, pulse wave analysis); Min, minute; TSST, Trier Social Stress Test.*

Primary Outcomes

Pulse-Wave Velocity – Arterial Stiffness

For assessment of arterial stiffness, the Vicorder (SMT Medical, Wuerzburg, Germany) was used to measure brachial-femoral PWV (bfPWV). Per device guidelines, cuffs were placed

around the right arm over the brachial artery and around the right thigh over the femoral artery as high up on the leg as possible. PWV (m/s) was calculated by dividing arterial path length by the pulse transit time. The distance used was the distance between the suprasternal notch and the umbilicus. To measure pulse transit time, the two cuffs were simultaneously inflated to a low (~50 mmHg) pressure, and a proprietary algorithm calculated the time between the foot of the proximal and distal pressure waveforms[146]. This method has excellent between-day reliability (ICC: 0.98), and has been validated against the non-invasive gold-standard cfPWV as well as magnetic-resonance based AS measures[146–148].

Indirect Calorimetry – Energy Expenditure

Indirect calorimetry using the dilution method (TrueOne 2400 Canopy System, ParvoMedics, Inc., Sandy, UT) was used to assess energy expenditure (kcal/kg/min) and was calibrated prior to each use. Data points were obtained continuously (breath-by-breath analysis), and was smoothed by averaging continuous 15-second segments of data. The first 5 minutes of data was excluded from data analysis because $\geq$ 5 minutes are needed to achieve steady-state metabolism, and to allow breathing and dilution rate to normalize[153]. Dilution rate is normally adjusted during the first five minutes of the test so that the fraction of expired carbon dioxide is between 1.0 and 1.2%. However, following consultation with the manufacturers, we adjusted the dilution so that the fraction of expired carbon dioxide during the first five minutes was targeted at 0.9. This protocol modification was necessary to account for the increased expelling of carbon dioxide which would be expected with the participants exposure to the lab stressor. The baseline period used for mixed model analysis was the 10-minute period (within the first 30 minutes of indirect calorimetry measurement) with the least

variance in the fraction of expired carbon dioxide, oxygen consumption, minute ventilation, and respiratory exchange ratio. EE was also assessed using the trapezoidal method for area under the curve (AUC). With this analysis, the first 10 minutes of data were removed, and AUC was calculated continuously until 60 minutes following the stressor/control condition. Energy expenditure measurements, including those obtained in our lab, demonstrate excellent test-retest reliability (ICC: 0.94), with a standard error of measurement of 125.6 kcal/day, and a mean detectable difference of 244.3 kcal/day[153].

Secondary Outcomes and Control Measures

Pulse-Wave Analysis – Central Hemodynamics

Central BP (cSBP, cDBP), peripheral BP (SBP, DBP), systolic wave reflection (systolic augmentation index) (sAI), and pulse rate were measured using the BP+ device (USCOM BP+, Sydney, Australia). Measurements were taken on the left upper arm following standard manufacturer guidelines.[201] Each measurement cycle was approximately 40 seconds, consisting of a brachial blood pressure recording then a 10-second suprasystolic recording. A corresponding aortic pressure waveform was generated using a validated transfer function which estimated cSBP. Arterial wave reflection was calculated from the suprasystolic waveform using the formula: sAI = (P3-P0)/(P1-P0), where P0 is the pressure the pulse onset, P1 is the highest pressure at the incident wave, and P3 is the highest pressure of the reflected wave. Oscillometric-based between-day reliability for cSBP is high (intraclass correlation coefficient: 0.90), and both cSBP (r=0.94) and sAI (r=0.71) have also been validated against gold-standard tonometry[202, 203].

Equivital – Autonomic Measures

Autonomic data consisted of heart rate variability (HRV), respiration rate, and galvanic skin response (GSR), which were assessed using the Equivital wireless physiological monitoring system (Equivital Inc, New York, NY) and analyzed in ADInstruments Labchart Labchart software (ADInstruments, Colorado Springs, CO). Data was measured continuously. Ten 5-minute segments of the data were averaged and subsequently used for analysis at the following timepoints: i) baseline (prior to start of experimental/control condition), ii) speech task (part 1 of condition), iii) arithmetic task (part 2 of condition), iv) immediately (0-5 minutes) after condition, v) 5-10 minutes after condition, vi) 10-15 minutes after condition, vii) 15-20 minutes after condition, viii) 25-30 minutes after condition, ix) 40-45 minutes after condition, and x) 55-60 minutes after condition. For measurements viii-x, data selections for analysis were chosen such that they occurred at times when the PWV and PWA devices were not inflating in order to reduce the autonomic data to be impacted by the inflations. Data was collected at the following sample rates: electrocardiogram (256 HZ), respiration rate (25.6 HZ), GSR (8 HZ). For HRV settings, the R-R interval threshold was set to 500 to 1600 ms and ectopic beats were excluded. Measures of HRV included standard deviation of R-R intervals (SDRR), the root-mean-square of successive differences of R-R intervals (RMSSD) and the low-frequency (LF) power, high-frequency (HF) power, and frequency power ratio (LF/HF). Based on visual inspection of Q-Q plots, the frequency-domain variables (LF, HF, LF/HF) had non-uniformity of error. Thus, we applied a natural logarithm for analyses of the frequency-domain data.

Actigraph - Accelerometry

Prior to participating in the laboratory experiments, participants wore an accelerometer (Actigraph GT3X+, Pensacola, FL, USA) to characterize their physical activity levels. Participants wore the accelerometer on their non-dominant wrist during an 8-day free living period. Using NHANES protocols[204], Hildebrand (in)activity cutoff points[205, 206], and the GGIR package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria) participants' daily average minutes spent in vigorous, moderate, light and (in)-activity were calculated. Epoch length was set at 5 seconds, non-wear detection resolution at 15 mins, and the non-wear detection window at 60 minutes.

Exposure Variables

Moderate-Intensity Aerobic Exercise

Following a 2.5 minute warm-up at a comfortable self-selected pace, participants engaged in 25 minutes of moderate-intensity aerobic exercise performed on a full-body LifeFitness 95x elliptical machine (LifeFitness, Rosemont, IL) with continuous, contralateral movements of arms and legs. Participants then completed a 2.5 minute cool-down period on the elliptical at a comfortable, self-selected pace. This intensity and modality of exercise was used because full-body moderate-intensity AE i) may most optimally promote whole-body shear-stress and endothelial function throughout the arterial tree[215], and ii) has been shown to counteract a number of psychophysiological indices of mental stress[216, 217]. Moderate-intensity was defined as 55-70% of Heart Rate Reserve as calculated by the Karvonen Method: [(Age-predicted HR_{max} – HR_{rest}) x (%Intensity)] + HR_{rest}., where age-predicted HR_{max} was determined by 220-age[45]. HR_{rest} was determined after five minutes of quiet supine rest on

the familiarization visit using a chest-worn HR monitor (Polar, Bethpage, NY, USA). To ensure appropriate exercise intensity, HR and rate of perceived exertion (RPE) were monitored and recorded every three minutes throughout the exercise session. For HR, the chest-worn Polar HR monitor was used, while RPE was measured on the 6-20 Borg scale [46]. During the AE, participants were instructed to push and pull from both the arms and legs and to maintain a pace of $\geq$ 50 revolutions per minute. The intensity (resistance) of the AE was adjusted in order for the participant to remain within the objective (55-70% of HRR) and subjective (RPE of 11-16) intensity criteria[45]. If HR and RPE values were in conflict with respect to the intensity level, HR was prioritized as they key outcome in terms of whether or not elliptical resistance (intensity) was adjusted.

Experimental Condition: Trier Social Stress Test

The TSST, a short battery of mental stressors (speech task + mental arithmetic test) which has been shown to elicit a robust cardiovascular response[27, 28], was administered on the experimental lab visit. The TSST is a reliable and valid acute stressor that can robustly induce an acute physiological response. A modified version of the TSST was used in which the normally three-minute preparatory period (prior to the speech task starting) was shortened to two minutes due to the long duration of the entire test period as well as the added stress of having a metabolic canopy covering their body during exposure to the TSST. Additionally, participants typically use the 3-minute preparatory period to write down notes on a piece of paper to prepare thoughts and ideas for the interview/speech task. However, since participants were administered the TSST during the same time as the EE measurement, they were be able to move their arms or hands (e.g., they were under metabolic canopy**)**, and thus were not able to

write. Following the 2-minute preparatory period, participants were instructed to think of their ideal job and explain to the TSST administrator (someone who participants were unfamiliar with; e.g., was not involved in recruitment, data collection, etc.) why they were "the best candidate for their ideal job" in five minutes. Next, participants were given a surprise 5-minute mental arithmetic task and told they're responses would be monitored and compared to the norm. At the beginning of each minute of the 5-minute arithmetic task, participants were told to subtract the number 7 or 13 (alternated at each minute) from a new 4-digit number. Participants were also asked to answer at the pace of a metronome, which was surreptitiously increased for each minute of the test (min 1: 12 bpm; min 2: 14 bpm; min 3: 16 bpm; min 4: 18 bpm; min 5: 20 bpm). Other important aspects of the TSST included i) use of a video-camera (not recording but made to look like it was recording for added stress), ii) shining a bright light towards the subject, and iii) having the TSST administrator and 1-2 assistants wear white lab coats during TSST administration. Additional details of the TSST are reported in a 2017 review by Allen et al[32].

Control Condition: Neutral Task

For the control visit, a neutral task similar in structure to the TSST was administered. The protocol was time-matched with the TSST: 2--minute preparatory period, 5-minute speech period, and 5-minute arithmetic period. However, participants were told that their responses would not be critiqued or compared to other scores. For the speech period, participants were instructed to "talk about a boring or neutral activity such as sitting in a dull lecture, waiting for the bus, or sitting in a waiting room." For the math period, participants were instructed to count repeatedly from 1-10 at a slow, calm pace.

Sample Size

Sample size calculations were made using GPower 3.1.9. Prior studies have reported increases in PWV ranging from 0.29-0.57 $\pm$ 0.2-1.06 m/s[20, 94]. Based on the moderate effect seen in these studies, we expected to observe a similar moderate effect in the current study. With a moderate effect size, an alpha level set at 0.05, the power set at 0.8, 8 measurement time points and a moderate (0.5) correlation among repeated measures, the sample size was computed to be 16[187]. We inflated the sample to 23 participants to account for attrition and the chance of poor or incomplete data.

Statistical Analysis

Statistical analyses were performed using Jamovi (2022, Version 2.3.21.0) for linear mixed models and the pkr package for R (R Foundation for Statistical Computing, Vienna, Austria) for area under the curve analyses (AUC). The significance level was set *a priori* for all statistical procedures at alpha = 0.05, however interactions between group and time were considered significant at the alpha < 0.1 level because of the increased power needed to detect interactions. Raw data are presented as mean (SD) and mixed model data are presented as mean (95% CI). Effect sizes(ES) were calculated as Cohen's *d* where $\leq$ 0.2, 0.2-0.3, 0.4-0.8, and $\geq$ 0.8 were defined as trivial, small, moderate, and large, respectively[187]. The corresponding author (GZ) had full access to the data in the study and was responsible for the integrity of the data set and the data analysis. Based on the cross-over study design of the experimental studies, linear mixed model regression with random effects were used to test the effects of the TSST and control conditions on PWV and EE. Effects were specified as random for time (slope) and subject (intercept), and fixed for condition. The models were adjusted for baseline values

as specified by Kenward and Roger[207]. EE data was also tested using the trapezoidal method for AUC. Paired samples T-tests with mean differences and 95% CIs were used to test differences in AUC between conditions. PWV and EE were also corrected for mean arterial pressure and lean muscle mass due to the pressure-dependency of arterial stiffness [145, 208] and the influence of body composition on metabolic rate[153], respectively.

Results

Participants

The CONSORT diagram is presented in **Supplement Figure 7S.1**. Twenty-four participants were recruited, of which 20 [20.0 (1.90) years, 60% female, 24.2 (4.5 kg/m^2)] completed the study. Participants identified as white (n=11) and non-white (n=9; 4 Asian; 4 Black; 1 Hispanic). Of the four participants who dropped out, attrition was attributed to time constraints (n=1), 1 due to musculo-skeletal injury (n=1), respiratory illness (n=1). The other individual that dropped out did so after the first laboratory visit due to unspecified health issues, however data was collected for their first (control) visit.

Baseline Data

Table 7.1 displays demographics and participant characteristics.

Table 7.1. Participant characteristics.

	Mean/n	SD/%
Biological Sex (M/F)	8 M/ 12 F	40/60
Age (years)	20.0	1.9
Height (cm)	171.7	9.8
Weight (kg)	70.6	12.1
BMI (kg/m^2)	23.9	4.5
%BF	24.1	11.9
Perceived Stress (PSS Score)	13.7	7.0
Activity Status		
MVPA (min/day)	78.2	39.0
VPA (min/day)	4.8	10.0
MPA (min/day)	73.5	32.7
LPA (min/day)	141.9	43.5
Sedentary (hrs/day)	12.7	1.7

Abbreviations**:** *BF, body fat; cm, Centimeters; F, Female; hrs, hours; kg, kilograms; LPA, light physical activity, M, Male; m, meters; MPA, moderate physical activity; MVPA, moderate-vigorous physical activity; PSS, Perceived Stress Scale; VPA, vigorous physical activity.*

Control Measures

Acute Changes in Perceived Stress

Data from the 1-10 Likert Scale (administered immediately before and immediately after the experimental and control conditions) indicated that the experimental condition (mean difference: ↑3.25; $p<0.001$), but not the control condition (mean difference: ↑0.16; $p=0.19$), resulted in an increase in perceived stress.

Moderate-Intensity Aerobic Exercise

Objective and perceived exercise intensity assessments indicated that the exercise was of moderate intensity with an average HRR of 69.2 $\pm$ 8.33 %, and an average RPE of 12.1 $\pm$ 1.3. The exercise exposure also did not result in different pre-stress physiological responses

between each of the two conditions, as there was no statistically significant difference in change scores for PWV from Baseline 1 (pre-exercise) to Baseline 2 (post-exercise) between conditions (p=0.11) (**Figure 7.2**).

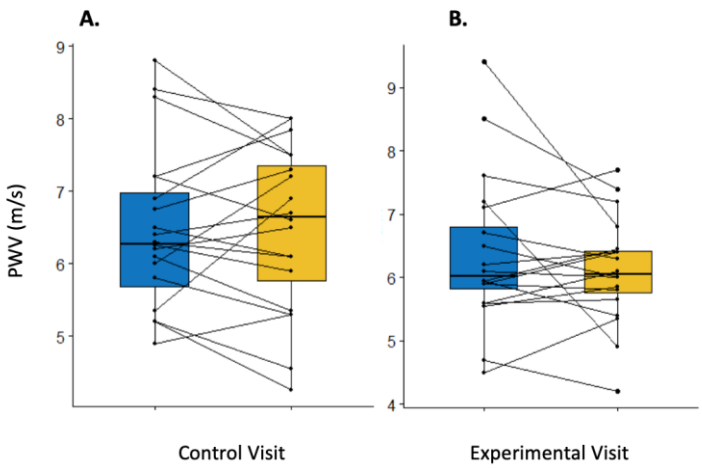

Figure 7.2. Arterial stiffness measures before and after exercise. Baseline 1 and Baseline 2 pulse-wave velocity assessments taken before (blue box) and after (yellow box) aerobic exercise on the (A) Control and (B) Experimental visits. Changes from pre- to post-exercise within and between conditions were not statistically different (p<0.05). Abbreviations: *PWV, Pulse-wave velocity.*

Cardiovascular Outcomes

Primary Cardiovascular Outcome: Arterial Stiffness

Arterial stiffness PWV measures adjusted for mean arterial pressure are presented in **Table 7.2** and **Figure 7.3.** There was a significant, small effect size condition x time interaction (B=0.47 m/s, d=0.23, 95%CI: 0.21, 0.72) with the greatest change between conditions occurring from pre- to immediately post-condition exposure. The acute mental stress condition caused a greater increase in PWV from pre to immediately post stress [↑0.43 m/s (7%)] compared to the control condition which exhibited a decrease in PWV [↓0.05 m/s (-1%)].

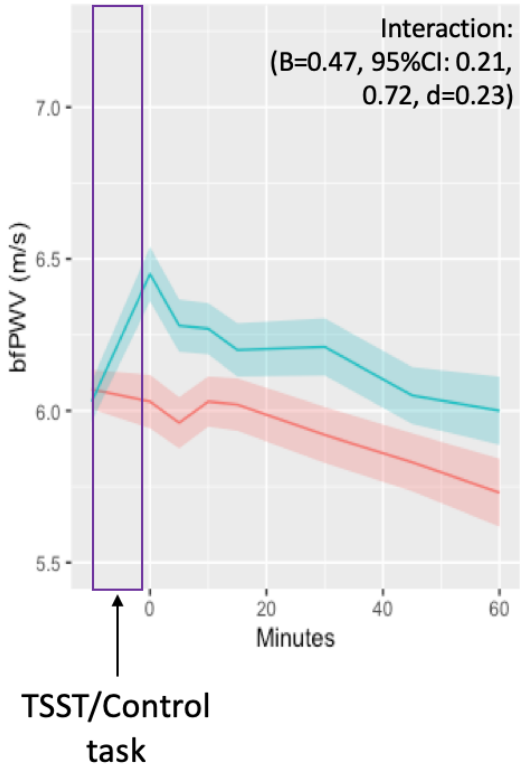

Figure 7.3. Pulse-wave velocity before and after condition exposure. Control (red line) and experimental (green line) condition responses are shown. Plotted data are the estimated marginal means from the linear mixed models with the shaded region representing the standard error. Eight measurement timepoints were at -10 minutes (Baseline 3/pre-condition), and at 0, 5, 10, 15, 30, 45, and 60 minutes post-condition. Abbreviations: *bfPWV, brachial-femoral pulse-wave velocity; m/s, meters per second; TSST, Trier Social Stress Test.*

Secondary Cardiovascular Outcomes: Blood Pressure and Hemodynamics

BP and central hemodynamic measures are presented in **Table 7.1.** There were small interaction effects of condition and time for SBP (B=6.83 mmHg, 95%CI: 3.00, 10.65, d=0.22) and cSBP (B=7.39 mmHg, 95%CI: 3.84, 10.94, d=0.26) with the greatest changes between conditions occurring between pre- to immediately post-condition exposure. For DBP, cDBP, sAI, sPR there were main effects of condition (ranging from trivial to moderate in effect size) and trivial main effects of time.

Table 7.2. Cardiovascular results. Pre and immediately post-condition data are displayed, as well as results from the linear mixed model.

		PWV m/s	SBP mmHg	DBP mmHg	cSBP mmHg	cDBP mmHg	sAI %	HR bpm
Means								
CON	PRE	6.29	107.00	61.40	96.80	63.30	32.50	74.00
	POST	6.24	107.00	63.10	95.90	64.30	36.00	74.70
EXP	PRE	5.79	108.00	62.80	96.70	64.40	28.70	76.40
	POST	6.22	114.00	66.30	103.00	68.10	32.70	81.00
Standard Deviations								
CON	PRE	1.05	10.40	7.80	9.11	8.54	18.60	11.80
	POST	1.08	10.90	7.45	8.17	7.66	18.60	11.60
EXP	PRE	1.01	10.70	7.25	9.20	7.87	17.00	10.80
	POST	1.14	12.60	7.46	11.60	7.92	17.70	14.50
Condition Effect								
	β	0.24	3.16	2.51	2.58	2.45	-2.62	3.97
	P	<0.001	<0.001	<0.001	<0.001	<0.001	0.016	<0.001
	ES	0.41	0.40	0.34	0.36	0.32	-0.15	0.40
	LCI	0.16	2.19	1.585	1.68	1.51	-4.74	2.75
	UCI	0.31	4.12	3.44	3.47	3.39	-4.99	5.19
Time Effect								
	β	0.19	3.36	2.79	2.83	2.56	-3.66	3.01
	P	0.009	0.001	<0.001	<0.001	<0.001	0.001	<0.001
	ES	0.14	0.20	0.19	0.18	0.17	-0.11	0.15
	LCI	0.02	1.24	0.96	0.84	0.70	0.53	0.51
	UCI	0.36	5.48	4.62	4.81	4.42	7.94	5.51
Interaction Effect								
	β	0.47	6.83	2.37	7.39	3.18	2.33	3.64
	P	0.036	0.038	0.579	0.006	0.531	0.287	0.873
	ES	0.23	0.22	0.08	0.26	0.11	0.03	0.09
	LCI	0.21	3.00	-1.29	3.84	-0.54	-6.04	-1.18
	UCI	0.72	10.65	6.03	10.94	6.90	10.71	8.47

Abbreviations: *B, beta coefficient; Con, control; cDBP, central diastolic blood pressure; cSBP, central systolic blood pressure; DBP, diastolic blood pressure; ES, effect size; EXP, experimental; LCI, lower confidence interval; p, p-value; bfPWV, pulse-wave velocity; sAI, systolic augmentation index, SBP, systolic blood pressure; sPR, systolic pulse rate; UCI, upper confidence interval.*

Energy Outcomes

Primary Energy Outcome: Energy Expenditure

Energy expenditure measures adjusted for lean mass are presented in **Table 7.2.** There were no condition x time interaction effects. There was a small main effect of condition (B= 0.0005 kcal/kg/min, 95%CI: 0.0003, 0.0008, d=0.33), with the experimental condition having a 0.0008 kcal/kg/min greater EE compared to the control condition. There was also a small main effect of time (B=0.0011 kcal/kg/min, 95%CI: 0.0006, 0.0016, d=0.31) with the largest increase in EE (0.001 kcal/kg/min) occurring from Time 1 to Time 2 (from pre to during condition exposure).

With the AUC analysis, the EE in the experimental condition was not significantly different than the control condition (mean difference: 0.04 kcal/kg, 95%CI: -0.08, 0.01, p=0.12, d=0.27). Post-Hoc analyses showed that when the AUC analyses was limited only to the baseline period and the first 35 minutes (rather than full 60 min post-condition period), the difference in EE responses between the experimental and control conditions remained non-significant (mean difference: 0.02 kcal/kg, 95%CI: -0.05, 0.01, p=0.19, d-0.22). EE AUC responses averaged across participants for the experimental and control conditions are displayed in **Figure 7.4**.

Secondary Energy-Related Measures

Secondary (VO_2/kg, METS, RQ) energy-related measures are shown in **Table 7.3**. For the secondary energy-related variables, there was a trivial time x condition interaction for RQ (B=0.04, 95%CI: 0.01, 0.07, d=0.18) with the greatest changes in measurements occurring from pre-condition to during condition. For VO_2/kg, there were small main effects of condition

(B=0.13 ml/kg/min, 95%CI: 0.07, 0.19, d=0.32) and time (B=0.22 ml/kg/min, 95%CI: 0.12, 0.33, d=0.30). For METS, there were also small main effects of condition (B=0.04, 95%CI: 0.02, 0.05, d=0.32) and time (B=0.06, 95%CI: 0.03, 0.10, d=0.30). Ancillary energy-related measures (fractional content of carbon dioxide, fractional content of oxygen; resting energy expenditure; carbon dioxide production, minute ventilation, oxygen consumption) are presented in **Supplement Table 7S.1.**

Table 7.3. Energy results. Pre and during stress data are presented, as well as the analysis from the linear mixed models.

		EE kcal/kg/min	VO2/kg ml/kg/min	METS	RQ
Means					
CON	PRE	0.0161	3.37	0.96	0.70
	DURING	0.0167	3.51	1.00	0.75
EXP	PRE	0.0161	3.38	0.97	0.70
	DURING	0.0175	3.68	1.05	0.79
Standard Deviations					
CON	PRE	0.0021	0.43	0.12	0.06
	DURING	0.0021	0.48	0.14	0.05
EXP	PRE	0.0015	0.31	0.09	0.06
	DURING	0.0020	0.42	0.12	0.06
Condition Effect					
	β	0.00	0.13	0.04	0.01
	P	<0.001	<0.001	<0.001	0.21
	ES	0.33	0.32	0.32	0.09
	LCI	0.00	0.07	0.02	0.00
	UCI	0.00	0.19	0.05	0.01
Time Effect					
	β	0.00	0.22	0.06	0.07
	P	<0.001	0.001	<0.001	<0.001
	ES	0.31	0.30	0.30	0.64
	LCI	0.00	0.12	0.03	0.06
	UCI	0.00	0.33	0.10	0.09
Interaction Effect					
	β	0.00	0.16	0.05	0.04
	P	0.343	0.574	0.574	<0.001
	ES	0.13	0.12	0.12	0.18
	LCI	0.00	-0.04	-0.01	0.01
	UCI	0.00	0.36	0.10	0.07

Abbreviations: *B, beta coefficient; Con, control; EE, energy expenditure; ES, effect size; EXP, experimental; LCI, lower confidence interval; METS, metabolic equivalents; RQ, respiratory quotient; p, p-value, UCI, upper confidence interval; VO_2/kg, relative oxygen consumption*.

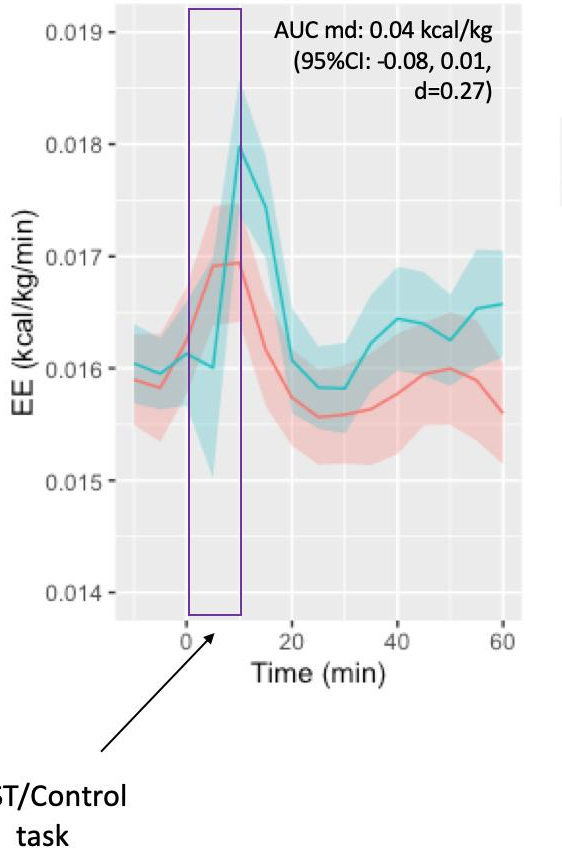

Figure 7.4. The area under the curve for the energy expenditure response. Data is averaged across participants for the control (red line) and experimental (green line) condition. Abbreviations: *95% CI, 95% confidence interval; AUC, area under the curve; EE, energy expenditure; kcal, kilocalories; kg, kilograms; md, mean difference; min, minute; TSST, Trier Social Stress Test.*

Heart Rate Variability, Galvanic Skin Response, and Respiration Rate

There were time x condition interactions for HF (B=0.13, 95%CI: -0.14, 0.40, d=0.06), LF/HF (B=-0.14, 95%CI: -0.34, 0.06, d=-0.08), respiration rate (B=-1.37 BrPM, 95%CI: -4.11, 1.36, d=-0.06), GSR (B=-0.24, 95%CI: -1.05, 0.57, d=-0.03) and HR (B=7.14 bpm, 95%CI:2.51, 11.77, d=0.18). There were main effects of both condition and time for LF ($p<0.05$). For RMSSD and SDRR, there were main effects of condition and time, respectively (both $p<0.05$). Full analyses for HRV, GSR, and respiration rate are reported in **Supplement Table 7S.1.**

Discussion

This study assessed how a prior bout of moderate-intensity aerobic exercise impacts the EE and arterial stiffness responses to a short psychosocial laboratory stressor compared to a neutral control task. Findings suggest that stress-induced elevations in arterial stiffness are not abated with prior aerobic exercise, and that aerobic exercise may partially blunt the energetic response to acute stress.

Limitations and Strengths

Limitations and strengths are provided to help contextualize findings. First, a relatively homogenous sample of young, healthy adults was recruited, which limits generalizability to other populations (e.g. different ages and health/disease statuses). However, it is first imperative to study the biological responses to mental stress in a sample which limits the potential for confounding of age and disease processes that strongly influence cardiovascular and metabolic physiology. Second, the current study was not powered to investigate effects of biological sex. Future research is warranted to investigate potential sex differences in the arterial stiffness and EE responses to acute mental stress. Third, the TSST was not administered by the same individuals for each experimental condition. However, the same two individuals were the lead administrators of the TSST (depending on their availability) and they administered the stressor using the same script. Major strengths of the study were the development and implementation of a time-matched neutral, control condition, as well as the use of sensitive measurements of cardiovascular and metabolic constructs implicated in CVD pathophysiology.

Comparison to Literature

Arterial Stiffness

Several studies by Kume et al. have assessed how acute exercise may interact with the arterial stiffness response to acute mental stress, with results showing that post-stress exercise ameliorated elevations in heart-brachial PWV, brachial-ankle PWV, heart-ankle and cardio-ankle vascular index[25, 214, 218]. For heart-brachial PWV, which includes the proximal aorta and upper-limb muscular arteries (subclavian, brachial) similar to the bfPWV measurement used in the current study, Kume et al. showed that following initial stress-induced elevations in arterial stiffness, post-stress exercise in the form of three[218] and ten[214] minutes of post-stress bench stepping, as well as 10 minutes of cycling[25] induced decreases in PWV by ~0.2 m/s. Though these studies demonstrated that post-stress exercise may exert positive effects on arterial stiffness, comparisons between results are limited because these studies only recruited men, and they used a variety of types and durations of stressors.

Whereas these prior studies by Kume et al. assessed post-stress exercise, the current study is the first study that we are aware of to test the arterial stiffness response to acute mental stress *after* a prior exercise bout. Our findings showed that the arterial stiffness response to acute mental stress is not abated by prior exercise [↑0.43 m/s (7%)]. However, whereas Kume et al. used a non-exercise resting protocol as a control condition, the current study tested the post-exercise stress response against a neutral control task. This distinction is important because simple psychosocial activities - irrespective of mental stress perception – are expected to stimulate a physiological response. Nevertheless, exercise may best temper the arterial stiffness response if completed after rather than prior to stress exposure.

At first glance, the current findings may not seem to depict a promising picture regarding the potential for prior exercise to moderate arterial stiffness stress reactivity. However, compared to the current study, our prior work (Experiment 1) using the same stress protocol in the same population (e.g. different individuals, same inclusion and exclusion criteria) demonstrated nearly double the increase in bfPWV [↑0.81 m/s (13%)] and a 0.21 greater effect size when prior exercise was not performed. This is also in line with evidence demonstrating that prior exercise promotes lesser blood pressure reactivity [216] and mitigates transient endothelial dysfunction [133] following exposure to acute mental stress. Mechanistically, exercise may prime the vasculature to be better able to cope with the stressors as a function of improved autonomic (central) and endothelial (local) functioning[116–120]. In terms of autonomic function, acute aerobic exercise may benefit cardiovascular reactivity by helping regulate the autonomic nervous system, e.g. increasing parasympathetic nervous system tone[11, 220]. In terms of endothelial function, continuous, moderate-intensity aerobic exercise likely also dampens cardiovascular stress reactivity by increasing blood-flow induced shear-stress[133, 221]. Shear stress is a beneficial frictional force between the red blood cells and endothelium which stimulates the nitric-oxide system and subsequently elevates endothelium-dependent arterial vasodilatory capacity[221]. Such acute cardio-protection in the face of a stressor, may, over time, partially account for the decreased risk of CVD that is observed among individuals (independent of age) with higher levels of cardiorespiratory fitness[121–123].

Energy Expenditure

While several studies have demonstrated acute increases in EE in response to acute mental stress exposure[13–16], no studies that we are aware of have assessed the influence of post-exercise mental stress on EE. In line with our hypothesis, the current study showed that compared to a neutral control condition, exposure to acute mental stress did not result in a significant elevation in EE, with both conditions resulting in a 0.001 kcal/kg/min increase in EE. Since metabolic rate is strongly governed by the autonomic nervous system, exercise-mediated dampening of EE may be a result of a beneficial influence on sympatho-vagal balance, as was described in the context of arterial stiffness above. Acute exercise also provokes hormonal responses that may influence physiological stress-reactivity[222]. For example, acute exercise has been reported to moderate reactivity of cortisol – a hormone tightly tied to EE since it helps regulate glucose and lipid metabolism[51, 217].

The idea that aerobic exercise contributes to dampened stress reactivity is also in line with the constrained energy theory, which postulates that while total daily EE may be somewhat hard-wired and genetically pre-determined, allocations of energy may be uniquely allocated depending on physical activity and exercise levels (**Figure 7.5**)[126]. More specifically, the theory posits that elevations in physical activity-related EE may reduce non-essential metabolic processes including autonomic, neuroendocrine, and inflammatory actions that are linked with CVD risk[126–128]. In line with this schema, contrary to our current results (d=0.13 effect size increase in EE, $p>0.05$), our prior work using the same stress protocol in a similar population demonstrated a significant post-stress EE response (d=0.31 effect size increase in EE, $p<0.05$) when prior exercise was *not* performed. Thus, in addition to autonomic and

hemodynamic factors, exercise-related dampening of physiological stress reactivity may also be related to how energy resources may be uniquely allocated depending on lifestyle factors such as exercise and/or physical activity levels.

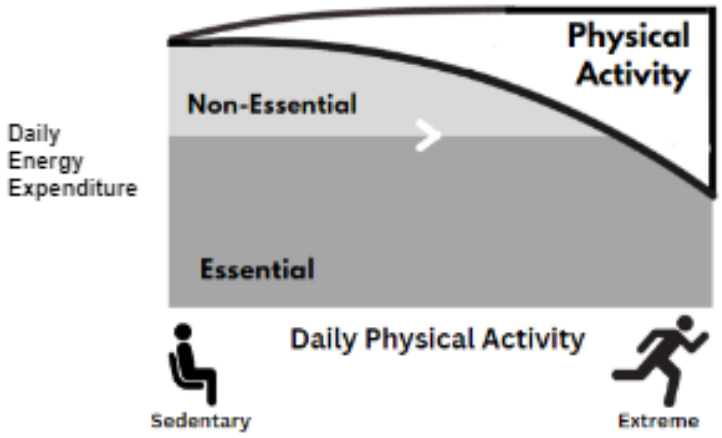

Figure 7.5. Schematic illustrating the constrained energy theory (adapted from Pontzer, 2018)[128]. Essential and non-essential refer to energy expenditure. For example, with increased physical activity, essential energy expenditure (e.g. digestion, immune response to pathogens, etc.) may be largely maintained while non-essential energy expenditure, which is maladaptive in excess (e.g. autonomic and neuroendocrine stress-reactivity, inflammation), is ameliorated, thereby reducing CVD risk.

Implications

Mental stress is an unavoidable aspect of the human experience and contributes to CVD risk[180]. However, whether cardiometabolic stress reactivity is modifiable through simple lifestyle factors is relatively understudied, particularly in the context of CVD risk. Stress-related CVD risk could arguably be reduced through three primary strategies: i) reduce stress exposure, ii) improve psychological and/or biological resilience in the face of a given stressor, and/or iii) - as alluded to in the brief discussion of the constrained energy theory above – elevate physical activity levels to re-allocate energy resources away from stress-reactivity and subsequent

inflammation which may contribute to CVD risk[197, 211]. The current study does not directly address the constrained energy theory (**Figure 7.5**). However, assessing the results alongside our prior non-exercise stress study does suggest that activity-related energy allocation, as suggested in the constrained energy theory, may moderate cardiometabolic stress reactivity and could be implicated in stress-related cardiovascular pathophysiology. While the current study did not directly test each of the biomarkers and causal pathways in **Figure 7.6**, findings do highlight that the variables shown in this figure may be involved in the relationship between repeated exposures to acute mental stress and CVD risk over time. Specifically, **Figure 7.6** illustrates the putative biological framework linking interactions between acute aerobic exercise and acute stress-related effects on arterial stiffness and energy expenditure in the context of CVD risk.

From a practical, behavioral standpoint, recent efforts, including by our group, have demonstrated that in addition to total or net exposure to 24-hour activity behaviors (physical activity, sedentary behavior, sleep), behavioral timing – especially in relation to other lifestyle factors - may be particularly critical in terms of CVD risk[223–226]. Similarly, findings from the current study have the potential to inform lifestyle strategies and public health recommendations for mitigating stress-related CVD risk. For example, engaging in prior aerobic exercise may be a key stress-related CVD risk prevention/reduction strategy for individuals anticipating exposure to severe prolonged or repeated stress (e.g. prior to a stressful academic examination, job interview, care-giving period for a loved one, or shift for a first-responder/emergency worker).

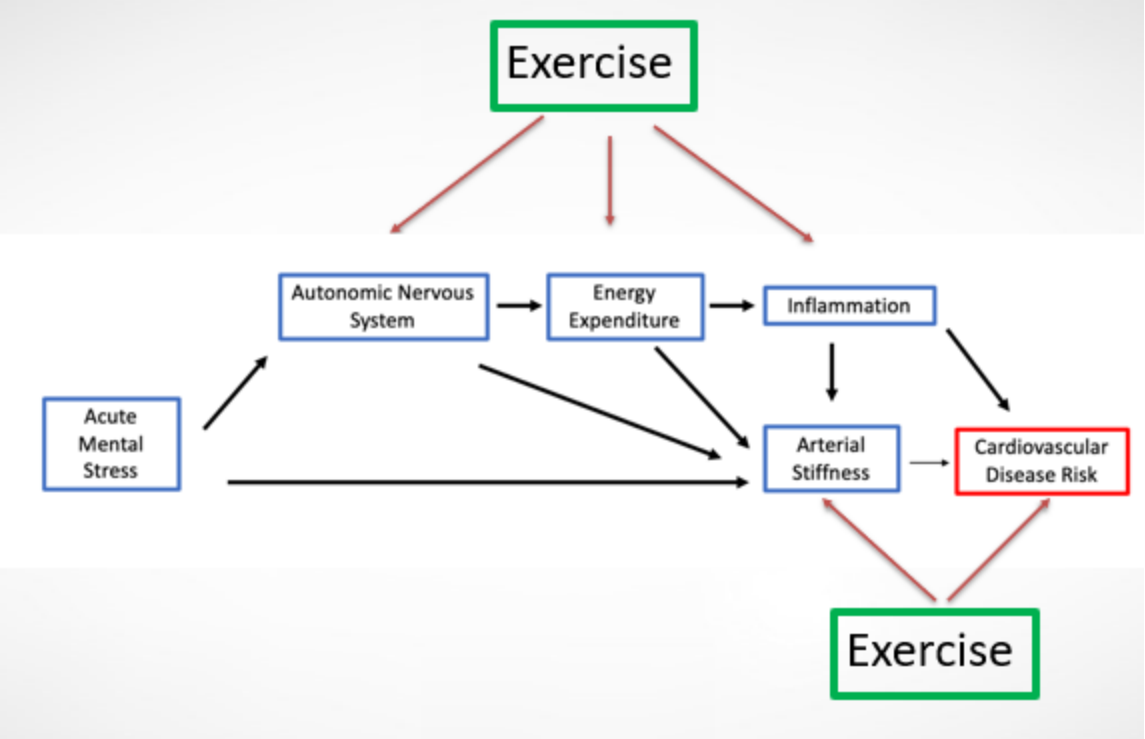

Figure 7.6. Conceptual framework using a directed acyclic graph (DAG) for plausible pathway linking interactions between acute aerobic exercise and acute stress-related effects on arterial stiffness and energy expenditure in the context of CVD risk. Abbreviations: *ANS, autonomic nervous system; AS, arterial stiffness; CVD, cardiovascular disease; EE, energy expenditure; MS, mental stress (acute).*

Conclusion

Compared to a neutral control condition, exposure to acute mental stress following an aerobic exercise bout did not provoke significant physiological increases EE. While increases in arterial stiffness were observed following post-exercise stress exposure, this response was likely tempered by the prior exercise bout. Arterial stiffening and EE responses may be key players in the relationship between mental stress and CVD risk. Future work is particularly warranted to explore this relationship further within the framework of the constrained energy theory, which posits that exercise may divert energy use away from stress-reactivity and

subsequent CVD risk development. Findings also suggest that aerobic exercise may be a feasible lifestyle-based health strategy to off-set stress-related CVD risk.

Experiment 2 Supplement

Table 7S.1. Ancillary energy data. Pre and during stress data are presented, as well as the analysis from the linear mixed models.

		VO2 ml/min	VCO2 ml/min	VE L/min	FEO2 %	FECO2 %	REE kcal/D
Means							
CON	PRE	236.00	164.00	25.20	19.90	0.83	1586.00
	DURING	245.00	185.00	25.60	19.80	0.91	1671.00
EXP	PRE	236.00	165.00	25.30	19.90	0.83	1588.00
	DURING	257.00	204.00	25.10	19.70	1.02	1768.00
Standard Deviations							
CON	PRE	43.30	28.10	4.22	0.09	0.05	284.00
	DURING	43.00	34.00	4.22	0.10	0.07	291.00
EXP	PRE	37.90	21.80	3.62	0.08	0.05	242.00
	DURING	46.20	35.80	3.72	0.09	0.06	311.00
Condition Effect							
	β	7.45	6.59	0.00	-0.04	0.03	52.22
	P	<0.001	<0.001	0.99	<0.001	<0.001	<0.001
	ES	0.26	0.25	0.00	-0.33	0.39	0.26
	LCI	3.28	2.77	-0.37	-0.05	0.02	23.48
	UCI	11.62	10.41	0.38	-0.02	0.05	81.00
Time Effect							
	β	15.33	29.91	0.14	-0.08	0.14	133.60
	P	0.002	<0.001	0.106	<0.001	<0.001	<0.001
	ES	0.30	0.60	0.03	-0.37	0.74	0.37
	LCI	7.97	22.76	-0.51	-0.11	0.11	81.84
	UCI	22.69	37.06	0.78	-0.05	0.16	185.40
Interaction Effect							
	β	12.11	18.93	-0.50	-0.08	0.10	96.71
	P	0.65	0.009	0.434	0.011	<0.001	0.477
	ES	0.07	0.21	-0.06	-0.23	0.35	0.14
	LCI	-2.02	5.96	-1.76	-0.14	0.06	-0.80
	UCI	26.25	31.91	0.77	-0.03	0.15	194.20

Abbreviations: *B, beta coefficient; Con, control; ES, effect size; EXP, experimental; $FECO_2$, fractional content of carbon dioxide; FEO_2, fractional content of oxygen; LCI, lower confidence interval; p, p-value, REE, resting energy expenditure; UCI, upper confidence interval; VCO_2, carbon dioxide production, VE, minute ventilation; VO_2, oxygen consumption.*

Table 7S.2. Mixed model analysis for autonomic nervous system data from the Equivital Wireless Physiological Monitoring System. Frequency-domain measures underwent log transformation due to heteroscedasticity.

		RMSSD	SDRR	LF Log	HF Log	LF/HF Log	Resp Rate (BrPM)	GSR (uS)	HR (bpm)
Condition Effect									
	β	-3.60	-1.96	-0.07	-0.08	0.01	0.03	-0.20	5.91
	P	0.014	0.171	0.011	0.01	0.559	0.427	0.038	<0.001
	ES	-0.15	-0.08	-0.15	-0.15	0.03	0.00	-0.12	0.65
	LCI	-6.46	-4.75	-0.12	-0.14	-0.03	-0.37	-0.38	4.84
	UCI	-0.74	0.84	-0.02	-0.02	0.06	0.89	-0.01	6.97
Time Effect									
	β	-5.92	-11.04	0.01	-0.23	0.24	-1.99	1.67	12.66
	P	0.171	<0.001	<0.001	0.003	<0.001	<0.001	0.007	<0.001
	ES	-0.06	-0.11	0.01	-0.13	0.25	-0.12	0.21	0.49
	LCI	-17.03	-23.26	-0.17	-0.42	0.13	-3.87	0.74	9.61
	UCI	5.19	1.18	0.19	-0.03	0.35	-0.10	2.61	15.71
Interaction Effect									
	β	8.64	0.16	-0.01	0.13	-0.14	-1.37	-0.24	7.14
	P	0.087	0.193	0.84	0.017	0.013	0.012	0.011	<0.001
	ES	0.08	0.00	-0.01	0.06	-0.08	-0.06	-0.03	0.18
	LCI	-3.84	-12.04	-0.23	-0.14	-0.34	-4.11	-1.05	2.51
	UCI	21.11	12.35	0.21	0.40	0.06	1.36	0.57	11.77

Abbreviations*: B, beta coefficient; BPM, beats per minute; BrPM, breaths per minute; ES, effect size; GSR, galvanic skin response; HF Log, logarithm transformed high-frequency band; HR, heart rate; LCI, lower confidence interval; LF Log, logarithm transformed low-frequency band; LF/HF Log, logarithm transformed ratio of low: high frequency bands; UCI, upper confidence interval; p, p-value*; *RMSSD, root mean square of successive differences between R-R intervals; SDRR, standard deviation of R-R intervals; uS, micro-Siemens.*

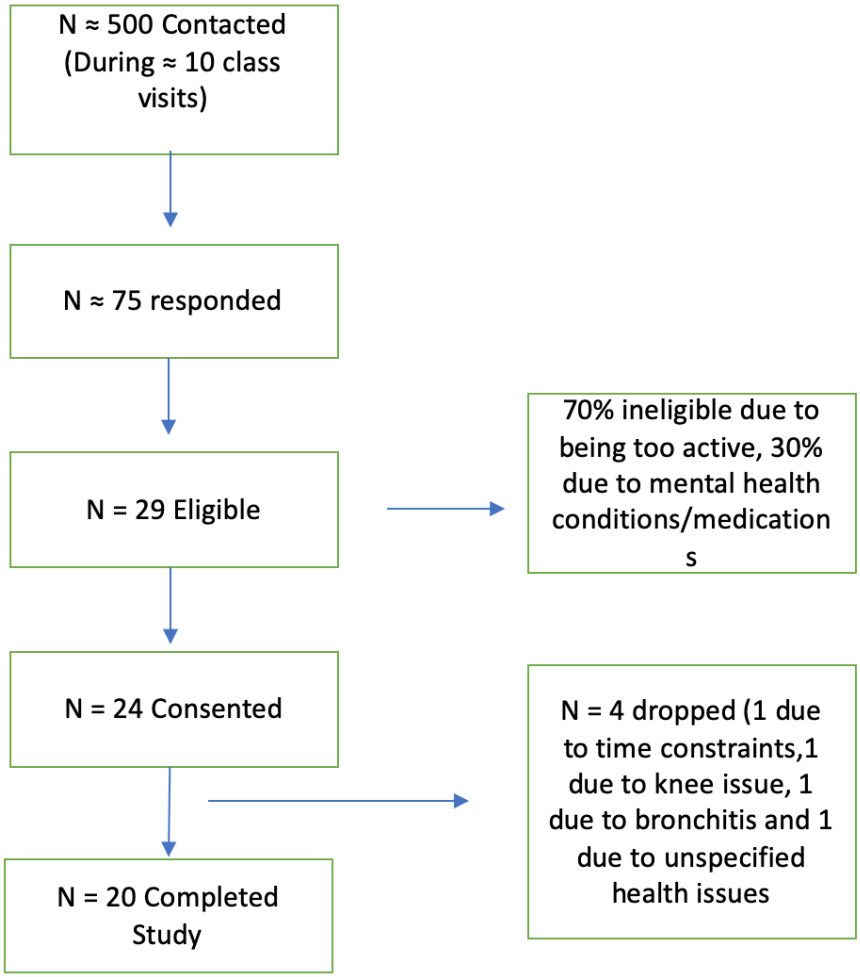

Figure 7S.1. Participant flow-chart. Consolidated Standards of Reporting Trials (CONSORT) diagram shows participant recruitment, attrition, and study completion.

CHAPTER 8: DISSERTATION SUMMARY

Preamble

This chapter summarizes the main findings from the current dissertation. In addition to highlighting the findings from each study, this chapter also provides a critical comparison of results derived from the two experimental studies. Given the similar participant samples (e.g. same inclusion and exclusion criteria) and study protocols between these experiments, this chapter enables a comparison of post-stress cardiometabolic reactivity with and without a prior aerobic exercise bout. Finally, this chapter describes the potential implications of the current findings including logical next steps for future research.

Key Findings

Chronic psychological stress is a known contributor to elevated cardiovascular disease (CVD) risk, and severe levels of mental stress are commonplace in today's society[1]. The initial meta-analysis of the existing literature demonstrates that exposure to acute mental stress causes arterial stiffness to increase, potentially leading to CVD risk over time. The first laboratory experiment then showed that, compared to a neutral control condition, exposure to acute mental stress caused both significant, albeit transit increases in arterial stiffness and EE. EE in particular is an understudied biological variable which may be implicated in the relationship between acute stress and CVD risk over time. The second laboratory study then showed that, compared to a neutral control condition, exposure to acute mental stress following an aerobic exercise bout did not provoke significant physiological increases EE.

However, compared to the first experimental study, the prior exercise introduced in the second study appears to have dampened the arterial stiffness and energy expenditure responses to acute mental stress.

Key Figures Consolidating Experimental Studies

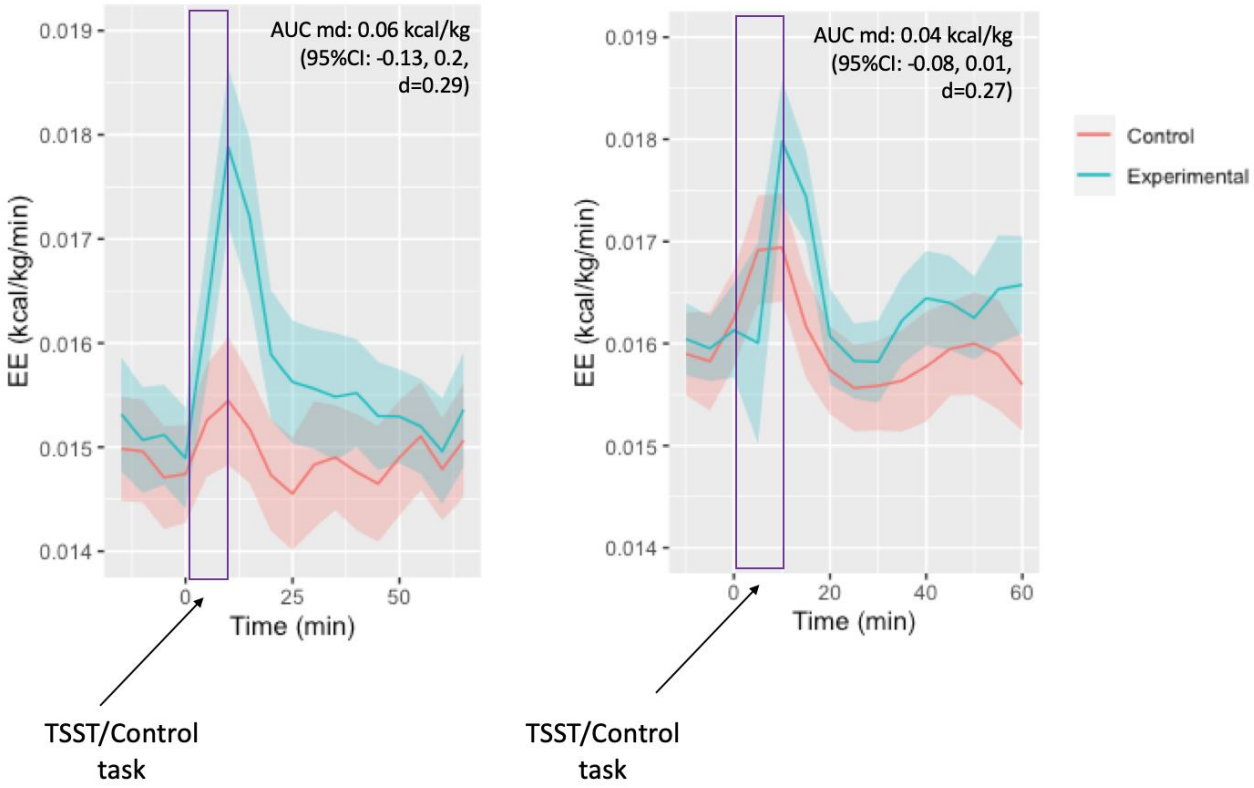

Figure 8.1. Energy expenditure area under the curve. (A) Experiment 1 (no prior exercise) and (B) Experiment 2 (with prior exercise). Green = experimental condition; Red=control condition. Shaded areas denote standard error. Abbreviations: *EE, energy expenditure, kcal, kilocalories; min, minutes.*

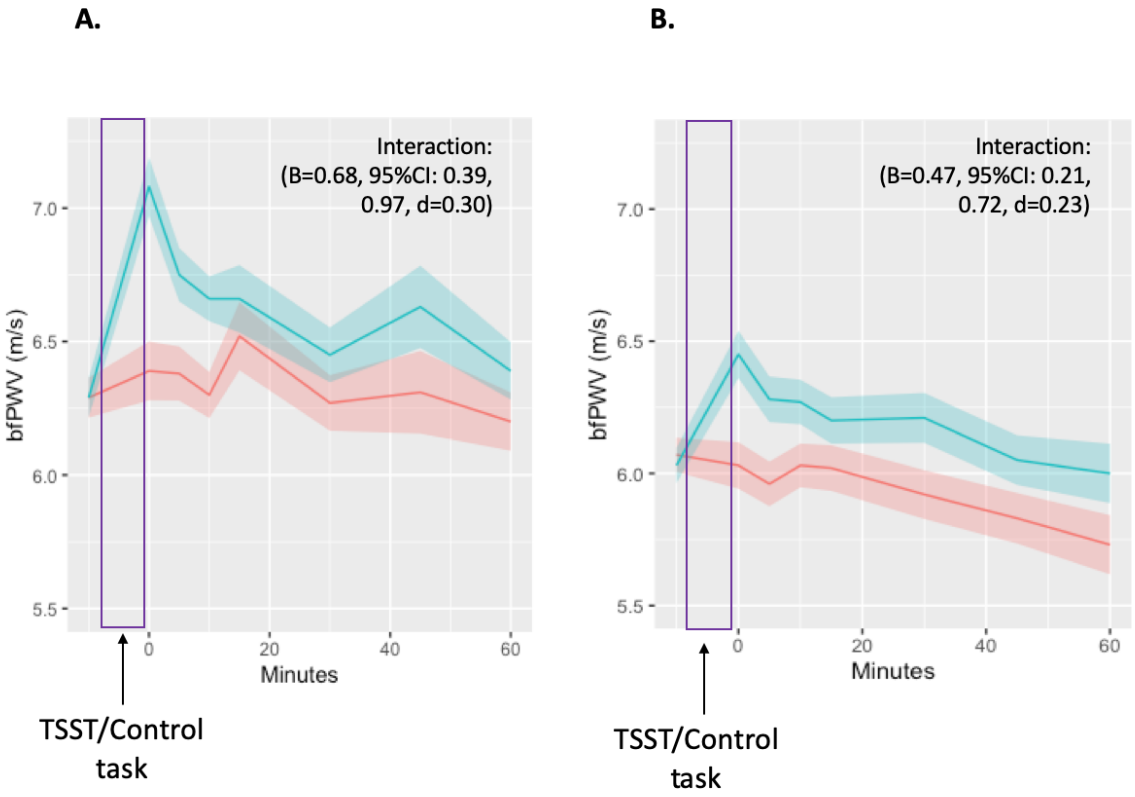

Figure 8.2. Pulse-wave velocity before and after condition exposure. (A) Experiment 1 (no prior exercise) and (B) Experiment 2 (with prior exercise). Control (red line) and experimental (green line) condition responses are shown. Plotted data are the estimated marginal means from the linear mixed models with the shaded region representing the standard error. Eight measurement timepoints were at -10 minutes (Baseline), and at 0, 5, 10, 15, 30, 45, and 60 minutes post-condition. Abbreviations: *bfPWV, brachial-femoral pulse-wave velocity; m/s, meters per second; TSST, Trier Social Stress Test.*

Implications

Key implications are detailed in **Table 8.1**. Additionally, **Figure 8.3** illustrates the putative biological framework linking interactions between acute aerobic exercise and acute stress-related effects on arterial stiffness and energy expenditure in the context of the constrained energy theory and its implications for stress-mediated CVD risk.

Table 8.1. Dissertation implications.

What did we know?
• Chronic stress is associated with CVD risk. • Acute stress stimulates the cardiovascular and autonomic nervous systems which may partially explain the relationship between chronic stress and CVD risk.
What did we not know?
• The effects of acute mental stress exposure on arterial stiffness • The effects of acute mental stress exposure on energy expenditure • The impact of a prior aerobic exercise bout on post-stress cardiovascular and metabolic responses
What have we learned?
• Acute mental stress exposure transiently increases arterial stiffness. • Acute mental stress exposure transiently increases energy expenditure. • While not abated, stress-induced increases in arterial stiffness are dampened with a prior exercise bout • With prior aerobic exercise, the energy expenditure response to acute stress seems to be largely abated.
Why is this new information useful?
• Arterial stiffening and energy expenditure responses may be key players in the relationship between mental stress and CVD risk. • Improved mechanistic understanding of stress-induced changes in arterial stiffness and energy expenditure will help inform prevention and treatment strategies for stress-related CVD risk. • Aerobic exercise may be a promising and feasible lifestyle strategy in the context of minimizing stress-related CVD risk. • Findings highlight the potential importance of exercise timing relative to an individual's 24-hour day (e.g. in the context of anticipated mental stress exposure). • The constrained energy theory posits that daily energy expenditure may be largely hard-wired and that the healthful effects of physical activity may be partially due to a re-allocation of energy (diverting energy resources away from maladaptive stress-reactivity and subsequent inflammation) which may lessen CVD risk. • Dampening of arterial stiffening and energy expenditure processes in response to acute stress and post-exercise stress respectively provides preliminary physiological support for the constrained energy model
What do we need to know next?
• Further explore the stress-CVD relationship further within the framework of the constrained energy theory. • Investigate if longer, and/or more severe stress bouts lead to more sustained increases in arterial stiffness and energy expenditure. • Explore how other lifestyle behaviors and lifestyle-associated factors) of cardiovascular function may also influence the effects of repeated or prolonged exposure to acute stress on CVD risk.

Abbreviations: *CVD, Cardiovascular Disease*

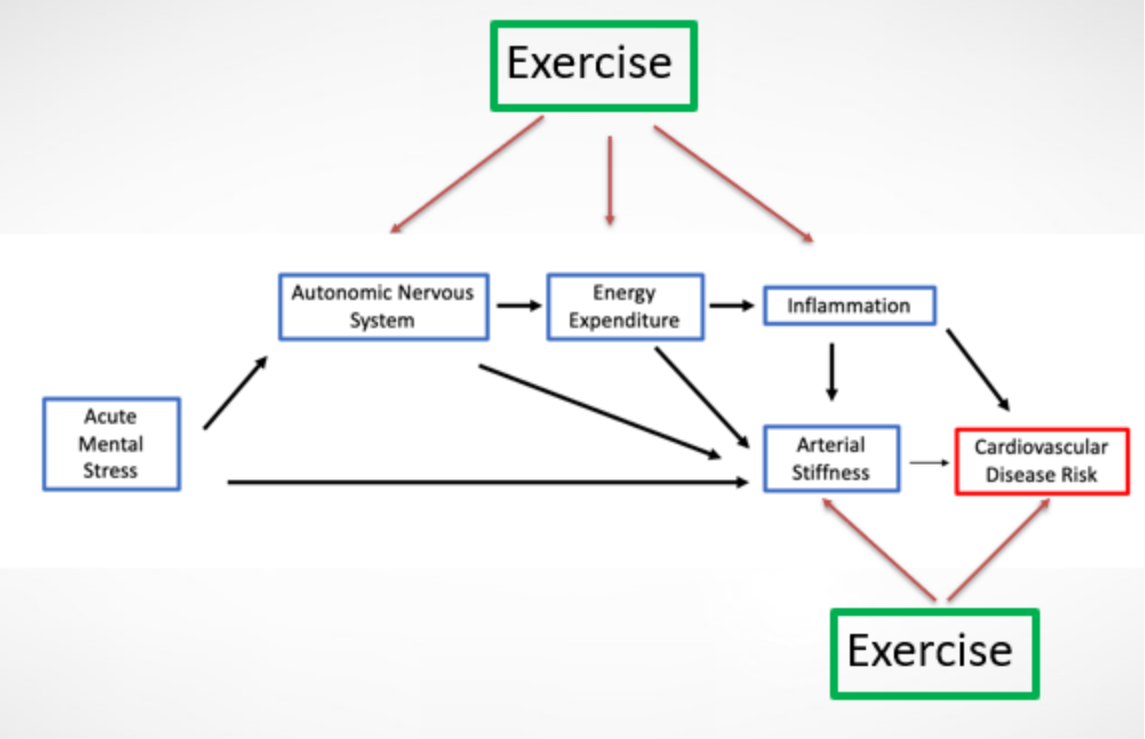

Figure 8.3. Directed acyclic graph (DAG) for plausible pathways linking interactions between aerobic exercise and stress-related effects on arterial stiffness and energy expenditure in the context of the constrained energy theory and cardiovascular disease risk. Abbreviations: *ANS, autonomic nervous system; AS, arterial stiffness; CVD, cardiovascular disease; EE, energy expenditure; MS, mental stress (acute).*

Recommendation for Future Research

Arterial stiffening and energy expenditure responses may be key players in the relationship between mental stress and CVD risk. Future work is particularly warranted to explore this relationship further within the framework of the constrained energy theory, which posits that exercise may divert energy use away from stress-reactivity and subsequent CVD risk development. For example, future studies should seek to include assessment of inflammatory markers (e.g. cytokines, interleukins). Future studies are also warranted to explore how other lifestyle behaviors (e.g. exercise, mindfulness, diet, sleep) and lifestyle-associated factors (e.g. psychological resilience, cardiorespiratory fitness) of cardiovascular function may also influence

the effects of repeated or prolonged exposure to acute stress on CVD risk. Better understanding the pathophysiological chain of events implicated in the stress-CVD paradigm is necessary to inform stress-related CVD prevention and treatment efforts.

APPENDIX 1: PUBLICATIONS AND PRESENTATIONS

Publications Arising from this Thesis (in Anticipated Order of Publication)

1. Systematic Review and Meta-Analysis: The effects of acute mental stress on arterial stiffness

2. Original Research (Experimental Study 1): The energy expenditure and arterial stiffness response to, an acute mental stressor

3. Original Research (Experimental Study 2): Effects of a prior aerobic exercise bout on arterial stiffness and energy expenditure following exposure to an acute mental stressor

Other Publications During PhD

1. Castro N, **Zieff G***, Bates LC, Pagan Lassalle P, Higgins S, Faulkner J, Lark S, Skidmore P, Hamlin MJ, Signal L, Williams MA, Stoner L. A Cross-Sectional Investigation of Preadolescent Cardiometabolic Health: Associations with fitness, physical activity, sedentary behavior, nutrition, and sleep. *Children*. 10(2):336. PMID: 36832464. (*Co-first author)

2. **Zieff G**, Stone K, Paterson C, Fryer S, Diana J, Blackwell J, Meyer ML, Stoner L. 2023. Pulse-wave velocity assessments derived from a simple photoplethysmography device: agreement with a referent device. *Frontiers in Cardiovascular Medicine*. 10: 1108219. PMID: 36824455.

3. Castro N, Bates LC, **Zieff G**, Pagan Lassalle P, Faulkner J, Lark S, Hamlin M, Skidmore P, Signal L, Williams MA, Higgins S, Stoner L. Adiposity in preadolescent children: associations with cardiorespiratory fitness. *PLOS ONE*. 17(10):e0275982. PMID: 36288267.

4. Hackney AC, **Zieff G**, Lane AR, Register-Mihalik JK. (2022) Marathon running and sexual libido in adult men: Exercise training and racing effects. *Journal of Endocrinological Science*. 4(1):10-12. PMID: 36068871.

5. Callahan C, Stoner L, **Zieff** G, Register-Mihalik JK. 2022. The Additive Benefits of Aerobic Exercise and Cognitive Training Post-Concussion: Current Clinical Concepts. *Journal of Athletic Training*. (Online ahead of print: doi: 10.4085/1062-6050-0186.22). PMID: 35984726.

6. **Zieff G,** Stoner L, Frank B, Gaylord S, Battle S, Hackney AC. 2022. Aerobic exercise, mindfulness meditation, and stress-reduction in high-stress, college-based young adults. *Journal of American College Health*. doi: 10.1080/07448481.2022.2076103. PMID: 35613415.

7. Fryer S., Paterson C., Stoner L., Brown M, Faulkner J, Turner L, Martínez Aguirre-Betolaza A, Zieff G, & Stone, K. 2022. Leg fidgeting improves executive function following prolonged sitting with a typical Western meal: A randomized controlled cross-over trial. *International Journal of Environmental Research and Public Health.* 19(3): 1357. PMID: 35162381.

8. Paterson C, Fryer S, Stone K, **Zieff G**, Turner L, Stoner L. 2021. The effects of acute exposure to prolonged sitting, with and without interruption, on peripheral blood pressure among adults: A systematic review and meta-analysis. *Sports Medicine*. 52(6): 1369-1383. PMID: 34932203.

9. Stone K, Fryer S, Faulkner J, Meyer ML, Heffernan K, Kucharska-Newton A, **Zieff G**, Paterson C, Matsushita K, Hughes TM, Tanaka H, Stoner L. 2021. Associations of lower-limb atherosclerosis and arteriosclerosis with cardiovascular risk factors and disease in older adults: The atherosclerosis risk in communities (ARIC) study. *Atherosclerosis*. 340: 53-60. PMID: 34799100.

10. Pagan Lassalle P, Meyer ML, Conners R, **Zieff G**, Rojas J, Faghy MA, Vermeesch AR, Joseph RP, Stoner L. 2021. Targeting sedentary behavior in minority populations as a feasible health strategy during and beyond COVID-19: On behalf of ACSM-EIM and HL-PIVOT. *Translational Journal of the American College of Sports Medicine*. 6(4):e000174. PMID: 34746382.

11. K Stone, Fryer S, Faulkner J, Meyer ML, Heffernan K, **Zieff G**, Paterson C, Lambrick D, Stoner L. 2021. The aortic-femoral arterial stiffness gradient demonstrates good between-day reliability. *Hypertension Research*. 44(12): 1686-1688. PMID: 34548651.

12. Conners R, Bates LC, Lassalle PP, **Zieff G**, Whitehead PN, Stevens S, Killen L, Cochrum R, Rodebaugh KL, Faghy M, Stoner l. Current and Future Implications of COVID-19 among Youth Wheelchair Users: 24-Hour Activity Behavior. *Children*. (2021). *Children*. 8(8)690.

13. Anderson KC, **Zieff G**, Paterson C, Stoner L, Weltman A, Allen JD. 2021. The effect of acute exercise on pre-prandial ghrelin levels in healthy adults: a systematic review and meta-analysis. *Peptides*. 145: 170625.

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23. **Zieff G,** Bates LC, Kerr ZY, Moore JB, Hanson ED, Battaglini C. 2020. Targeting sedentary behavior as a feasible health strategy during COVID-19. *Translational Behavioral Medicine.* doi: 10.1093/tbm/ibaa101.

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25. Paterson C, Fryer S, **Zieff G,** Stone K, Credeur DP, Barone Gibbs B, Padilla J, Parker JK, Stoner L. 2020. The effects of acute exposure to prolonged siting with and without interruption on vascular function among adults: A meta-analysis. *Sports Medicine*. 50(11):1929-1942.

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2. Stoner L, **Zieff G**, Vermeesch A, Pomeroy A, Pagan Lassalle P. COVID-19 and Exercise is Medicine for Underserved Communities: Lessons Learned, Future Challenges, and Recommendations (Symposium). American College of Sports Medicine National Conference. San Diego, CA (2022).

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APPENDIX 2: DEFINITION OF KEY TERMINOLOGY

Table A.1. Definitions of key terminology.

Term	Definition
Arterial Stiffness (AS)	The biological process of reduced elasticity and increased rigidity within the artery, which is associated with aging and atherosclerosis
Energy Expenditure (EE)	The amount of energy (kilocalories) expended
Eustress	A positive stressor (e.g. one that promotes beneficial adaptations in an organism such as aerobic exercise)
Heart Rate Variability (HRV)	A measure of autonomic function based on the variability in the R-R intervals of electrocardiography
Laboratory Stressor	A laboratory-based stressful stimuli created and administered by researchers to investigate psychophysiological reactivity to acute mental stress
Mental Stress	A broad term describing the psychological perception of negative, stressful stimuli
Pulse-wave Velocity (PWV)	The speed of the forward pressure-wave in the arterial tree following ventricular ejection; the gold-standard measurement of arterial stiffness
Resting Metabolic Rate (RMR)	The minimum energy expenditure needed to sustain basic physiological functioning (e.g. excluding thermic effect of activity and digestion)
Trier Social Stress Test (TSST)	A common laboratory stressor which typically combines a mental arithmetic test and speech task

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