ACUTE MENTAL STRESS AND ARTERIAL STIFFNESS: A COMPARISON BETWEEN HISPANIC AND NON-HISPANIC WHITE ADULTS

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ABSTRACT

Jacklyn Rojas: Acute Mental Stress and Arterial Stiffness: A Comparison Between Hispanic and Non-Hispanic White Adults
(Under the direction of Malia Blue)

Hispanics face a disproportionate burden of cardiovascular disease (CVD) risk factors, including mental stress, when compared to non-Hispanic white adults (NHWA). However, the mechanisms underlying these disparities are not fully understood. This study aimed to investigate the cardiovascular response to acute mental stress in Hispanic and NHWA. The randomized crossover design included six participants (all female; four Hispanics) and assessed arterial stiffness using pulse wave velocity as the primary outcome measure. Hispanics exhibited the greatest increase in PWV during the control condition, while NHW individuals showed the greatest increase during the experimental condition. State stress measures also demonstrated significant differences before and after both test conditions. These findings suggest a counterintuitive cardiovascular response to acute stress in Hispanic adults when compared to NHWA. This study highlights the need for further research to understand the complex relationship between mental stress and cardiovascular risk in different populations, in particular, Hispanic adults.

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LIST OF ABBREVIATIONS

AS	Arterial stiffness
ANS	Autonomic nervous system
CO	Cardiac output
CV	Cardiovascular
CVD	Cardiovascular disease
HR	Heart rate
HPA	Hypothalamic-pituitary-adrenal axis
NHW	Non-Hispanic white
PNS	Parasympathetic nervous system
SDOH	Social determinants of health
SRS	Stress response system
SNS	Sympathetic nervous system
TSST	Trier Social Stress Test

CHAPTER 1. INTRODUCTION

THESIS STRUCTURE

This thesis comprises 6 chapters. Chapter 1 (this chapter) provides background information and rationale for this dissertation. Chapter 2 is a literature review, which briefly outlines the significance of the proposed research, and Chapter 3 then provides rationale for each study design aspect. Chapter 4 is an in-depth review of the methodology, including information about the experimental design, measures, outcome(s), variables, etc. Chapter 5 provides the results of this study including information on the participants, baseline data, outcomes, and analysis. Finally, Chapter 6 is a discussion of the key findings, including the implications of the findings and suggestions for further research.

THESIS RATIONALE

Importance of Topic: Hispanics are the largest growing minority group in the U.S. and carry a disproportionately heavy burden of cardiovascular disease (CVD) risk factors including high cholesterol, diabetes mellitus, and, of particular interest, mental stress, compared to non-Hispanic white adults. [1-4] Due to the lack of research addressing the health disparities faced by Hispanic adults, the mechanisms by which they arise are not yet fully understood. [5] This study strives to begin closing the gap in the literature on Hispanics by comparing the CV response to mental stress between Hispanic and non-Hispanic white adults.

What is Known: Mental stress is an unavoidable aspect of life that can either be acute or chronic in nature, and adaptive or maladaptive. Repeated acute and chronic stress are both associated with increased CVD risk. [6-13] One plausible mechanism by which increased CVD risk may ensue from mental stress is autonomic nervous system (ANS) and vascular dysfunction. [14-16] Vascular health can be assessed via arterial stiffness (AS) as estimated by pulse wave velocity (PWV) and further understood via pulse wave analysis (PWA). Autonomic function can be assessed via heart rate variability (HRV). Additionally, while it is unethical to induce chronic stress on participants, acute stress can be reliably produced/measured in a laboratory setting for a relatively short period of time, making it both an accessible and ethical tool for researchers to induce a stressor. [17-18]

Gap in Knowledge: It is not currently fully understood how acute mental stress may lead to increased CVD risk, and whether Hispanics and NHW adults' CV systems respond differently to acute stress. This study is needed to begin to understand how acute mental stress impacts CVD risk and play a role in increasing Hispanic-centric research.

OVERALL OBJECTIVE AND APPROACH

The long-term goal of this thesis is to facilitate the understanding of the effect of acute mental stress on cardiovascular reactivity in ethnic minorities, such that development of strategies to prevent CVD occurrence via non-invasive predictive measures of health are possible. To support this goal, the overall objective of this project is to observe and compare the arterial stiffening response to a laboratory psychosocial stressor in both Hispanic and non-Hispanic white adults (NHWA). This objective is unique and crucial to understanding the mechanisms by which acute stress impacts CV responses, as most studies investigating mental acute stress and CVD do not include nor compare the responses on Hispanics to NHW adults.

Aim 1. To determine if a difference exists in the PWV/PWA response to an acute mental stressor bertween Hispanic and NHW adults. **Aim 1 Rationale.** Hispanic adults experience disproportionate levels of mental stress and CVD risk factor occurrence. Identifying a disparity in a CVD precursor can mitigate CVD risk. **Summary.** Fulfilling this aim will begin to broaden the understanding of the mechanisms by which CVD risk factors such as mental stress disproportionately affect Hispanic adults when compared to NHW.

SPECIFIC AIMS

Aim 1: To determine if a difference exists in the AS response to an acute mental stressor between Hispanic and NHW adults. **Aim 1 Strategy:** PWV/PWA was measured before and after both the TSST and control interventions. The results attained from this study will be compared using linear mixed models. **Aim 1 Hypothesis:** Hispanics will have an exaggerated AS response to the TSST when compared to the NHW adults.

INNOVATION AND SIGNIFICANCE

Expected Outcomes. There will be a significant increase in AS after the mental stressor for both groups, and there will be a greater increase in Hispanic adults compared to NHWA. **Innovation**. No other study (to the author's knowledge) has investigated whether a link exists between an acute mental stressor and AS. Further, no other study has compared the AS response to an acute mental stressor between ethnicities; in particular, Hispanic and NHW adults. **Significance.** Completion of this project will provide insight as to whether developing a strategy for dealing with acute stress would be of value when considering how to mitigate CVD risk. Further, this project will inform future studies that wish to delve deeper into investigating the mechanisms by which CVD risk accrues and differs in Hispanics versus NHW.

CHAPTER 2. LITERATURE REVIEW – SIGNIFICANCE

OVERVIEW

The overarching problem tackled in this project is depicted in **Figure 1.** The variables in the figure below are key factors that were considered in in designing this proposal. Each consideration will be outlined below in Table 1, and I will clearly state why this is a key consideration and how a resolution was determined.

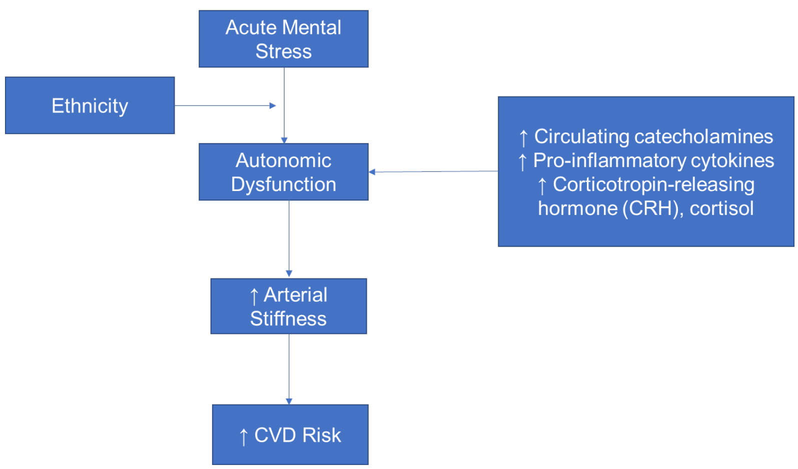

Figure 1. The relationship between variables considered in this thesis.

Key Message

ETHNICITY MODERATES THE AUTONOMIC AND CARDIOVASCULAR RESPONSE TO AN ACUTE MENTAL STRESSOR. POTENTIAL AUTONOMIC DYSFUNCTION AND INCREASE IN ARTERIAL STIFFENING LEADS TO INCREASED CVD RISK.

Table 1. Key points in literature review..

Topic	Page(s)
Health disparities, the social determinants of health and stress	15-17
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HEALTH DISPARITIES, THE SOCIAL DETERMINANTS OF HEALTH AND MENTAL STRESS

The following section will define and provide a brief overview on the social determinants of health, health disparities, and mental stress as it relates to this thesis.

Health disparities are defined as “differences in the incidence, prevalence, mortality, and burden of diseases and other adverse health conditions that exist among specific population groups in the United States.” [19] It is known that ethnic minorities are at higher risk for numerous pathologies, such as cardiovascular disease (CVD) and its risk factors, than their NHW counterparts. While the existence of health disparities has been known for decades, the mechanisms are not yet fully understood. This is due in part to the fact that the emergence of health disparities is likely multifactorial and highly complex in nature. However, there have been a group of factors linked to health outcomes and disparities, known as the social determinants of health (SDOH). The SDOH are the "conditions in the environment where people are born, live, learn, work, play, and age, that affect a wide range of health, functioning, and quality-of-life outcomes and risks.” [20-21] The quality and stability of these conditions are influenced by factors such as financial standing, social support networks, and mental stress. This thesis is particularly interested in examining stress and its impact on physiological responses.

The word “stress” is used in a variety of contexts, and as such, has become quite an ambiguous term. For the purposes of this thesis, however, the definition of stress is “a real or interpreted threat to the physiological or psychological integrity of an individual, that results in physiological and/or behavioral responses." [22] Psychological stress, also referred to as mental stress, is the primary variable of interest in this model. Therefore, the reader should consider any uses of "stress" to mean mental stress, unless otherwise stated.

Stress is a ubiquitous, inescapable fact of life that can be acute or chronic in nature. To a certain extent, both acute and chronic stress can result in positive adaptations that benefit an individual's life and window of tolerance. Too much stress of either kind, however, results in negative psychological and physiological outcomes, as will be further detailed in a later section. It is known that ethnic and racial minorities face greater exposures to stress than their white counterparts [23], and many models of health disparities argue that stress plays a central role in the distribution of disease among different populations. [16, 24] However, most research has only considered health outcomes as a factor of chronic stress. Acute mental stress must not be forgotten about, as it still contributes to a person's overall stress exposure—which, especially since the election of Donald Trump in 2016, has become exacerbated in minority communities. [25-27] Specifically, Hispanic Americans have expressed that their situation has worsened significantly. [28] For example, 66% of Hispanic Americans report they are worried about their loved ones (or themselves) being deported, and 40% of Hispanics have experienced offensive and discriminatory incidents because of their ethnicity. Incidents reported include being criticized for speaking Spanish in public, being told to go back to their home country, and being called derogatory names. [28] These incidents are not a burden non-Hispanic white adults have to carry. The lack of research on Hispanic populations, who face a disproportionate amount of mental stress—a well-established risk factor for CVD—was crucial in developing this thesis' research question.

The following sections of this thesis will provide insight into "normal" stress response systems' functioning and mechanisms by which increased mental stress can contribute to CVD risk, such as ANS dysfunction, advanced vascular aging, and arterial stiffening. First, however, it is important to further elaborate on the rationale for recruiting Hispanic adults by discussing the disparities in CVD risk factors observed in said population.

HISPANICS ARE DISPROPORTIONATELY AFFLICTED WITH CVD RISK FACTORS

It has been well established that Hispanic populations carry a significant and unique burden of CVD risk when compared to their NHW counterparts. [1, 2, 29–31] For example, incidence of high cholesterol (HC) is significantly higher. According to the National Health and Nutrition Examination Survey (NHANES) data, average LDL cholesterol levels for NHW men and women in the U.S. were 141.7 and 119.1 mg/dL respectively, compared to an average of 161.4 and 134.1 mg/dL in Mexican Americans. [32] Despite the higher prevalence of HC, only 49% of Hispanic individuals affected by it were aware of their condition, and alarmingly, less than a third were receiving treatment. [29] Unsurprisingly, individuals who do not receive treatment for HC have an increased risk of developing CVD. [33] Further, the risk of being diagnosed with diabetes mellitus is 66% higher for Hispanics compared to NHW, and there is a higher mortality rate in Hispanics with DM when compared to NHW adults. [2]

The stark differences in the burden of CVD risk in Hispanics compared to NHW adults must not be ignored. Hispanics in the US mainland makeup the largest and fastest growing population in the U.S.[31], projected to make up 30% of the U.S. by 2050. [34, 35] Yet, research on Hispanic populations is grossly sparse. One of the numerous gaps in Hispanic-centric literature is an understanding of how these disparities in CVD risk factors arise. Targeted research that is conscious of the unique challenges presented to the Hispanic community is necessary to elucidate the complex manners in which disparities may arise, and can lead to further improvements in the overall CV public health burden in the U.S.

THE STRESS RESPONSE SYSTEM: PHYSIOLOGY

As briefly mentioned before, one plausible method for increased CVD risk is the disproportionate exposure to stress and resulting ANS dysfunction. Consequently, the following section will provide definitions and concepts related to stress physiology. The body’s physiological systems exist in a pre-programmed state, known as *homeostasis,* where functionality is optimized, and health is maintained. This optimal state is constantly challenged in numerous ways, and these challenges are called “stressors.” Stressors can be internal or external, perceived or real. As mentioned earlier, they can be categorized as psychological, environmental, or physiological, and result in an inability of the body to function normally. [36] An example of an “actual” stressor is seeing a grizzly bear in your home, whereas a “perceived” stressor would be waiting in your car during a traffic jam for several hours. In the former, your physical safety is threatened; in the latter, your sanity is threatened. Regardless of type, when the body experiences a stressor of any kind, there is a coordinated chain of events that ensues. These chains of events are orchestrated by the Stress Response System (SRS).

The SRS is comprised of the sympathetic branch (SNS) of the ANS and the hypothalamic-pituitary-adrenal (HPA) axis. [10] When the body experiences a stressor, the SNS is activated, while the parasympathetic nervous system (PNS) is inhibited. Activation of the SNS triggers the release of catecholamines, which bind to their respective receptors and results in a cascade of physiological responses. Heart rate, blood pressure, vasoconstriction, and cardiac output all increase as a result of this process. [37] Further, SNS activation results in the release of corticotrophin-releasing hormone (CRH) from the hypothalamus, which stimulates another cascade of physiological responses, culminating with the release of cortisol from the HPA axis. [38] The release of cortisol augments ANS activity—specifically, the earlier discussed CV responses to stress: increased heart rate, cardiac output, etc. Figure 2 helps to visually tie the chain of events just described together. Evidently, the ANS and the HPA axis work hand in hand to deal with a stressor, which is why they are considered the components of the SRS. When the stress load is too great for the SRS to handle, the body enters a state known as "allostatic overload," and several pathophysiologies, including ANS dysfunction, are observed.

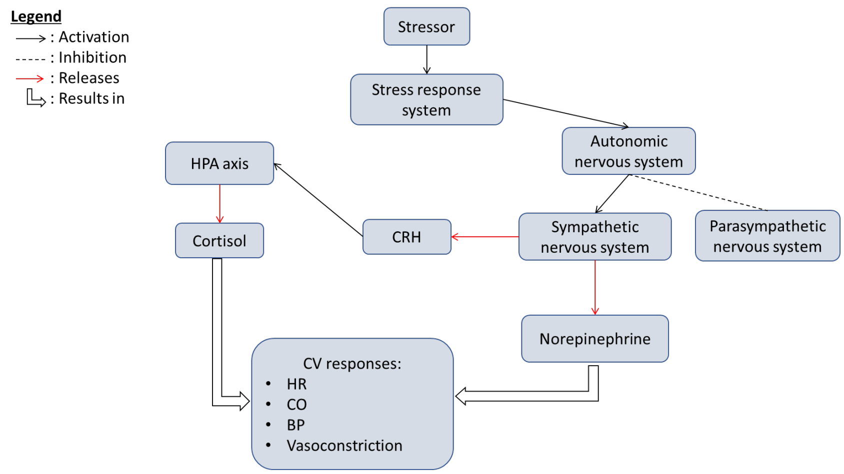

Figure 2. The stress response system is activated and results in cardiovascular reactivity.

THE HEALTH CONSEQUENCES OF STRESS-INDUCED ALLOSTATIC OVERLOAD

When the body is adequately able to cope with a stressor, the SRS is activated for an appropriate amount of time, and then turns off. This is known as allostasis - the active process by which the body responds to daily events and maintains homeostasis. [39] When the body is unable to cope with the stressor, a state of allostatic overload (AO) results, which is characterized by four types of responses: repeated "hits" from multiple stressors, lack of adaptation, prolonged response, and abnormally low physiologic response over time. [40] These responses are summarized in **Figure 3** below. As a result of AO, various pathophysiological conditions are observed, including immune dysfunction [41], [42], inflammation [43], and tumor growth. [44] The condition this thesis is particularly interested in due to its prevalence, subtle onset, and disproportionate toll on Hispanic communities (which will be discussed in a later section), is increased CVD risk.. A strong longitudinal association between chronic stress and CVD risk has been established in the literature [12, 43, 46, 47], however, the mechanisms by which this increased risk comes about is not yet fully understood. One plausible biological mechanism by which increased stress and AO result in increased CVD risk is ANS dysfunction.

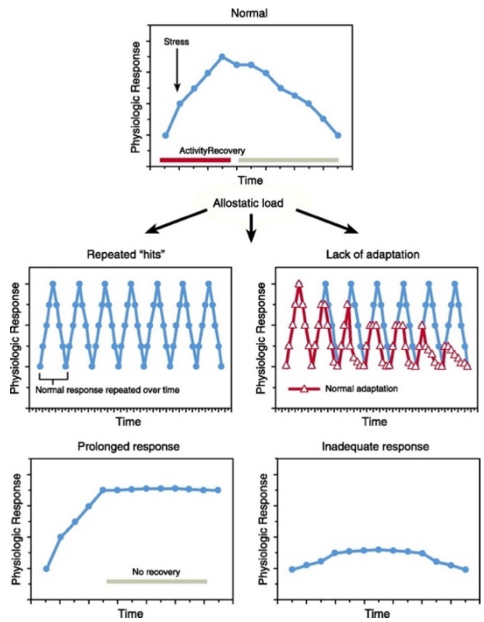

Figure 3. The potential physiologic responses to stressors.

THE IMPACT OF ANS DYSFUNCTION ON VASCULAR PHYSIOLOGY

As discussed earlier in this literature review, the body's "normal" physiologic response to a stressor is orchestrated in part by the ANS, which stimulates the release of catecholamines and CRH. Under normal functioning conditions, the parasympathetic branch of the ANS can moderate SNS activity to return the body back to a resting state. However, when faced with allostatic overload, the ability of the PNS to moderate the SNS response is diminished during both a stress exposure and rest. For the purposes of this review, this phenomenon is referred to as ANS dysfunction. Blood vessel walls are innervated by both PNS and SNS fibers and, as a result of ANS dysfunction, the body experiences a detrimental effect on vascular function and structural integrity. [48-49] The literature has demonstrated that enhanced SNS activity induces increased endothelial dysfunction (ED) [7], [50]–[52], a precursor to atherosclerosis. [13, 50, 52] However, the majority of studies have shown the link between SNS activity and ED by collecting data on traditional measures such as heart rate (HR), blood pressure (BP), and flow-mediated dilation (FMD). Another variable worth investigating is arterial stiffness as it is both associated with CVD risk and affected by acute and chronic stress. [8, 9, 13, 50, 53, 54]

VASCULAR STRUCTURE, FUNCTION, AND AGING

The following section will provide a brief overview of arterial stiffness and vascular aging as it relates to increased CVD risk. Vascular aging refers to changes in the mechanical and physical properties of the vascular wall and is mainly determined by the happenings in the endothelial extracellular matrix (ECM). [55] The ECM is made up of contractile smooth muscle cells and elastic fibers that is constantly undergoing remodeling to maintain health. With age and disease, this remodeling system is disrupted, resulting in the fragmentation of elastin fibers and increased synthesis of the (much stiffer) collagenous fibers. These two processes result in an overall decrease of elasticity and compliance of vessels; i.e., arterial stiffening. [56]

Arterial stiffness (AS) is estimated via pulse-wave velocity (PWV). As the arterial wall becomes stiffer due to the remodeling mentioned above, PWV increases, and reduces the ability of the arteries to buffer increasing pressure and energy as needed. This higher level of blood pulsatility may injure the smaller vessels in the brain and kidneys as they are not equipped to handle the increased energy. The heart is affected by AS as well, not by increased energy, but by alterations in wave reflection timings and decreases in coronary perfusion. Consequently, organ damage is observed. [57] These events have significant implications in the context of CVD risk. For example, one meta-analysis of 17 longitudinal studies found that an increase in PWV was associated with an increased risk of a CV event, CV mortality, and all-cause mortality. [56] Evidently, using PWV to infer about AS is an excellent non-invasive measure of CVD risk.

As alluded to earlier, AS has been demonstrated to be affected by acute and chronic stress. Of particular interest to this thesis, one study demonstrated that an acute laboratory stressor induces increased AS via PWV. However, not much other research has been done investigating acute stress and

AS; much less whether the response differs between ethnicities. Due to the limited knowledge on the AS response to an acute stressor, the limited knowledge on the mechanisms behind CVD risk disparities and the strong stress-CVD risk and AS-CVD risk associations, this thesis argues it is critical to investigate the AS response to an acute stressor as it relates to ethnicity. The following sections will describe considerations for measuring AS via PWV and for measuring the acute stress response.

PWV AS AN ESTIMATE OF ARTERIAL STIFFNESS

This section will describe how PWV can be employed as a measure of arterial stiffness in this study. While there are many non-invasive methods that can be employed for measuring AS such as applanation tonometry, oscillometry, and doppler ultrasound, the present study used oscillometry. The reasons for this are detailed later in the *Methodology* section of this thesis. With oscillometry, AS can be measured using various segments of the arterial tree including carotid-femoral PWV, brachial ankle PWV, brachial femoral PWV (cf, ba, bf-PWV). The gold-standard is cf-PWV because of its clinical relevancy, reproducibility, and simplicity. [58] However, to measure cf-PWV would require the use of a balloon pad placed around participants' neck, which is often cited as uncomfortable for participants. Further, the balloon used for cf-PWV would interfere with other outcomes, such as brain blood flow, that the co-investigator, Madeline Rheault (MR), was collecting as part of this study. Carotid-femoral PWV values are closely related to those values obtained from bf-PWV measurements. [59] Therefore, to prevent participant discomfort and confounding of study outcomes, bf-PWV was chosen as appropriate for this study.

THE TRIER SOCIAL STRESS TEST IS IDEAL FOR THIS STUDY

In this section, the most common stress-evoking modalities will be briefly reviewed in terms of their procedure and effectiveness at evoking a stress response. The first stress-induction test to be discussed is the Socially Evaluated Cold Pressor Test (SECPT), which is both common and extensively validated as a method of eliciting a stress response. The SECPT combines both physiological and psychological stressors by having participants immerse one hand in ice water while being socially evaluated, i.e., watched, by the experimenter and video camera. [60] Another stress-induction modality is the Maastricht Acute Stress Test (MAST). The MAST also combines a physiological and psychological stressor by having participants alternate between immersing their hand into ice water and engaging in a mental arithmetic task. The MAST protocol induced similar changes in BP immediately after a stressor as the previously mentioned SECPT and the Trier Social Stress Test. [61] The TSST is an ecologically valid stressor that can robustly induce an acute stress response under controlled conditions such as a laboratory setting. The TSST is a purely psychological stressor that incorporates social evaluation and unpredictability as participants are asked to speak in front of a neutral audience and complete a spontaneous mental arithmetic test. Due to the reliable, robust and modifiable nature of the TSST, it is considered the gold standard for inducing human experimental stress. [17]

While the SECPT and MAST protocols are fine choices for inducing stress in general, the TSST is the ideal choice for the purposes of the present study. The TSST elicits solely a psychological stressor, whereas both the SECPT and MAST protocols induce a physical stressor (ice water) as well. Employing either the SECPT or MAST protocol in this study would add a confounding variable (pain) and would make it difficult, if not impossible, to determine whether the CV response observed was due to the psychological or physical stressor. As a result, this thesis chose the TSST as the stress-inducing protocol of choice.

LITERATURE REVIEW SUMMARY

WHY IS THIS STUDY NEEDED?

Research targeting the impact of acute stress on CV health outcomes among all populations, but especially in Hispanics, is limited. This study will help to elucidate mechanisms by which acute stress may or may not lead to increased CVD risk, which can then lead to randomized control trials which can help create and promote specific, effective public health strategies for reducing CVD risk.

WHAT IS KNOWN

Hispanics are the fastest growing population in the U.S., predicted to represent 30% of the U.S. by the year 2050. Further, Hispanics carry a significantly heavier burden of CVD risk factors, including acute stress, than their NHW counterparts.

WHAT IS NOT KNOWN

It is not fully understood how acute stress may affect CV outcomes, and how this may translate to the development of CVD risk. Further, it is not known if the CV stress response differs between ethnicities—of particular interest, between Hispanics and NHW adults.

CRITICAL NEED

There is a critical need to develop strategies specific to high-risk populations, such as Hispanics, to dampen the accrued CVD risk over time.

WHAT THIS STUDY WILL ADD

This study will provide a starting ground for researchers to think critically about the ways in which acute stress may impact CV health for NHW and Hispanic adults.

CHAPTER 3. LITERATURE REVIEW – RATIONALE FOR APPROACH

OVERVIEW

This section will outline all considerations made while designing the study to provide better understanding into our methodological choices.

POPULATION AND SAMPLING CONSIDERATIONS

The researcher sought to recruit exclusively participants who identified as either non-Hispanic white or Hispanic to allow for fulfilment of study aims. Further, only young, healthy individuals (defined as free of cardiometabolic and anxiety-related mental health disorders) of both sexes were recruited to minimize confounding variables. People who engage in smoking and who had a body mass index (BMI) greater than 30 were not eligible to participate in the study due to the impact smoking and obesity has on vascular function. Finally, individuals who were pregnant were ineligible to participate in the study because of the impact hormone fluctuations have on the outcome measures of interest. This information is summarized in **Tables 2 and 3**.

Table 2. Inclusion criteria.

Criteria	Method	Rationale
Age 18-35	Screening interview	To ensure normal physiological responses
Non-Hispanic white or Hispanic	Screening interview	To obtain participants that self-identify as Hispanic or Non-Hispanic White

Table 3. Exclusion criteria.

Criteria	Method	Rationale
Pregnant	Screening interview	Women who are pregnant have heterogenous hormonal profiles. Including them in the study would add unwanted confounding variables.
Current tobacco or nicotine user	Screening interview	Smoking tobacco/nicotine containing products actively negatively impact CV

		health. This study is interested in observing the AS response to stress in a healthy population.
Currently taking medication that alters CV functioning	Screening interview	This study is interested in the unadulterated AS response to stress; medications known to affect CV functioning, such as beta blockers, will dampen the CV response.
History of CVD or anxiety disorders	Screening interview	Individuals with CVD and anxiety disorders have disrupted systems physiology; this study needs healthy participants for the aims to be properly fulfilled
Abbreviations: CV, cardiovascular; AS, arterial stiffness; CVD, cardiovascular disease		

STUDY DESIGN CONSIDERATIONS

Several study designs were considered in addressing the research question. These designs are summarized in **Table 4** and are discussed below.

Table 4. Study design considerations.

Consideration	Choices	Selection	Explanation
Study design	Observational study Interventional study	Observational study	Interventional study would not have been a possibility as this would require asking a participant to come to the laboratory when they experience an acute mental stressor.
Control group	Compare a mentally stimulating task to a mentally challenging task Compare a "boring" task to a mentally challenging task	Compare a "boring" task to a mentally challenging task	A mentally stimulating task could have still resulted in a change in PWV comparable to the one seen in response to the mentally challenging one

INTERNAL VALIDITY CONSIDERATIONS

Investigators followed established screening procedures to recruit a homogenous sample group. A group relatively similar in age, activity, and health was recruited to simplify analysis and increase the validity of experimental conclusions. The measures taken to maintain internal validity are further detailed in **Table 5.**

Table 5. Internal validity.		
Consideration	Explanation	Control Procedure
Screening procedures	Ensures homogeneous sample, eliminates potential covariates	During recruitment, participants were required to provide their age, medical history, and ethnicity/race.
Standardized window for data collection	Prevents within and between subject variation in cardiovascular and hormonal measures due to daily circadian rhythms	All measurements occurred between the hours of 4:00 and 5:30 PM.
Standardized data collection settings	Prevents errors in PWV calculation	During the familiarization visit, all path lengths were measured using the same segmometer.
Standardized environment (quiet, well-lit room)	Prevents fluctuations in outcome measures not solely due to experimental procedures	During the experimental and control visits, curtains were drawn to alert others a study visit was in progress. All the lights were on.

PRE-ASSESSMENT GUIDELINES CONSIDERATIONS

To ensure data was collected under standardized conditions, pre-assessment guidelines (PAG) were established: participants abstained from alcohol and moderate to vigorous physical activity (MVPA) 24 hours prior to testing, and from food and caffeine 4 hours prior to testing. The time of day at which testing occurred was also standardized as much as possible, both within and between subjects. Finally, the researcher confirmed the participants were experiencing a “normal” stress level (defined as scoring less than 50 mm on the visual analog scale) at the beginning of the visit. If the participant failed to meet one or more of the PAG, their visit was rescheduled. **Table 6** summarizes these pre-assessment guidelines.

Table 6: Pre-assessment guidelines		
Guideline	Explanation	Control Procedure
Standardized beginning time for all experimental sessions	Prevent activities of daily living from interfering with data quality	All experimental sessions commenced between 3:00 – 5:30 PM
No moderate to vigorous PA in the 24 hours before testing	Prevent prolonged effects of moderate-vigorous PA on CV system from influencing data	Individuals will be reminded two days prior to an experimental session to refrain from vigorous physical activity for at least 24 hours prior to each visit.
Prevention of alcohol and caffeine consumption before experimental sessions	Prevent effects on CV system and data (e.g., hydration status, heart rate)	Individuals were reminded two days prior to an experimental session to refrain from caffeine and alcohol consumption for at least 12 and 24 hours prior to each visit, respectively.
Prevention of food consumption before experimental sessions	Prevent within- and between-subject variation in CV data due to insulin release and/or different meals composition.	Individual will be reminded the day before an experimental session to refrain from food consumption for at least 4 hours prior to each visit
Normal stress level at intake	A high-stress state will impact the primary outcomes of this study and confound the stress response. Important to ensure "normal" stress level for a given participant	Use of the visual analog scale (VAS) prior to visit to ensure they score < 50mm, which would constitute a normal stress level.
Abbreviations: PA, physical activity; CV, cardiovascular		

SEX AS A BIOLOGICAL FACTOR

This study was not powered to assess sex differences. Women who were experiencing or had experienced menopause were unable to participate in this study due to the related changes to their cardiovascular system. The women in this study underwent all testing during the beginning of the follicular phase of their menstrual cycle, because estradiol levels were likely to be lowest. Estradiol is likely to be a covariate of PWV. [62], [63] Information on birth control was not collected.

ETHNICITY/RACE

Participants were included if they either self-identified as Hispanic or non-Hispanic white. The research team aimed to recruit an equal number of participants from each ethnic group.

EXTERNAL VALIDITY / GENERALIZABILITY

External validity can be maintained by constructing a sample that represents the population of interest. In this case, we were interested in assessing Hispanic and NHW adults around the University of North Carolina at Chapel Hill (UNCCH). To do this, investigators sampled from students across the university and surrounding towns, so that the sample can match the desired demographics as closely as possible. All studies balance external and internal validity measures, and this study is included. This study focused on internal validity to establish consistent results of a mechanism, the function of which can then be explored in other populations.

MEASUREMENT CONSIDERATIONS

To fill the knowledge gaps identified in this thesis, the investigator explored changes in vascular, hemodynamic, and autonomic measures such as arterial stiffness, central systolic blood pressure (cSBP) and heart rate variability (HRV). The measures chosen are commonly used within the literature and in the clinical setting to investigate vascular and hemodynamic function. See **Table 7** for a summary of the rationale behind the outcomes of choice.

Table 7. Rationale for outcomes.

Construct	Choices	Selection	Explanation
Arterial stiffness	PWV (bf-PWV, cf-PWV, ba-PWV, aPWV) Carotid β-stiffness index	PWV (bf-PWV)	PWV is the gold standard measure of arterial stiffness. Assessing central arterial stiffness through bf-PWV allows researchers to take multi-time point measurements without having cf-PWV confound the results of other measures that were taken during this study.

Pulse wave analysis: BP and waveform qualities	pSBP vs. cSBP pAIx vs. sAIx	cSBP, sAIx	cSBP and sAIx are both highly reliable and have predictive value for CV events
ANS activity	HR HRV GSR Blood catecholamines	HRV	HRV is a robust, reproducible physiological marker that reflects cardiac autonomic systems' functioning.
Stress level (state)	Likert scale Visual analog scale (VAS) Questionnaires Biological markers (cortisol levels)	Likert and VAS	Biological markers require invasive procedures (use of needles) which may introduce a confounding source of stress. The Likert and VAS is noninvasive and less time-consuming than quesionnaires.

Abbreviations: PWV, pulse wave velocity; a-PWV, aortic; bf-PWV, brachial-femoral; ba-PWV, brachial-ankle; cf-PWV, carotid-femoral; ANS, autonomic nervous system HR, heart rate; HRV, heart rate variability; GSR, Galvanic skin response; VAS, visual analog scale; pSBP, peripheral systolic blood pressure; cSBP, central systolic blood pressure; pAIx, peripheral augmentation index; sAIx, systolic augmentation index.

MEASURE 1: PULSE WAVE VELOCITY/ARTERIAL STIFFNESS (PRIMARY OUTCOME)

Rationale for Estimating Arterial Stiffness

The term "arterial stiffness" describes the structural change that is observed when the arterial wall is composed of more load bearing than elastic components. [56] Extensive research has demonstrated that AS is independently associated with an increased risk of CVD. [55], [58], [64], [65] There is also evidence demonstrating that AS and endothelial dysfunction increases after a bout of acute mental stress. [50, 52-53, 66] Considering that this thesis aimed at investigating the relationship between mental stress and cardiovascular reactivity, estimating AS was indicated.

Methodological Options for Estimating Arterial Stiffness and Rationale for Choosing PWV

While systemic AS must be estimated, local and regional AS can be measured directly through both invasive and noninvasive methods/measures. [56], [58], [67] Only noninvasive methods were considered to ease both researcher and participant burden. Pulse wave velocity is the most frequently used and widely researched non-invasive measure of arterial stiffness. Pulse wave velocity is extremely useful due to its predictive value for CV events and is simple, robust, and highly reproducible. [56, 58, 64, 67-68] For these reasons, PWV was chosen as the measure of interest for this thesis.

PWV can be a single point or two-point measure, and it can be used to determine local or regional stiffness. Due to the lack of evidence linking single point measures of PWV to CV outcomes, two-point measures were utilized in this thesis. [64] Additionally, since measuring local stiffness is highly time-intensive and requires a great degree of technical expertise [58], measures of regional stiffness were chosen for this thesis. Further, PWV can be measured using several different methodologies, including magnetic resonance imaging (MRI), ultrasonography, applanation tonometry, and oscillometry. MRI was eliminated as an option because it requires high technical expertise, the use of expensive equipment, and is time intensive. Ultrasonography was eliminated as an option because it is used to assess local, not regional, stiffness. [64] Finally, applanation tonometry was eliminated as an option because it does not produce reliable results when used at the femoral artery. [69] Oscillometry was elected as the methodology of choice for several reasons. Firstly, oscillometry allows for precise measures that can be taken rapidly and in succession. Secondly, oscillometry allows for simultaneous measures of central blood pressure via pulse wave analysis (PWA). As a result, the use of oscillometry minimized both researcher and participant burden.

Carotid-femoral PWV (cf-PWV), one of many regional measures of stiffness, is considered the "gold-standard." However, measuring cf-PWV involves palpation of the carotid artery, which requires technical expertise and can be uncomfortable for participants. Further, cf-PWV measures are highly pressure-dependent, and can be attenuated by the presence of carotid plaque. Contrarily, brachial-femoral PWV (bf-PWV) measures induce very little participant discomfort and has very strong repeated measures agreement with cfPWV [70] As a result, bf-PWV was chosen as the regional measure of AS.

Principles and Key Considerations for PWV Measurements

The Vicorder (SMT Med) device works by inflating two oscillometric cuffs simultaneously to a sub-diastolic pressure and then recording the time it takes a pulse wave to travel between the proximal and distal cuff. The Vicorder is validated for PWV measures in a semi-reclined measure; as a result, the participants laid on a blue wedge that situated them about 30 degrees above supine level. The Vicorder device calculates PWV via a proprietary algorithm that can calculate the time between the foot of the proximal pressure waveform to the foot of the distal pressure waveform, and each measurement cycle lasts 60 seconds. To collect bf-PWV data, the proximal cuff was placed at the brachial artery and the distal cuff was placed at the femoral artery. Data was collected in triplicate and entered into an Excel file. For data reduction, recorded pressure waveforms from the brachial cuff will be automatically averaged via an Excel formula after the recording is completed by the Vicorder software. The Excel formula will help with data reduction by averaging the two closest numbers. In terms of interpreting the data, a clinically meaningful change in arterial stiffness is considered a change of 1 m/s. [71]–[73] This will be referenced again in the results chapter of this thesis.

MEASURE 2: HEART RATE VARIABILITY (SECONDARY OUTCOME)

Rationale for Measuring Heart Rate Variability

Considering that this thesis study is investigating the CV reactivity, which is regulated via ANS activity, measuring ANS activity is indicated. One measure commonly used to quantify ANS activity is heart rate variability (HRV). HRV is defined as a measure of the cyclic variations of beat-to-beat (RR) intervals [74], [75] which are modulated in real-time by the ANS. [76] Consequently, HRV is an excellent indicator of cardiac autonomic function. Further, reduced HRV is associated with increased risk of CVD and adverse CV events, including myocardial infarction and sudden cardiac death. [75], [77], [78] Perhaps most importantly, use of HRV as a marker of ANS activity has been widely used in in studies employing laboratory psychosocial stressors. [79]–[82] For these reasons, HRV was selected as the measure to assess ANS activity in this thesis study.

Methodological Options for Measuring HRV

As mentioned previously, HRV captures the beat-to-beat variation in the duration of the R-R interval.[78] The two primary approaches to measuring HRV include time domain and frequency domain methods. While frequency domain methods provide more detailed information than the time domain methods, the analysis is much more complex, requiring either the use of Fourier transformations or autoregressive modeling. [78] Analysis of time domain measures are much more accessible and, as a result, are more frequently used. [74] Further, time domain methods allow the researcher to select intervals of interest between successive normal QRS complexes. As a result, measuring HRV using time-domain methods was selected for this thesis study.

Principles and Key Considerations for Measurements of Heart Rate Variability

Time-domain HRV data collection has historically been done by running a continuous electrocardiogram (ECG), which requires the placement of electrodes around the chest and abdomen area. [78] The present study used the Mindware Mobile Impedance Cardiograph (New Bio Technology LTD) device to run a 7-lead continuous ECG. This device was chosen because it allows for simultaneous collection of other measures (e.g., Galvanic skin response, respiration rate) that the co-investigator needed to analyze for their study. Further, the Mindware (NBT LTD) device computes root mean square of successive differences (RMSSD) between normal heartbeats automatically, which is the most common statistical method used to analyze time domain measures of HRV. Consequently, using the Mindware (NBD LTD) device was the most appropriate for this study.

MEASURE 3: PULSE WAVE ANALYSIS (SECONDARY OUTCOME)

Pulse wave analysis (PWA) is a non-invasive technique that deconstructs pulse pressure waveforms into various measures used to characterize CV systems' functioning. [83] Two of the most reported outcomes of PWA are central systolic blood pressure (cSBP) and systolic augmentation index (sAIx). Studies suggest that cSBP is a better predictor of CV events and all-cause mortality [71], [84] than peripheral systolic blood pressure (pSBP) and is also highly reliable. [85] Further, studies suggest that sAIx may be a useful marker of arterial stiffness and CVD risk. [86]–[88] Due to its clinical relevancy, PWA was chosen as an outcome. Similar to assessing PWV, PWA can be measured via tonometry, PPG, and/or oscillometry. Oscillometry was chosen, specifically the BP+ (USCOM) device, since this device required little technical expertise and allowed for simultaneous measurements of the primary outcome, PWV.

MEASURE 4: STATE STRESS LEVEL (SECONDARY OUTCOME)

State stress levels can be characterized using a variety of data collection methods, including self-reported questionnaires, Visual Analog Scales (VAS), and behavioral observations. The present study elected to measure state stress levels before and after the study conditions using a 0-10 Likert and Visual Analog Scale. Both scales are reliable and valid, and as a result, have been widely used in research involving the measurement of pain and stress. [89]–[92] The Likert scale asked participants to rate their state stress level on a scale of 0 to 10, where 0 meant "No stress," and 10 meant "Worst possible stress." Similarly, the VAS asked participants to rate their state stress level from "No stress" to "Worst possible stress," however, participants rated this by making a mark on a horizontal line with no associated numbers. These scales were chosen because they are time-efficient and easy to administer, thereby reducing researcher and participant burden.

POTENTIAL CHALLENGES & ALTERNATIVE STRATEGIES

There were several potential challenges that can occur during this experimental study. One of the potential challenges included the chance for an extremely distressful experience when participating in the TSST. To avoid this, investigators were very friendly with all the participants from recruitment to the end of their time in the study. This helped the participant feel they were in a safe environment, despite the stressor.

Another potential challenge was the need for participant to use the bathroom or fidget during the visits, which would negatively impact the accuracy of the outcome measures. One strategy employed to avoid this was to ask them if they need to use the bathroom several times before beginning their rest period. Another strategy used to avoid excessive movement during the visit was to emphasize the importance of stillness for the accuracy of our measures and allow for some time before the rest period to move as much as they needed to. This successfully prevented interruptions during the study visits.

Finally, another potential challenge was unmet recruitment targets. Due to the pandemic, our inclusion criteria, and the fact that UNCCH is a predominantly white institution (PWI), we may fail to acquire our desired sample size. To prevent this, investigators promoted the study to several communities across UNCCH and Duke’s campus.

CHAPTER 4. METHODOLOGY

OVERVIEW

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines. Ethical approval was obtained by the University of North Carolina at Chapel Hill Institutional Review Board (IRB #22705). All participants provided written informed consent prior to study participation.

PARTICIPANTS AND RECRUITMENT

Eight individuals were recruited (1 Hispanic male, 5 Hispanic females, 2 NHW females) between 18-35 years of age from the UNC-CH population. Eligibility was assessed using a Qualtrics survey and participants were scheduled using Microsoft Bookings. No incentives were provided for participation. The UNC-CH Institutional Review Board approved all methods, and all participants provided written informed consent prior to participation.

EXPERIMENTAL DESIGN

This thesis implemented a randomized crossover study design in which study outcomes were measured before and after either an acute psychosocial stressor (TSST) or a time-matched neutral condition (control). Participants reported to the Applied Physiology Laboratory (APL) on three occasions, first for familiarization, and the following two for the study interventions. **Figure 4** summarizes the experimental design. The intervention order was randomized using an online number randomizer (www.randomizer.org).

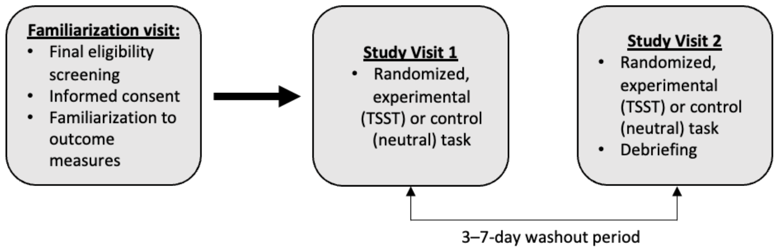

Figure 4. Layout of experimental design.

During the familiarization visit, the investigator performed a final eligibility screening before obtaining informed consent. Signed consent forms were uploaded to the study's SharePoint (Microsoft) and the hard copy was immediately shredded to maintain participant confidentiality. Then, the participants were familiarized with the laboratory environment and study devices. The participants laid supine briefly for measurement of the arterial path length needed to calculate bf-PWV using a segmometer, then the participant was brought to a semi-reclined position. The Vicorder cuffs were placed on the participants' right brachial and femoral arteries. The BP+ cuff was placed on the participants' left brachial artery. The investigator then took two test measurements with the Vicorder and BP+ cuffs inflating simultaneously. The investigator removed all equipment and then answered any questions the participants had. Finally, the participants scheduled their next two visits using Microsoft Bookings before leaving the APL. The investigator ensured the visits were scheduled 3—7 days apart and at the same time of day to allow for a wash-out period and control for diurnal fluctuations.

At the beginning of both the experimental and control visits, participants were asked to provide a urine sample to assess hydration status. Urine specific gravity (USG) was obtained via refractometry (10440, American Optical Corp, Keene, NH). If the participant was dehydrated (defined as specific gravity < 1.0025), [93] they were instructed to drink one cup of water. Simultaneously, the investigator confirmed that the participant followed the required pre-assessment guidelines and were at a "normal" stress level (less than 50mm on VAS and 5 on Likert scale). If the participant failed to meet one or more of the requirements, they were asked to reschedule. After placing the Vicorder and BP+ cuffs on the participant, they laid down for twenty minutes of quiet rest in a semi-reclined position. At the end of the rest period, baseline CV measures were recorded in triplicate. Following baseline measures, the participant began either the TSST or time-matched neutral task. Immediately post-cessation of the task, participants filled out the Visual Analog and Likert scales. CV measures were also taken immediately after the task, as well as 5, 10, 15, 30, 45, and 60 minutes post—task. **Figure 5** summarizes the experimental timeline.

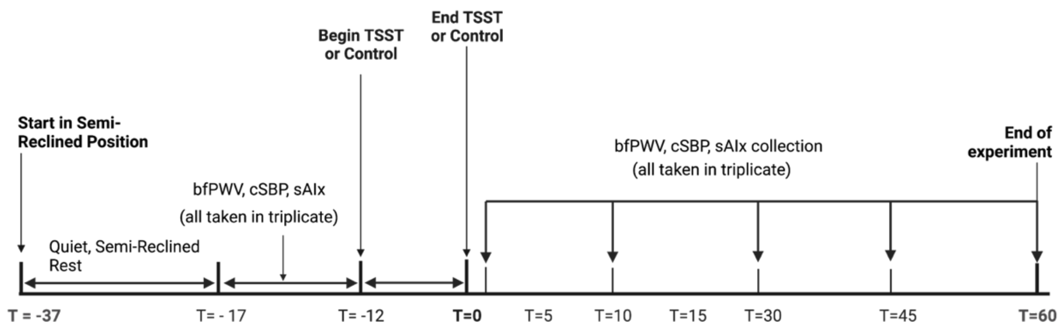

Figure 5. Layout of experimental timeline.

EXPERIMENTAL CONDITION: TRIER SOCIAL STRESS TEST

Just before starting the TSST, the research team unveiled a camera and a tripod work light, which was shone brightly on the participant. The TSST administrator – strategically, someone wearing a white lab coat and unfamiliar to the participant – then explained the first task: the participant was instructed to imagine they were at an interview for their ideal job and had to give a five-minute speech describing why they are the best candidate to a panel of judges. Participants were told they were being recorded and that their speech would be evaluated by professionals trained in public speaking. They were allotted two minutes to mentally prepare their speech and were then instructed to begin speaking. At the end of the five minutes, the participants were told to imagine the panel of judges were requiring them to complete a mental math test, where they had to subtract a set interval from a large number in time with a metronome. Participants were told that their incorrect answers were being recorded to be compared to the norm. This math test was five minutes long, with the set interval, large number, and metronome pace changing (unbeknownst to them) every minute. For example: for the first minute, they would subtract 7 from 4568 with the metronome set to 12 beats per minute (bpm), the second minute they would subtract 13 from 9149 at 14bpm, etc.

To avoid the potential for a diffused stress response, the TSST administrator gave occasional reminders to the participants that they should not be moving, smiling, or laughing during both the speech and math test. If the participant failed to speak for more than a few seconds, the administrator would intervene with phrases such as “you must continue speaking,” and “answer to the beat of the metronome.” At the end of the math test, the lights and camera were quickly stored away, and their stress responses (via Likert and VAS) were recorded on REDCap. Outcome measures (PWV, PWA) were started immediately post-TSST and continued for the next 60 minutes.

It is important to note that participants were not actually being recorded and compared to the norm. This was detailed to them at the end of their time in the study via a debriefing form, which will be discussed in a later section. It is also important to note that this study employed a modified TSST. Traditionally, the TSST allots 3 minutes for participants to prepare their speech and allows participants to write down their thoughts on paper. This study shortened the preparation period to 2 minutes to account for the length of the study visit and did not employ the use of pen/paper as this would disturb the rest required for accurate outcome measures. Additional details of the TSST are reported in a comprehensive 2017 review by Allen et al. [17]

CONTROL CONDITION: NEUTRAL TASK

The control condition employed was a neutral task, time-matched and similar in structure to the TSST. For the speech task, participants were told they were to think about a boring or neutral experience they had for 2 minutes, and then speak about the experiences they thought about for 5 minutes.

Examples provided included sitting in a dull lecture or waiting for the bus to arrive. Then, the participants were instructed to count at a self-selected pace from one to ten, and then starting over again from one. This counting task continued for 5 minutes. At the end of the counting task, their state stress levels were recorded (via Likert and VAS) on REDCap. Outcome measures (PWV, PWA) were started immediately post-control condition and continued for the next 60 minutes.

Several key distinctions took place during the control visit. For example, the neutral task administrator was someone who was familiar to the participant and was not wearing a white lab coat. Additionally, there was no camera or light shone on the participant. Participants were also told they were not being compared to the norm. Finally, the neutral task administrator did not give stern reminders to continue speaking during the tasks.

DEBRIEFING

Before leaving the APL for the last time, the investigator sat down to review the IRB-approved debriefing form with the participants. The investigator explained that the intention of the psychosocial task was indeed to elicit a physiological stress response, and that they were not actually being recorded or evaluated. The investigator briefly explained what the TSST was and the rationale for choosing that task specifically. Further, the investigator explained that withholding information regarding the TSST was required to avoid negatively impacting the study results. Finally, the investigator explained that the participants had the right to withdraw their data if they wished and obtained their signature confirming their choice. Participants were given a copy of the debriefing form for future reference. The signed debrief form was uploaded to the study's SharePoint (Microsoft) and immediately shredded to maintain participant confidentiality.

EXPERIMENTAL OUTCOMES

The Vicorder device (Wuerzburg, Germany) was used to record PWV. Each participant was entered as a "New Patient" under a code name reflecting the order in which they were recruited and the type of study visit (e.g., EMS 01 CON.) As per device guidelines, one cuff was placed over the right brachial artery and one cuff was placed over the femoral artery. The distance (D) between the suprasternal notch and umbilicus was measured using a segmometer and entered into REDCap by the investigator. This path length distance was entered into the Vicorder software, which captures the time it takes for the pulse wave to travel – referred to as transit time (TT) -between the segments of interest. The Vicorder uses these two values to calculate PWV, defined as D/TT. The investigator visually inspected waveforms before capturing a segment, then entered the corresponding PWV values directly into an Excel spreadsheet to be used for data analysis.

Pulse wave analysis outcomes (cSBP, sAIx) were measured using the BP+ device (USCOM BP+, Sydner, Australia). As per device guidelines, the oscillometric cuff was placed on the left brachial artery. Mirroring the naming convention for the Vicorder device, each participant was entered as a "New Patient" under a code name reflecting the order in which they were recruited and the type of study visit. PWV and PWA measures were taken simultaneously and in triplicate for each of the outcome measures, taken at baseline, T=0, 5, 10, 15, 30, 45, and 60. cSBP and sAIx were also directly entered into an Excel spreadsheet for data analysis.

SAMPLE SIZE

Sample size calculations were based on bf-PWV, the primary outcome measure of this study. Using an α-level of 0.05, an estimated power of 80% and a moderate effect size of 0.25, an estimate of 18 participants per group was required (G*Power Statistical Power Analysis Software v3.1). To account for potential data quality problems and attrition, this number was inflated to 20 per group, for a total of 40 participants enrolled.

STATISTICAL ANALYSIS

Numerous statistical methods exist for analyzing data in a crossover study design. For example, a mixed-design ANOVA could have been chosen for this thesis. However, mixed-designs ANOVA will result in considerable data loss if a participant drops out. Given the time commitment this study required, participant attrition was expected. Further, a mixed-design ANOVA does not have the capability of using continuous data, which the co-investigator collected. A linear mixed model, however, addresses all these issues: it accounts for missing data points, controls for covariates, allows for fixed and random effects, and is statistically robust. As a result, the investigator chose to implement a linear mixed model and used Jamovi (2022, Version 2.3.21.0) to conduct the statistical analysis.

CHAPTER 5. RESULTS

PARTICIPANTS

Participants were recruited between 17 October 2022 and 25 April 2023 through the Research for Me (RFM) @UNC platform, flyers (including a QR code linking to our RFM page), emails, newsletters, and short presentations to classes at UNC Chapel Hill. Eleven participants were screened for eligibility through the Qualtrics survey. Out of those eleven participants, two were ineligible, and nine were eligible to participate in the study. Out of those nine participants, eight scheduled their first study visit (familiarization) via our Microsoft Bookings page. One participant declined to participate due lack of financial compensation. Therefore, eight participants were enrolled in the study, of which six completed all three study visits. Two participants dropped out of the study due to scheduling difficulties. One of these two participants completed two study visits—the familiarization and experimental visits. Six subjects were included in the final analysis. See **Figure 6** for CONSORT flow and reasons for exclusion.

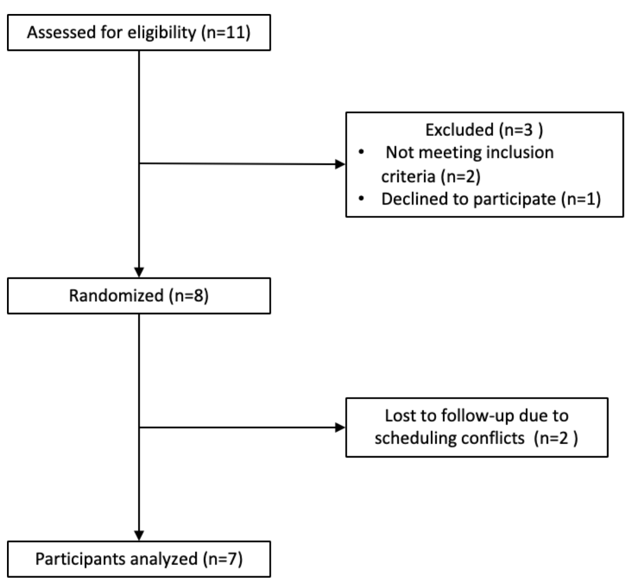

Figure 6: CONSORT diagram for recruitment and testing.

PARTICIPANT DEMOGRAPHICS

This section will outline the baseline demographic characteristics for the six participants – all female - included in the table below.

n=6	Hispanic	Non-Hispanic White
Ethnicity	4 (67%)	2 (33%)
Age	21.01 (2.79)	23.86 (5.59)
BMI (kg/m^2)	26.51 (3.08)	23.86 (2.09)

Table 8. Participant demographic information.

PHYSIOLOGICAL OUTCOME MEASURE RESULTS

The main effect of ethnicity on PWV was significant such that Hispanics had lower PWV on average than NHWA, (p=0.013, b=0.355, 95%CI: 0.183, 0.550). The interaction effect of study visit type on change in PWV was significant such that Hispanics had the greatest change in the control condition and NHWA had the greatest change in the experimental condition (p=<0.001, b=0.466, 95% CI: 0.260, 0.671). The interaction effect of study visit type on change in cSBP was significant such that Hispanics had the greatest change in the control condition and NHWA had the greatest change in the experimental condition (p=0.009, b=-5.344, 95%CI: -9.184, -1.504). There were no significant effects on sAIx. **Tables 9 – 11** provide further detail into the data observed.

	Ethnicity	PWV (m/s)	cSBP (mmHg)
Mean	Hispanic	5.10	94.3
	NHW	6.39	97.8
Median	Hispanic	5.35	94.0
	NHW	6.40	100
Standard deviation	Hispanic	0.797	5.73
	NHW	0.375	7.53

Table 10. Descriptive statistics by ethnicity.

Ethnicity	Condition	△ PWV (m/s)
Hispanic	Control	+0.33 (0.93)
	Experimental	+0.10 (0.84)
NHW	Control	-0.15 (0.28)
	Experimental	+0.90 (0.22)

Table 9. Changes in PWV by experimental group.

Ethnicity	Condition	△ cSBP (mmHg)
Hispanic	Control Experimental	+5.8 (1.32) +4.0 (2.45)
NHW	Control Experimental	-1.0 (1.02) +5.0 (1.00)

Table 11. Changes in cSBP by experimental group.

STATE STRESS OUTCOME MEASURE RESULTS

One paired samples t-test was performed to compare participants' Likert scale scores before and after the control condition. There was a significant difference in the scores before (M=2.83, SD=1.72) and after (M=1.33, SD=1.37) the control condition (p=0.045, d=1.09). One paired samples t-test was performed to compare participants' Likert scale scores before and after the experimental condition. There was a significant difference in the scores before (M=2.50, SD=0.55) and after (M=4.83, SD=0.98) the experimental condition (p=<0.001, d=2.86). One paired samples t-test was performed to compare participants' VAS scores before and after the experimental condition. There was a significant difference in the scores before (M=19.17, SD=8.26) and after (M=40.17, SD=15.22) the experimental condition (p=0.034, d=1.42). There were no significant differences found in the VAS scores in response to the control condition (p=0.180). A summary of these findings can be found in **Table 12.** Independent samples t-tests were performed to evaluate whether there was a difference in the change in the Likert and VAS scores between Hispanic and NHWA's. The results indicated no significant differences.

Condition	Outcome Measure	Time	Mean (SD)	p-value
Control	Likert	Pre Post	2.83 (1.72) 1.33 (1.37)	0.045
	VAS	Pre Post	26.67 (15.90) 16.20 (8.76)	0.180
Experimental	Likert	Pre Post	2.50 (0.55) 4.83 (0.98)	<0.001
	VAS	Pre Post	19.17 (8.26) 40.17 (15.22)	0.010

Table 12. Significant statistical results.

HARMS

The only potential harm in this study was the potential for mild embarrassment after the TSST. The debriefing form minimized this, and the investigator reassured the participants that they could come forward with any questions or concerns they had. Considering no participant opted to withdraw their data from the study, it is highly likely there were no long-term harms done.

CHAPTER 6. DISCUSSION

SUMMARY

Hispanics face a highly disproportionate burden of cardiovascular disease risk factors—in particular, mental stress—compared to non-Hispanic whites. [1], [2], [29]–[31] Despite the fact that this phenomenon is well-known, very little research has been done to understand the mechanisms by which disparities arise. To the author's knowledge, no study has investigated the relationship between acute mental stress and CVD risk in this context. As a result, this study compared the CV response to an acute mental stressor between Hispanic and Non-Hispanic white adults. The main finding of the present study was that Hispanics had the greater CV response to the neutral speaking task, whereas NHWA had the greater CV response to the stress-inducing speaking task. Future research should consider measuring stress appraisal in addition to stress response to further contextualize and potentially explain the counterintuitive results seen in Hispanic adults.

LIMITATIONS AND STRENGTHS

While every effort was made to increase generalizability and maintain internal validity, there were still limitations in this study that must be addressed. The most substantial limitation to this study is the small sample size. To have results that were highly powered, the investigator aimed to have a sample size of n=40 (20 Hispanics, 20 NHW). At the time of writing (June 2023), only 6 participants were included in the analyses. There were several contributing factors to the small sample size obtained – one of which includes the geographical area. The population at UNC-CH and surrounding areas are predominantly busy college students and young, working adults. As a result, the time commitment for this study likely dissuaded potential participants from enrolling. Further, due to time constraints and limited laboratory availability, visits were required to take place in the late afternoon. This limited availability, in conjunction with the requirement to abstain from caffeine and food for 12 and 4 hours, respectively, likely impacted study enrollment as well. Finally, excluding individuals with anxiety or panic disorders in a high-stress educational setting like UNC-CH may have also impacted study enrollment and eligibility. Consequently, conclusions drawn from this study are quite limited and must be interpreted with caution.

Another limitation to this study is that only healthy individuals were recruited. While this was done to maintain internal validity and decrease confounding variables, it is important to recognize that this was at the expense of generalizability to diseased populations. Therefore, the results obtained may not be generalizable to men, individuals over the age of 35, or those with cardiovascular disorders.

Despite the limitations outlined above, this study also had several strengths. For example, the investigator was well-versed in study procedures prior to collecting data from participants. The investigator had served as a research assistant in several other studies from the laboratory group using the exact devices (Vicorder, BP+). As a result, the investigator was highly qualified to inspect waveforms and check for quality during the study visits. Another strength of this study is that, although our sample size was low, the majority (57%) of participants were Hispanic. Finally, this study followed strict pre-assessment guidelines, which eliminated the potential for additional confounding variables.

COMPARISON TO LITERATURE

Due to the uniqueness of study protocol and aims, there are no exact studies the results can be compared to. However, since the TSST is a widely used laboratory stressor, comparisons can be made to the existing body of literature. This study found that, while Hispanics and NHWA’s responded to the TSST to different degrees, the TSST did in fact successfully induce mental stress, as evidenced by increases in study outcomes (PWV, cSBP). This can be considered a proof-of-concept and strengthen the existing body of literature supporting the use of the TSST to induce mental stress in a laboratory setting.

IMPLICATIONS

Surprisingly, this study found that Hispanic adults had the greatest CV response to the neutral speaking task as opposed to the mental stressor. When considering the potential implications of such an observation, it is important to recall the physiological responses to both acute and chronic mental stress, to provide further context into the study's results. As discussed earlier in this thesis, when the body is unable to adequately adapt to daily stressors, there are four different responses which may be observed (refer to **Figure 3**). One of these responses is a generalized dampening in the physiological reactivity to stressors. Given this context and the nature of the results obtained, it is biologically plausible that the Hispanic individuals in this study may have acquired the dampening response to mental stress from facing chronic stress their lives. Consequently, further research should investigate if this dampening does in fact exist in Hispanics adults by repeating this protocol, or a similar one, to a much larger degree.

CONCLUSIONS

The present study was a randomized crossover design aimed at investigating if a difference exists in the cardiovascular response to an acute mental stressor between Hispanic and non-Hispanic white adults. While generalizability of the results is limited due to the small sample size, evidence suggests that Hispanics' CV systems respond counterintuitively to laboratory induced acute mental stress. Given the role that mental stress plays in accruing CVD risk, the results of this study demonstrates that further research is warranted to understand the underlying mechanisms by which the interactions between mental stress and CVD risk may differ based on population.

REFERENCES

[1] C. J. Rodriguez *et al.*, "High Cholesterol Awareness, Treatment, and Control Among Hispanic/Latinos: Results From the Hispanic Community Health Study/Study of Latinos," *J. Am. Heart Assoc.*, vol. 4, no. 7, Jun. 2015, doi: 10.1161/JAHA.115.001867.

[2] S. Gomez, V. Blumer, and F. Rodriguez, "Unique Cardiovascular Disease Risk Factors in Hispanic Individuals," vol. 16, pp. 53–61, 2022, doi: 10.1007/s12170-022-00692-0.

[3] L. M. Pachter, C. H. Caldwell, J. S. Jackson, and B. A. Bernstein, "Discrimination and Mental Health in a Representative Sample of African-American and Afro-Caribbean Youth", doi: 10.1007/s40615-017-0428-z.

[4] L. L. Brown, U. A. Mitchell, and J. A. Ailshire, "Disentangling the Stress Process: Race/Ethnic Differences in the Exposure and Appraisal of Chronic Stressors Among Older Adults", doi: 10.1093/geronb/gby072.

[5] P. M. Shaw *et al.*, "Controversies and evidence for cardiovascular disease in the diverse Hispanic population," 2018, doi: 10.1016/j.jvs.2017.06.111.

[6] K. T. Jones, R. C. Shelton, J. Wan, and L. Li, "Impact of Acute Psychological Stress on Cardiovascular Risk Factors in Face of Insulin Resistance HHS Public Access", doi: 10.1080/10253890.2016.1231804.

[7] L. E. Spieker *et al.*, "Mental stress induces prolonged endothelial dysfunction via endothelin-A receptors," *Circulation*, 2002, doi: 10.1161/01.CIR.0000021598.15895.34.

[8] C. Vlachopoulos, F. Kosmopoulou, N. Alexopoulos, N. Ioakeimidis, G. Siasos, and C. Stefanadis, "Acute mental stress has a prolonged unfavorable effect on arterial stiffness and wave reflections," *Psychosom. Med.*, vol. 68, no. 2, pp. 231–237, 2006, doi: 10.1097/01.psy.0000203171.33348.72.

[9] L. JG, T. BA, L. X, F. CR, L. Z, and A. BH, "Acute psychological stress, autonomic function, and arterial stiffness among women," *Int. J. Psychophysiol. Off. J. Int. Organ. Psychophysiol.*, vol. 155, pp. 219–226, Sep. 2020, doi: 10.1016/J.IJPSYCHO.2020.06.015.

[10] T. G. Guilliams and L. Edwards, "The Stress Response System Chronic Stress and the HPA Axis: Clinical Assessment and Therapeutic Considerations".

[11] H. S. Kim and K. I. Cho, “Impact of chronic emotional stress on myocardial function in postmenopausal women and its relationship with endothelial dysfunction.,” *Korean Circ. J.*, vol. 43, no. 5, pp. 295–302, May 2013, doi: 10.4070/kcj.2013.43.5.295.

[12] H. Nabi *et al.*, “Increased risk of coronary heart disease among individuals reporting adverse impact of stress on their health: the Whitehall II prospective cohort study,” *Eur Heart J*, vol. 34, no. 34, pp. 2697–2705, 2013, doi: 10.1093/eurheartj/eht216.

[13] B. T. Mausbach *et al.*, “Association Between Chronic Caregiving Stress and Impaired Endothelial Function in the Elderly,” *J. Am. Coll. Cardiol.*, vol. 55, no. 23, pp. 2599–2606, Jun. 2010, doi: 10.1016/J.JACC.2009.11.093.

[14] J. R. Carter and D. S. Goldstein, “Sympathoneural and adrenomedullary responses to mental stress.,” *Compr. Physiol.*, vol. 5, no. 1, pp. 119–46, Jan. 2015, doi: 10.1002/cphy.c140030.

[15] B. S. McEwen and J. C. Wingfield, “What’s in a name? Integrating homeostasis, allostasis and stress”, doi: 10.1016/j.yhbeh.2009.09.011.

[16] L. I. Pearlin, “The Sociological Study of Stress,” *Journal of Health and Social Behaviorr*, Sep. 1989.

[17] A. P. Allen, P. J. Kennedy, S. Dockray, J. F. Cryan, T. G. Dinan, and G. Clarke, “The Trier Social Stress Test: Principles and practice,” *Neurobiol. Stress*, 2016, doi: 10.1016/j.ynstr.2016.11.001.

[18] M. A. Birkett, “The Trier Social Stress Test Protocol for Inducing Psychological Stress,” *J Vis Exp*, no. 56, p. 3238, 2011, doi: 10.3791/3238.

[19] “Health Disparities, NHLBI, NIH.” https://www.nhlbi.nih.gov/health/educational/healthdisp/ (accessed Jul. 08, 2022).

[20] P. Braveman and L. Gottlieb, “The Social Determinants of Health: It’s Time to Consider the Causes of the Causes,” *Public Health Rep.*, vol. 129, p. 19, 2014.

[21] “Social Determinants of Health - Healthy People 2030 | health.gov.” https://health.gov/healthypeople/priority-areas/social-determinants-health (accessed Aug. 14, 2022).

[22] R. Verma, Y. P. S. Balhara, and C. S. Gupta, “Gender differences in stress response: Role of developmental and biological determinants,” *Ind. Psychiatry J.*, vol. 20, no. 1, pp. 4–10, 2011, doi: 10.4103/0972-6748.98407.

[23] M. J. Sternthal, N. Slopen, and D. R. Williams, “RACIAL DISPARITIES IN HEALTH How Much Does Stress Really Matter? 1,” 2011.

[24] P. A. Thoits, “Extra Issue: What Do We Know? Key Findings from 50 Years of,” *Source J. Health Soc. Behav.*, vol. 51, pp. 41–53, 2010, doi: 10.1177/0022146510383499.

[25] Y. Hswen, Q. Qin, D. R. Williams, K. Viswanath, S. V. Subramanian, and J. S. Brownstein, “Online negative sentiment towards Mexicans and Hispanics and impact on mental well-being: A time-series analysis of social media data during the 2016 United States presidential election,” *Heliyon*, vol. 6, no. 9, p. e04910, Sep. 2020, doi: 10.1016/j.heliyon.2020.e04910.

[26] G. R. S. and C. Bennett, “Anti-immigrant campaign ads negatively impact Latinos’ mental health and make them feel unwelcome in the United States,” *Brookings*, Nov. 01, 2022. https://www.brookings.edu/blog/fixgov/2022/11/01/anti-immigrant-campaign-ads-negatively-impact-latinos-mental-health-and-make-them-feel-unwelcome-in-the-united-states/ (accessed Apr. 30, 2023).

[27] B. S. Jones *et al.*, “Trump-induced anxiety among Latina/os,” *Group Process. Intergroup Relat.*, vol. 24, no. 1, pp. 68–87, Jan. 2021, doi: 10.1177/1368430219889132.

[28] M. H. Lopez, A. Gonzalez-Barrera, and J. M. Krogstad, “FOR MEDIA OR OTHER INQUIRIES,” vol. 25, 2018, Accessed: Jul. 30, 2022. [Online]. Available: www.pewresearch.org.

[29] P. C. Balfour, J. M. Ruiz, G. A. Talavera, M. A. Allison, and C. J. Rodriguez, “Cardiovascular Disease in Hispanics/Latinos in the United States”, doi: 10.1037/lat0000056.

[30] K. Fei *et al.*, “Racial and Ethnic Subgroup Disparities in Hypertension Prevalence, New York City Health and Nutrition Examination Survey, 2013–2014,” *Prev. Chronic. Dis.*, vol. 14, no. 4, Apr. 2019, doi: 10.5888/PCD14.160478.

[31] J. Z. Willey *et al.*, “Race-ethnic differences in the relationship between lipid profile components and risk of myocardial infarction: the Northern Manhattan Study,” *Am Heart J*, vol. 161, no. 5, pp. 886–892, 2011, doi: 10.1016/j.ahj.2011.01.018.

[32] C. J. Rodriguez *et al.*, “Status of Cardiovascular Disease and Stroke in Hispanics/ Latinos in the United States: A Science Advisory From the American Heart Association FAHA on behalf of the American Heart Association Council on Epidemiology and Prevention, Council on Clinical Cardiology, and Council on Cardiovascular and Stroke Nursing”, doi: 10.1161/CIR.0000000000000071.

[33] M. Kyoung Kim, K. Han, H. Na Joung, K.-H. Baek, K.-H. Song, and H.-S. Kwon, “Cholesterol levels and development of cardiovascular disease in Koreans with type 2 diabetes mellitus and without pre-existing cardiovascular disease,” *Cardiovasc Diabetol*, vol. 18, p. 139, 2019, doi: 10.1186/s12933-019-0943-9.

[34] “Projections of the Population by Sex, Race, and Hispanic Origin for the United States: 2010 to 2050,” Population Division, U.S. Census Bureau.

[35] M. L. Avilés-Santa, U. Colón-Ramos, N. M. Lindberg, J. Mattei, F. J. Pasquel, and C. M. Pérez, “From Sea to Shining Sea and the Great Plains to Patagonia: A Review on Current Knowledge of Diabetes Mellitus in Hispanics/Latinos in the US and Latin America,” *Front. Endocrinol.*, vol. 8, p. 298, 2017, doi: 10.3389/fendo.2017.00298.

[36] K. T. Jones, R. C. Shelton, J. Wan, and L. Li, “Impact of acute psychological stress on cardiovascular risk factors in face of insulin resistance,” *Stress*, pp. 1–8, Sep. 2016, doi: 10.1080/10253890.2016.1231804.

[37] B. Chu, K. Marwaha, T. Sanvictores, and D. Ayers, “Physiology, Stress Reaction,” *StatPearls*, Sep. 2021, Accessed: Jun. 23, 2022. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK541120/

[38] S. Rotenberg and J. J. Mcgrath, “Inter-relation between autonomic and HPA axis activity in children and adolescents”, doi: 10.1016/j.biopsycho.2016.01.015.

[39] M. G. Stewart *et al.*, “Stress suppresses and learning induces plasticity in CA3 of rat hippocampus: a three-dimensional ultrastructural study of thorny excrescences and their postsynaptic densities,” *Neuroscience*, vol. 131, no. 1, pp. 43–54, 2005, doi: 10.1016/j.neuroscience.2004.10.031.

[40] B. S. McEwen, “Physiology and neurobiology of stress and adaptation: Central role of the brain,” *Physiol. Rev.*, vol. 87, no. 3, pp. 873–904, 2007, doi: 10.1152/physrev.00041.2006.

[41] T. G. Guilliams and L. Edwards, “Chronic stress and the HPA axis: Clinical assessment and therapeutic considerations,” *The Standard*, vol. 9, no. 2, pp. 1–12, 2010.

[42] D. A. Padgett and R. Glaser, “How stress influences the immune response,” *Trends Immunol.*, vol. 24, no. 8, pp. 444–448, 2003, doi: 10.1016/S1471-4906(03)00173-X.

[43] P. H. Black and L. D. Garbutt, “Stress, inflammation and cardiovascular disease,” *J. Psychosom. Res.*, vol. 52, no. 1, pp. 1–23, 2002, doi: 10.1016/S0022-3999(01)00302-6.

[44] P. H. Thaker *et al.*, “Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma,” *Nat. Med.*, vol. 12, no. 8, pp. 939–944, 2006, doi: 10.1038/nm1447.

[45] E. Generaal *et al.*, “Reduced hypothalamic-pituitary-adrenal axis activity in chronic multi-site musculoskeletal pain: partly masked by depressive and anxiety disorders,” *BMC Musculoskelet. Disord.*, vol. 15, no. 1, p. 227, Dec. 2014, doi: 10.1186/1471-2474-15-227.

[46] R. Rosmond, “Role of stress in the pathogenesis of the metabolic syndrome,” *Psychoneuroendocrinology*, vol. 30, no. 1, pp. 1–10, 2005, doi: 10.1016/j.psyneuen.2004.05.007.

[47] E.-R. SA and L. TT, “Psychosocial factors and cardiovascular diseases,” *Annu. Rev. Public Health*, vol. 26, pp. 469–500, 2005, doi: 10.1146/ANNUREV.PUBLHEALTH.26.021304.144542.

[48] B.-C. Yao, L.-B. Meng, M.-L. Hao, Y.-M. Zhang, T. Gong, and Z.-G. Guo, “Chronic stress: a critical risk factor for atherosclerosis,” *J. Int. Med. Res.*, vol. 47, no. 4, pp. 1429–1440, 2019, doi: 10.1177/0300060519826820.

[49] E. Amiya, M. Watanabe, and I. Komuro, “The Relationship between Vascular Function and the Autonomic Nervous System”, doi: 10.3400/avd.ra.14-00048.

[50] Y.-T. Xue *et al.*, “Investigating the role of acute mental stress on endothelial dysfunction: a systematic review and meta-analysis,” *Clin. Res. Cardiol.*, vol. 104, no. 4, pp. 310–319, Apr. 2015, doi: 10.1007/s00392-014-0782-3.

[51] K. A. D’Urzo *et al.*, “The impact of acute mental stress on brachial artery flow-mediated dilation in women diagnosed with depression,” *Int. J. Psychophysiol.*, 2019, doi: 10.1016/j.ijpsycho.2018.12.003.

[52] L. Ghiadoni *et al.*, "Mental stress induces transient endothelial dysfunction in humans," *Circulation*, 2000, doi: 10.1161/01.CIR.102.20.2473.

[53] C. Vlachopoulos *et al.*, "Divergent Effects of Laughter and Mental Stress on Arterial Stiffness and Central Hemodynamics," *Psychosom. Med.*, vol. 71, no. 4, pp. 446–453, May 2009, doi: 10.1097/PSY.0b013e318198dcd4.

[54] J. G. Logan, D. J. Barksdale, J. Carlson, B. W. Carlson, and P. J. Rowsey, "Psychological Stress and Arterial Stiffness in Korean Americans," *J. Psychosom. Res.*, vol. 73, no. 1, p. 53, Jul. 2012, doi: 10.1016/J.JPSYCHORES.2012.04.008.

[55] L. De *et al.*, "Vascular Aging and Arterial Stiffness", doi: 10.5935/abc.201700
91.

[56] A. M. Kucharska-Newton, L. Stoner, and M. L. Meyer, "Determinants of Vascular Age: An Epidemiological Perspective.," *Clin. Chem.*, vol. 65, no. 1, pp. 108–118, Jan. 2019, doi: 10.1373/clinchem.2018.287623.

[57] M. F. O'rourke, "Isolated Systolic Hypertension, Pulse Pressure, and Arterial Stiffness as Risk Factors for Cardiovascular Disease," *Curr. Hypertens. Rep.*, vol. 3, pp. 204–211, 1999.

[58] S. Laurent *et al.*, "Expert consensus document on arterial stiffness: methodological issues and clinical applications," *Eur. Heart J.*, vol. 27, no. 21, pp. 2588–2605, Sep. 2006, doi: 10.1093/eurheartj/ehl254.

[59] E. Kis *et al.*, "Measurement of pulse wave velocity in children and young adults: A comparative study using three different devices," *Hypertens. Res.*, vol. 34, no. 11, pp. 1197–1202, Nov. 2011, doi: 10.1038/hr.2011.103.

[60] L. Becker, U. Schade, and N. Rohleder, "Evaluation of the socially evaluated cold-pressor group test (SECPT-G) in the general population", doi: 10.7717/peerj.7521.

[61] G. M. Manzoni, T. E. Wilson, P. K. Stein, S. G. Crewther, R. Laycock, and A. L. Shilton, "The Maastricht Acute Stress Test (MAST): Physiological and Subjective Responses in Anticipation, and Post-stress," 2017, doi: 10.3389/fpsyg.2017.00567.

[62] K. Jaroslaw, M. Zenon, H. Magdalena, W. Slawomir, and L. Janusz, "Effect of endogenous estrogen on blood flow through carotid arteries," *Stroke*, vol. 32, no. 1, pp. 30–36, 2001, doi: 10.1161/01.STR.32.1.30.

[63] I. Peter *et al.*, "Association between Arterial Stiffness and Variations in Estrogen-Related Genes," *J. Hum. Hypertens.*, vol. 23, no. 10, pp. 636–644, Oct. 2009, doi: 10.1038/jhh.2009.1.

[64] R. R. Townsend *et al.*, "Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness," *Hypertension*, vol. 66, no. 3, pp. 698–722, Sep. 2015, doi: 10.1161/HYP.0000000000000033.

[65] A. Barseghian, T. Padro, J. Bernhagen, S.-H. Kim, and H.-L. Kim, "Pulse Wave Velocity in Atherosclerosis," *Front. Cardiovasc. Med. Wwwfrontiersinorg*, vol. 1, p. 41, 2019, doi: 10.3389/fcvm.2019.00041.

[66] C. Vlachopoulos, F. Kosmopoulou, N. Alexopoulos, N. Ioakeimidis, G. Siasos, and C. Stefanadis, "Acute Mental Stress Has a Prolonged Unfavorable Effect on Arterial Stiffness and Wave Reflections," *Psychosom. Med.*, vol. 68, no. 2, pp. 231–237, Mar. 2006, doi: 10.1097/01.psy.0000203171.33348.72.

[67] G. M. London and B. Pannier, "Arterial functions: How to interpret the complex physiology," *Nephrol. Dial. Transplant.*, 2010, doi: 10.1093/ndt/gfq614.

[68] S. Laurent, "Defining vascular aging and cardiovascular risk," *J. Hypertens.*, vol. 30, no. SUPPL. 1, 2012, doi: 10.1097/HJH.0b013e328353e501.

[69] L. M. Van Bortel *et al.*, "Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking," *J. Hypertens.*, vol. 19, no. 6, pp. 1037–1044, Jun. 2001, doi: 10.1097/00004872-200106000-00007.

[70] J. Poles *et al.*, "Overall and repeated measures agreement between brachial-femoral and carotid-femoral measures of pulse wave velocity in young and healthy individuals," *Blood Press. Monit.*, vol. 27, no. 5, pp. 334–340, Oct. 2022, doi: 10.1097/MBP.0000000000000614.

[71] C. Vlachopoulos, K. Aznaouridis, M. F. O'Rourke, M. E. Safar, K. Baou, and C. Stefanadis, "Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis," *Eur. Heart J.*, vol. 31, no. 15, pp. 1865–1871, Aug. 2010, doi: 10.1093/EURHEARTJ/EHQ024.

[72] A. P. Guerin, J. Blacher, B. Pannier, S. J. Marchais, M. E. Safar, and G. M. London, "Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure," *Circulation*, vol. 103, no. 7, pp. 987–992, Feb. 2001, doi: 10.1161/01.cir.103.7.987.

[73] S. Laurent *et al.*, "Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients," *Hypertens. Dallas Tex 1979*, vol. 37, no. 5, pp. 1236–1241, May 2001, doi: 10.1161/01.hyp.37.5.1236.

[74] M. Malik *et al.*, "Heart rate variability. Standards of measurement, physiological interpretation, and clinical use," *Eur. Heart J.*, vol. 17, no. 5, pp. 354–381, 1996, doi: 10.1093/oxfordjournals.eurheartj.a014868.

[75] R. Tiwari, R. Kumar, S. Malik, T. Raj, and P. Kumar, "Analysis of Heart Rate Variability and Implication of Different Factors on Heart Rate Variability," *Curr. Cardiol. Rev.*, vol. 17, no. 5, p. e160721189770, Oct. 2021, doi: 10.2174/1573403X16999201231203854.

[76] A. Montaño, F. Brown, D. P. Credeur, M. A. Williams, and L. Stoner, "Telemetry-derived heart rate variability responses to a physical stressor," *Clin. Physiol. Funct. Imaging*, vol. 37, no. 4, pp. 421–427, Jul. 2017, doi: 10.1111/cpf.12320.

[77] G. E. Billman, H. V. Huikuri, J. Sacha, and K. Trimmel, "An introduction to heart rate variability: methodological considerations and clinical applications," *Front. Physiol.*, 2015, doi: 10.3389/fphys.2015.00055.

[78] G. Billman, "Heart Rate Variability – A Historical Perspective," *Front. Physiol.*, vol. 2, 2011, Accessed: Apr. 17, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fphys.2011.00086

[79] A. Brugnera, C. Zarbo, M. P. Tarvainen, P. Marchettini, R. Adorni, and A. Compare, "Heart rate variability during acute psychosocial stress: A randomized cross-over trial of verbal and non-verbal laboratory stressors," *Int. J. Psychophysiol. Off. J. Int. Organ. Psychophysiol.*, vol. 127, pp. 17–25, May 2018, doi: 10.1016/j.ijpsycho.2018.02.016.

[80] M. Mestanik, A. Mestanikova, Z. Visnovcova, A. Calkovska, and I. Tonhajzerova, "Cardiovascular Sympathetic Arousal in Response to Different Mental Stressors," *Physiol Res*, vol. 64, pp. 585–594, 2015.

[81] J. F. Thayer and R. D. Lane, “The role of vagal function in the risk for cardiovascular disease and mortality,” *Biol. Psychol.*, vol. 74, no. 2, pp. 224–242, Feb. 2007, doi: 10.1016/j.biopsycho.2005.11.013.

[82] Y. Wang, X. Zhao, A. O’Neil, A. Turner, X. Liu, and M. Berk, “Altered cardiac autonomic nervous function in depression.,” *BMC Psychiatry*, vol. 13, p. 187, Jul. 2013, doi: 10.1186/1471-244X-13-187.

[83] J. P. Mynard, A. Kondiboyina, R. Kowalski, M. M. H. Cheung, and J. J. Smolich, “Measurement, Analysis and Interpretation of Pressure/Flow Waves in Blood Vessels,” *Front. Physiol.*, vol. 11, 2020, Accessed: Apr. 29, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fphys.2020.01085

[84] M. J. Roman *et al.*, “Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study,” *Hypertens. Dallas Tex 1979*, vol. 50, no. 1, pp. 197–203, Jul. 2007, doi: 10.1161/HYPERTENSIONAHA.107.089078.

[85] L. Stoner, D. Credeur, S. Fryer, J. Faulkner, D. Lambrick, and B. B. Gibbs, “Reliability of pulse waveform separation analysis: effects of posture and fasting,” *J. Hypertens.*, vol. 35, no. 3, pp. 501–505, Mar. 2017, doi: 10.1097/HJH.0000000000001182.

[86] A. Mahmud and J. Feely, “Arterial stiffness is related to systemic inflammation in essential hypertension,” *Hypertens. Dallas Tex 1979*, vol. 46, no. 5, pp. 1118–1122, Nov. 2005, doi: 10.1161/01.HYP.0000185463.27209.b0.

[87] T. Weber *et al.*, “Arterial stiffness, wave reflections, and the risk of coronary artery disease,” *Circulation*, vol. 109, no. 2, pp. 184–189, Jan. 2004, doi: 10.1161/01.CIR.0000105767.94169.E3.

[88] C. M. McEniery *et al.*, “Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT),” *J. Am. Coll. Cardiol.*, vol. 46, no. 9, pp. 1753–1760, Nov. 2005, doi: 10.1016/j.jacc.2005.07.037.

[89] A. D. Crosswell and K. G. Lockwood, “Best practices for stress measurement: How to measure psychological stress in health research,” *Health Psychol. Open*, vol. 7, no. 2, p. 2055102920933072, Jul. 2020, doi: 10.1177/2055102920933072.

[90] object Object, “Disordered eating behaviour is associated with blunted cortisol and cardiovascular reactions to acute psychological stress”, Accessed: Jun. 21, 2023. [Online]. Available: https://core.ac.uk/reader/9552661?utm_source=linkout

[91] J. Brazier and J. Ratcliffe, “Measurement and Valuation of Health for Economic Evaluation,” in *International Encyclopedia of Public Health (Second Edition)*, S. R. Quah, Ed., Oxford: Academic Press, 2017, pp. 586–593. doi: 10.1016/B978-0-12-803678-5.00457-4.

[92] A. W. K. Yeung and N. S. M. Wong, “The Historical Roots of Visual Analog Scale in Psychology as Revealed by Reference Publication Year Spectroscopy,” *Front. Hum. Neurosci.*, vol. 13, 2019, Accessed: Jun. 21, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fnhum.2019.00086

[93] L. E. Armstrong, “Assessing hydration status: the elusive gold standard,” *J. Am. Coll. Nutr.*, vol. 26, no. 5 Suppl, pp. 575S-584S, Oct. 2007, doi: 10.1080/07315724.2007.10719661.