BMJ Open

# Key dimensions of posttraumatic stress disorder and endothelial dysfunction: a mechanism-focused cohort study



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|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2020-043060 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 23-Jul-2020 |
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| Keywords: | Anxiety disorders < PSYCHIATRY, Adult psychiatry < PSYCHIATRY, Cardiology < INTERNAL MEDICINE |
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# Key Dimensions of Posttraumatic Stress Disorder and Endothelial Dysfunction: A Mechanism-Focused Cohort Study

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Word count: 4,000

## ABSTRACT

### Introduction

Both trauma exposure and posttraumatic stress disorder (PTSD) are associated with increased risk of cardiovascular disease (CVD), the leading cause of death in the United States. Endothelial dysfunction, a modifiable, early marker of CVD risk, may represent a physiological mechanism underlying this association and a promising target for intervention given its malleability. This mechanism-focused cohort study aims to investigate the relationship between PTSD (both in terms of diagnosis and underlying symptom dimensions) and endothelial dysfunction in a diverse, community-based sample of adult men and women.

### Methods and analysis

Using a cohort design, 160 trauma-exposed participants without a history of CVD are designated to the PTSD group (*n*=80) or trauma-exposed matched control group (*n*=80) after a baseline diagnostic interview assessment. Participants in the PTSD group have a current (past-month) diagnosis of PTSD, whereas those in the control group have a history of trauma but no current or past psychiatric diagnoses. Endothelial dysfunction is assessed via flow-mediated vasodilation (FMD) of the brachial artery and circulating levels of endothelial cell-derived microparticles (EMPs). Two higher-order symptom dimensions of PTSD—fear and dysphoria—are measured objectively with a fear conditioning paradigm and attention allocation task, respectively. Autonomic imbalance, inflammation, and oxidative stress are additionally assessed and will be examined as potential pathway variables linking PTSD and its dimensions with endothelial dysfunction. Participants are invited to return for a 2-year follow-up visit to re-assess PTSD and its dimensions and endothelial dysfunction in order to investigate longitudinal associations.

### Ethics and Dissemination plan

This study is conducted in compliance with the Helsinki Declaration and University of California, Los Angeles Institutional Review Board. The results of this study will be disseminated via articles in peer-reviewed journals and presentations at academic conferences and to community partners.

**Trial registration number**

NCT03778307; Pre-results

**Keywords:** Trauma; posttraumatic stress disorder; mental health; endothelial dysfunction; cardiology

## ARTICLE SUMMARY

### Strengths and limitations of this study

- This is the first study to assess endothelial dysfunction, an early marker of CVD risk, using both functional (FMD) and cellular (EMP) approaches in a sample of trauma-exposed individuals.
- This study is the first to investigate whether PTSD and its dimensions are related to change in endothelial dysfunction over time.
- This study comprehensively examines whether objective measures of underlying dimensions of PTSD—fear and dysphoria—are related to endothelial dysfunction.
- Whereas previous research on PTSD and endothelial dysfunction has been conducted in predominantly male samples of veterans and police officers, we have a diverse, community-based sample of men and women with and without current PTSD who have been exposed to a range of traumas.
- Although the approach of assessing key constructs in the research laboratory permits more controlled measures of these constructs, it limits the generalizability of findings to participants' experiences in real-world settings.

## INTRODUCTION

Despite advances in prevention and intervention, cardiovascular disease (CVD) remains the leading cause of death in the United States.[1] Growing research demonstrates longitudinal links between trauma exposure, posttraumatic stress disorder (PTSD), and CVD, suggesting trauma and PTSD as novel targets for reducing CVD risk. Trauma is common and wide-ranging in nature. The vast majority of individuals (50-90%) experience a trauma in their lifetime, including natural disaster exposure, physical assault, and unwanted sexual contact.[2 3] PTSD is the quintessential trauma-related disorder, characterized by symptoms of re-experiencing of the trauma, avoidance of trauma reminders, negative alterations in cognition and mood, and hyperarousal.[4] PTSD has been associated with heightened risk of incident CVD, even after accounting for numerous risk factors.[5-11] Trauma exposure, independent of trauma-related psychopathology, has also been linked to elevated CVD risk.[5 12-14]

Given this evidence, experts in clinical psychology and cardiology have called for CVD risk surveillance after trauma.[15] To support such efforts, it is critical to identify intermediary mechanisms linking posttraumatic stress and CVD risk that can guide monitoring and intervention approaches. Endothelial dysfunction may be one such intermediary mechanism. Endothelial cells, which form the inner lining of blood vessels, are one of the first physiological indicators of reduced capacity to respond to cardiovascular demand, and endothelial dysfunction is implicated in the pathophysiology of atherosclerosis and CVD.[16 17] For every 1% decrease in brachial artery flow-mediated vasodilation (FMD; a functional measure of endothelial dysfunction), risk of CVD events increases by 9-13%.[18-20] Endothelial dysfunction can also be assessed by quantifying circulating levels of endothelial cell-derived microparticles (EMPs), direct measures of endothelial cell injury involved in atherosclerosis risk.[21-24] Endothelial

dysfunction can be detected in CVD-free individuals,[25] and it is a malleable risk marker responsive to intervention,[26 27] making it an optimal target for prevention and risk mechanism research.

Initial studies in select samples (e.g., women aged 40-60 years, male veterans or police officers) suggest that trauma exposure and elevated PTSD symptoms are associated with lower FMD.[28-30] PTSD has also been associated with biomarkers of endothelial dysfunction,[31 32] although no studies have considered specific markers of cellular injury (EMPs). Additionally, work in trauma-exposed individuals reveals that the greater the PTSD symptom severity, the lower the FMD, even among individuals without PTSD diagnoses.[28] Existing research has been limited by treating PTSD as a homogeneous diagnosis. PTSD has great heterogeneity in how symptoms manifest,[33] and understanding which aspects of PTSD particularly affect intermediary mechanisms will inform monitoring efforts and intervention development. Broadly, PTSD consists of two higher-order dimensions: fear and dysphoria.[34-36] Fear reflects an alarm response to perceived danger, whereas dysphoria represents low positive affect and anhedonia.[34]

PTSD dimensions hold promise for distinguishing specific versus nonspecific aspects of the disorder.[34] Whereas dysphoria is considered an auxiliary component of PTSD that is shared with depression, fear is considered a core component of PTSD.[37] Longitudinal network analyses indicate that fear responses are central aspects of acute and chronic posttraumatic stress responses, whereas dysphoria symptoms are secondary responses that develop over time.[35] Furthermore, impaired fear learning and inabilities to suppress fear in safe situations are key pathological processes in PTSD.[38] Fear conditioning paradigms provide a translational framework for examining dysregulated fear responses.[39] These responses can be measured using objective psychophysiological measures, including skin conductance (SC) responses and fear-

potentiated startle (FPS).[40-42] PTSD is characterized by psychophysiological responses indicative of dysregulated fear responding, including difficulties with safety signal processing and fear inhibition.[43-45] Additionally, some trauma-exposed individuals without PTSD exhibit psychophysiological responses akin to individuals with PTSD.[46 47] Thus, even trauma-exposed individuals without a PTSD diagnosis may experience trauma-related psychophysiological fear that could explain their lower FMD.[28]

Psychophysiological fear responses are promising targets for understanding links between PTSD and CVD risk, as they may engage key biological mechanisms. For example, repeated bouts of psychophysiological reactivity may promote autonomic dysregulation[48-50] and elevated systemic inflammation and oxidative stress,[51-53] which can have deleterious effects on cardiovascular health.[54] Research has yet to comprehensively examine mechanisms by which psychophysiological fear—or another PTSD dimension—may relate to endothelial dysfunction.

**Objectives**

This study examines links between PTSD and its dimensions with endothelial dysfunction in trauma-exposed men and women (see Figure 1 for conceptual model). First, we examine the association of PTSD diagnosis with endothelial dysfunction, assessed via functional (FMD) and cellular (EMP) measures. We hypothesize that participants with versus without PTSD will have lower FMD and, secondarily, greater EMP levels. Second, we investigate which PTSD dimensions are most strongly associated with endothelial dysfunction. We predict that stronger psychophysiological fear responses (measured by FPS and SC) will be associated with lower FMD and, secondarily, greater EMP levels. Although we focus on the core dimension of fear, we investigate the dysphoria-endothelial dysfunction relation to comprehensively assess potential intervention targets. Third, we explore autonomic imbalance, inflammation, and

oxidative stress as potential mechanisms. Finally, we examine whether PTSD and its dimensions predict change in endothelial dysfunction over 2 years.

## METHODS AND ANALYSIS

### Brief Study Overview

This mechanism-focused cohort study examines primarily cross-sectional and, secondarily longitudinal, associations. Investigating the PTSD diagnosis-endothelial dysfunction relation utilizes a case-control approach, whereas analyses of PTSD dimensions are conducted in the full sample. The sample will include 160 men and women without CVD from the Los Angeles area (80 individuals with PTSD and 80 trauma-exposed controls). Group designations are determined via diagnostic interview assessment.

### Participants

Eligible participants are ≥18 years old, fluent English speakers, and trauma-exposed. Participants in the PTSD group have a current PTSD diagnosis based on the past-month Clinician-Administered PTSD Scale for *DSM-5* (CAPS-5),[55] whereas controls have trauma exposure but no psychiatric diagnoses and a CAPS-5 total score <25.[56] Exclusions include (a) CVD history (physician-diagnosed myocardial infarction, angina, heart failure, peripheral artery disease, stroke, transient ischemic attack, atrial fibrillation, ventricular arrhythmias); (b) current psychotropic medication use (except benzodiazepines taken as-needed); (c) current bipolar, psychotic, or moderate or severe substance use disorder; (d) mild or severe cognitive impairment (Mini-Mental State Exam (MMSE) score ≥18);[57] (e) acute, unstable, or severe medical disorder or pregnancy; or (f) needing immediate psychiatric intervention (e.g., active suicidality).

### Recruitment and Enrollment

The study timeline, with measures administered at each assessment, is depicted in Figure 2. Participants are recruited via online advertisements, University of California, Los Angeles (UCLA) staff listservs, flyers throughout the community, and word-of-mouth referrals. Interested individuals are phone-screened to assess initial eligibility. After obtaining verbal consent, research personnel assess inclusion and exclusion criteria: English fluency, CVD history, psychotropic medication use, lifetime exposure to 17 types of traumas (using the Life Events Checklist for *DSM-5* (LEC-5)),[58] and past-month PTSD symptoms with respect to the trauma participants identify as most distressing (using the PTSD Checklist for *DSM-5* (PCL-5)).[59] The most distressing trauma is categorized as combat, non-combat interpersonal violence (e.g., assault), or non-interpersonal violence (e.g., accident),[60] and time since trauma is recorded. Eligible individuals are scheduled for a diagnostic interview assessment.

**Procedures**

***Diagnostic Interview Assessment***

Highly trained research personnel administer standardized clinical interviews. Interviewers have at least a Bachelor's degree and complete extensive training that includes self-study, didactics, tests to correctly diagnose cases from audio recordings, and role-playing. After obtaining written informed consent, the interviewer conducts a brief clinical interview to build rapport and review information about the participant's history and current functioning.

The first diagnostic interview administered is the CAPS-5,[55] the gold standard in PTSD assessment.[61] It is a structured interview querying individuals' past-month experiences of the 20 *DSM-5* PTSD criteria.[61] Symptoms are anchored to an index trauma self-identified by participants as their most distressing event. Responses for each symptom are scored with respect to frequency and intensity and combined into a severity score ranging from 0 (Absent) to 4

(Extreme/incapacitating). Symptom severity scores are used to determine a current PTSD diagnosis (according to *DSM-5* criteria) and summed to create a total severity score. Presence and history of other psychopathology are evaluated using the Structured Clinical Interview for *DSM-5,* Research Version (SCID-5-RV),[62] the gold standard in psychopathology assessment. Mood disorder episodes and psychotic symptoms are assessed for all participants, whereas anxiety, substance abuse, eating, and obsessive-compulsive disorders are assessed as needed based on screening questions. Interviewers then assess participants' medical history and health behaviors, including family history of CVD, personal history of medical conditions, medication use, smoking, and physical activity. If cognitive impairment is suspected, the MMSE is administered.[57]

Participants conclude the visit by completing several valid and reliable questionnaires. After reporting on sociodemographics, participants complete measures of childhood adversity: the Childhood Trauma Questionnaire[63] (measuring child abuse and neglect); the Childhood Experiences of Care and Abuse[64] antipathy and neglect items (anchored to participants' primary caregiver); the Conflict Tactics Scale[65] (measuring family violence); and a food insecurity measure based on the U.S. Department of Agriculture's Food Security Scale.[66] Participants report on PTSD symptoms (anchored to the CAPS-5 index trauma) using the PCL-5[59 67] and depressive symptoms using the Patient Health Questionnaire-8.[68] Participants describe their sleep with the Pittsburgh Sleep Quality Index (PSQI)[69] and PSQI Addendum for PTSD[70] (measuring past-month sleep patterns and frequency of disruptive nocturnal behaviors characteristic of PTSD, respectively); Insomnia Severity Index[71] (measuring insomnia); and RU-SATED[72] (measuring sleep health).

Propensity scores are used in recruitment to improve balance of sociodemographic and trauma-related characteristics across the PTSD and trauma-exposed control groups.[73] Scores are calculated using age, gender, race/ethnicity, trauma type, time since trauma, and two-way interactions among these variables. Matching during recruitment is based on quintiles of the propensity score distribution, with scores re-estimated weekly throughout recruitment. Participants are recruited if there is no more than 1-3 unmatched individuals from their group in the same quintile.

Results of the diagnostic interview assessment are reviewed by interviewers and supervised by a licensed clinical psychologist. Participants are informed of their eligibility via phone and scheduled for a laboratory visit approximately 2-3 weeks post-interview if eligible.

***Baseline Laboratory Visit***

Participants are instructed to avoid exercise, food and drink (other than water), smoking, and use of other tobacco, marijuana, or cannabidiol-containing products at least eight hours before this visit. Participants refrain from taking medications and vitamins (except diabetes medication, blood thinners, statins, and birth control) for at least 48 hours.

Participants are asked to arrive at the UCLA research laboratory by 8am. They are escorted to a study room for initial measurements and specimen collection; assessments are conducted by a research nurse. Height, weight, waist and hip circumferences, and left arm circumference are measured, and a spot urine sample is collected. An appropriately sized blood pressure cuff is then placed on the left arm. After a 5-minute rest, three seated blood pressure measures are taken 1 minute apart using a validated device (Omron HEM907XL). Blood is then drawn into serum-separating, citrate, and EDTA tubes. The citrate tube is centrifuged immediately and processed to measure EMPs. The remaining tubes are processed for a

comprehensive metabolic panel, lipid panel, and HbA1c; plasma aliquots are stored at -80°C for inflammatory marker assays.

Participants are then escorted to a temperature-controlled room to measure brachial artery FMD using a high-resolution, semi-automatic ultrasonography system (UNEXEF38G).[74-76] Testing occurs between 8-10am to account for circadian effects on FMD and is conducted according to published guidelines.[77 78] Participants lie supine, and a blood pressure cuff is placed around the right forearm. After a 15-minute rest, a baseline measure of brachial artery diameter is obtained. The UNEXEF38G system has a high-resolution linear artery transducer, coupled to computer-assisted analysis software that uses an automated edge detection system for measurement. The cuff is then inflated to 200mmHg (or 50mmHg above systolic pressure, if higher than 150mmHg) for 5 minutes. The image of the brachial artery is measured continuously until 2 minutes after cuff deflation.

After FMD measurement, participants are provided with a snack before completing behavioral tasks assessing posttraumatic fear and dysphoria. Task order is counterbalanced. Prior to completing the fear conditioning paradigm used to assess posttraumatic fear, participants undergo a heart rate response to deep breathing (HRDB) protocol; both tasks involve psychophysiological measurements. HRDB tests autonomic function, specifically parasympathetic tone.[79] For this task, participants lie supine. Respiration is measured with a respiration belt. ECG is measured with electrodes placed above the left and right collar bones and on the left lower forearm and recorded with the Biopac MP160 ECG wireless module. ECG and respiration data are collected during a 1-minute resting baseline and 10-second respiratory cycle (5-second inhale, 5-second exhale), repeated six times.[79]

Posttraumatic fear is assessed using an established fear conditioning protocol with two phases: Fear Acquisition and Extinction.[43 80] During Fear Acquisition, participants complete a 4-trial habituation phase in which two colored shape conditioned stimuli (CS) and a 108dB, 40-millisecond broadband noise startle probe alone (NA) are presented to familiarize participants. Next, participants undergo a 36-trial conditioning phase, with 3 blocks of 12 trials: 4 reinforced CS (CS+), 4 nonreinforced CS (CS-), and 4 NA trials. CS are presented on a computer monitor for 6 seconds; the startle probe is delivered binaurally via headphones on every trial after 6 seconds. During conditioning, only the CS+ is reinforced with an unconditioned stimulus (US) on every trial. The US is a 250-millisecond airblast of 140psi intensity directed at the larynx, which has consistently produced robust FPS.[43 80] On the CS+ trials, the US co-terminates with the CS+ 0.5 seconds following the startle probe; CS- trials terminate immediately after the startle probe. After a 10-minute break, participants undergo the 72-trial Fear Extinction phase: 6 blocks of 4 trials of each type (CS+, CS-, NA). During Extinction, the CS+ is presented without the US. Inter-trial intervals are randomized to be 9-22 seconds for Fear Acquisition and Extinction.

Psychophysiological fear responses are collected using Biopac MP160 electromyography (EMG) and SC wireless modules; ECG is also recorded. FPS is measured through corrugator and orbicularis EMG. Startle magnitude is assessed as the peak amplitude of EMG contraction 20-200ms following the startle probe. SC data are collected using electrodes on the hypothenar surface of the non-dominant hand to measure sympathetic arousal. Psychophysiological data are filtered, rectified, and smoothed using MindWare software. Several indicators of psychophysiological fear responses are calculated, including: 1) FPS to the CS+ during early extinction (fear load[80]), 2) FPS to the CS+ during late extinction, and 3) FPS to the CS- during late fear acquisition. These measures reflect expression of conditioned fear, fear inhibition, and

fear discrimination.[80-82] FPS is calculated by subtracting startle magnitude to the NA from startle magnitude to the CS in each block to account for individual differences in startle magnitude and habituation. Comparable scores for SC response are secondary predictors. Although frequently used to index fear, SC response may be a more nonspecific measure of arousal that is not as closely tied to fear neurocircuitry as FPS.[82-84] SC response is calculated by subtracting SC prior to stimulus onset from maximum SC during CS presentation.

Posttraumatic dysphoria is assessed with an established and reliable eye-tracking paradigm adapted for dysphoria.[85] Attention allocation is measured using a remote high-speed eye-tracker (EyeLink Portable Duo). The task consists of free-viewing 4x4 matrices of NimStim[86] happy and sad faces. Trials begin with a fixation cross, shown until a 1000-millisecond fixation is recorded, to verify trials begin with gaze fixated at the matrix's center. Matrices are presented for 6000 milliseconds, followed by a 2000-millisecond inter-trial interval. Participants view 60 different matrices, presented in 2 blocks of 30 each, with a 60-second break between blocks. Eye-tracking data are used to define fixations as 100 milliseconds+ of stable fixation within 1-degree visual angle. Dwell time for Areas of Interest (AOIs)—one each for sad and happy faces—are calculated for each matrix. Total dwell time for each AOI averages dwell times across the 60 matrices.

***Follow-up Visit***

Approximately two years after the baseline laboratory visit, participants return to UCLA for a follow-up. All participants are invited to return; we aim to re-evaluate at least 80 individuals (40+ each in the PTSD and control groups). At follow-up, participants repeat all procedures from the baseline laboratory visit. Research personnel administer the CAPS-5 to assess past-month PTSD and the SCID-5 to assess psychopathology experienced since baseline.

Medical history, medications, health behaviors, PTSD and depressive symptoms, and sleep are also queried.

**Outcome Measures**

***Main Outcomes***

**Brachial Artery FMD.** Our primary outcome is brachial artery FMD. As described above, pulsed Doppler velocity signals are obtained at baseline and after cuff deflation using the UNEXEF38G ultrasonography system. FMD is the percent difference in brachial artery diameter, before and after occlusion.

**EMPs.** Circulating EMPs expressing CD62E and CD31 (endothelial cell activation and apoptosis, respectively)[24] assessed using flow cytometry are secondary outcome measures. EMP sample preparation is completed within two hours of blood draw, as previously described.[87-90] Citrated blood is centrifuged at 160g at 4℃ for 10 minutes to obtain platelet-rich plasma, which is further centrifuged at 1500g at 4℃ for 6 minutes to yield platelet-poor plasma. Fifty microliters of platelet-poor plasma are each incubated in two sets: 1) 4μL of brilliant violet 421 (BV421)-conjugated monoclonal antibody to CD62E (BD), 4μL of phycoerythrin (PE)-conjugated monoclonal antibody to CD31 (BD), 4μL of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody to CD42b (BD), and 2) 4μL of PE-conjugated monoclonal antibody to CD31, 4μL of FITC-conjugated Annexin V (BD). EMPs are the number of particles with size <1.5μm 1) positively labeled by CD62E, 2) positively labeled by CD31 and negatively labeled by CD42b, and 3) positively labeled by CD31 and Annexin V. Allophycocyanin (APC)-conjugated monoclonal antibody CD45 (a pan-leukocyte marker; BD) is used to exclude contamination by leukocyte microparticles.[88] Negative controls include the appropriate FITC-

labeled, PE-labeled, BV421-labeled, and APC-labeled isotype-matched IgG. EMPs are quantified as the number of EMPs per μL (ThermoFisher Attune NxT Flow Cytometer).

**Potential Pathway Variables**

We are exploring autonomic imbalance, inflammation, and oxidative stress as potential mechanisms linking PTSD and its dimensions with endothelial dysfunction. Average respiratory sinus arrhythmia (RSA) amplitude across the cycles of the HRDB task—defined as the difference between the end of expiration and end of inspiration in heart rate—reflects parasympathetic-mediated cardiac control.[79 91] Blood-based inflammatory markers are assayed from plasma, including high-sensitivity C-reactive protein measured by ELISA (R&D Systems). Interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-α, and interferon-gamma are measured in a multiplex assay utilizing a V-PLEX Custom Human Cytokine Proinflammatory Panel on the Meso Scale Discovery electrochemiluminesence platform. Oxidative stress is assessed by measuring urinary F2-isoprostanes using an ELISA (Oxford Biomedical Research).

**Statistical Analyses**

We will first conduct cross-sectional associations of PTSD and its dimensions with endothelial dysfunction; longitudinal associations will be considered in exploratory analyses. For cross-sectional analyses, two sample t-tests will compare participants with and without PTSD on 1) FMD and 2) EMPs expressing CD62E and CD31. We will extend these analyses using linear regression models adjusting for covariates (described below). We will consider total PTSD severity score as a continuous predictor. Second, objective measures of posttraumatic fear (e.g., fear load based on FPS) will be the exposures in cross-sectional linear regression analyses of FMD and EMP outcomes. Additionally, we will explore objective measures of posttraumatic dysphoria as predictors of endothelial dysfunction. The approach for these analyses will mirror

that for posttraumatic fear, with exposures operationalized as total dwell times for the sad and happy AOIs.

For longitudinal analyses, we will first examine how measures of PTSD and its dimensions at baseline predict change in FMD using linear regression. Second, we will examine how changes in PTSD and its dimensions over follow-up predict change in FMD using linear regression. Analyses will consider EMPs as secondary outcomes. For longitudinal analyses, FMD at the follow-up assessment will be the outcome, and FMD at the baseline assessment will be included as a covariate.

To examine if autonomic imbalance, inflammation, and oxidative stress explain associations of PTSD and its dimensions with endothelial dysfunction in cross-sectional and/or longitudinal analyses, we will estimate total and direct effects of measures of PTSD and its dimensions on endothelial dysfunction and test for significant indirect effects using bootstrapping methods.[92]

For all analyses, we will examine a series of adjusted models. The base adjusted model will include age, gender, race, and ethnicity; subsequent models will sequentially adjust for: 1) other sociodemographics (e.g., education, marital status); 2) CVD medical risk factors (e.g., diabetes, hypertension); 3) medications (e.g., medication for hypertension, hyperlipidemia); and 4) anthropometrics and health behaviors (e.g., body mass index, smoking). To avoid overfitting, we will fit group lasso regression, which performs variable selection and regularization of regression coefficients, to identify important predictors of endothelial dysfunction and improve model interpretability.[93]

**Sample Size Estimation and Power**

Analyses are powered to our primary outcome, FMD, and we used the smallest effect size from prior research to be conservative in our calculations. Specifically, we estimated our effect size based on Cohen's *d*=0.48 for the difference in percent change in FMD in patients with versus without probable PTSD.[28] Power analysis for a two sample *t*-test was conducted to determine a sufficient sample size using an alpha of .05, power of .80, a medium effect size (Cohen's *d*=0.48), two tails, and equal allocations of participants to the PTSD and trauma control groups. Based on these assumptions, the desired sample size was 70 participants per group. We conservatively selected 80 participants per group.

Given our hypothesis that psychophysiological fear is the key PTSD dimension that will relate to endothelial dysfunction, we considered power for this analysis based on our primary exposure for this aim: FPS. We assumed a standard deviation (SD) of 70 for FPS scores[80] and a SD of 3.5% for FMD.[28] With *N*=160 and a two-sided significance level of .05, we have 80% power to detect a change of 0.011% in FMD for each unit of FPS score increase.[94] This is a small effect size,[28] so our sample is large enough to model psychophysiological fear and FMD in adjusted models.

**Ethics and dissemination**

This study is conducted in compliance with the UCLA Institutional Review Board and the Helsinki Declaration. Participants sign informed consent and Health Insurance Portability and Accountability Act Authorization for Research forms at enrollment. Research personnel review the study in detail and answer questions. Individuals are informed that participation is optional and that they may withdraw at any time. After individuals are fully informed about study procedures, participants who elect to proceed with the study sign the consent document, as do research personnel. The findings of this study will be disseminated online (e.g.,

ClinicalTrials.gov, Open Science Framework), presented at academic conferences, and published in peer-reviewed journals.

**Patient and public involvement**

Patients and the public were not involved in the design or conduct of the study. We plan to share findings with the public through dissemination to community partners.

## DISCUSSION

To our knowledge, this is the first study to comprehensively investigate the ways in which PTSD and dimensions of posttraumatic stress relate to endothelial dysfunction, one of the earliest modifiable precursors to CVD. To distill pathways by which posttraumatic stress may lead to CVD risk, we focus on psychophysiological fear—a key dimension of PTSD—although we consider objective measures of dysphoria, a more nonspecific dimension. This study incorporates state-of-the-art measures of endothelial dysfunction by investigating both brachial artery FMD and, secondarily, EMPs. No investigation of trauma-exposed individuals has considered functional and cellular measures of endothelial dysfunction as we do here. Furthermore, unlike prior research with predominantly white, male samples,[27 28] this study will include men and women of diverse racial/ethnic backgrounds who have been exposed to a range of traumas, increasing the generalizability of findings.

Despite these strengths, there are possible limitations. All assessments are conducted in the laboratory, which offers a controlled setting that enhances internal validity but may limit the generalizability of findings to real-world settings. Additionally, although we use state-of-the-art measures of endothelial dysfunction, neither metric indicates endothelial function reactivity to trauma-related cues. Further research using provocation measures or assessing cardiovascular reactivity in naturalistic settings is needed to address this issue.

PTSD predicts incident CVD, but the field needs intervention targets and intermediary mechanisms to determine if PTSD interventions can offset CVD risk. This study will test whether endothelial dysfunction could be an early subclinical mechanism by which PTSD increases CVD risk, and whether posttraumatic fear or another dimension could be the target to offset that risk in vulnerable, trauma-exposed individuals.

## AUTHOR CONTRIBUTIONS

**Contributors:**

JAS, DS, YN, AL, OAA, RE, TH, and KW-T contributed significantly to the planning, conception, design, and successful funding of this study. SC, KR, JT, JAS, and KW-T are contributing significantly to the acquisition of data. SC, KR, and JAS drafted the initial version of this manuscript. AKM, DS, YN, AL, OAA, RE, TH, and JAS will be involved in the analyses and interpretation of the data. All authors revised the draft critically for important intellectual content and gave final approval for this version of the manuscript to be submitted for publication.

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Shiloh Cleveland and Kristina Reed contributed equally to this work.

## FUNDING STATEMENT

This study is supported by National Heart, Lung, and Blood Institute (NHLBI) grant number R01HL139614.

**Disclaimer:**

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## COMPETING INTERESTS

There are no competing interests declared for this study.

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## FIGURE LEGENDS

Figure 1. Conceptual model for the study. The aim examining whether PTSD diagnosis is related to endothelial dysfunction is indicated by the dashed arrows. The aim examining associations of posttraumatic fear and dysphoria with endothelial dysfunction is indicated by the solid arrow. Mechanisms examined in exploratory analyses are printed above and below the solid arrow.

Figure 2. Study timeline. CAPS-5=Clinician-Administered PTSD Scale for *DSM-5*. CVD=cardiovascular disease. FMD=flow-mediated dilation. LEC-5=Life Events Checklist for *DSM-5*. PCL-5=PTSD Checklist for *DSM-5*. SCID-5=Structured Clinical Interview for *DSM-5*.

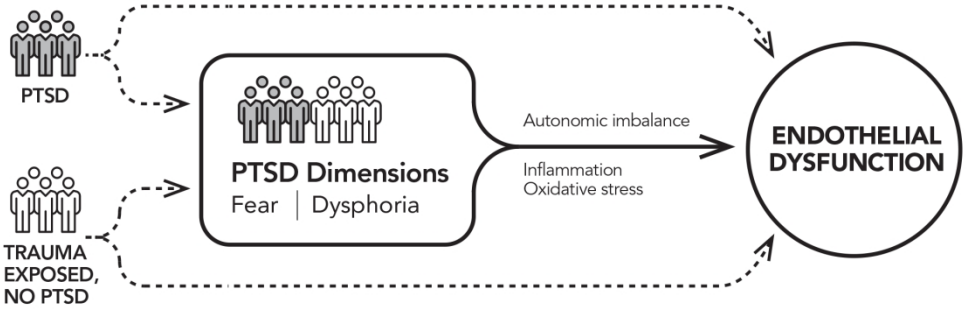


Figure 1. Conceptual model for the study. The aim examining whether PTSD diagnosis is related to endothelial dysfunction is indicated by the dashed arrows. The aim examining associations of posttraumatic fear and dysphoria with endothelial dysfunction is indicated by the solid arrow. Mechanisms examined in exploratory analyses are printed above and below the solid arrow.

176x61mm (300 x 300 DPI)

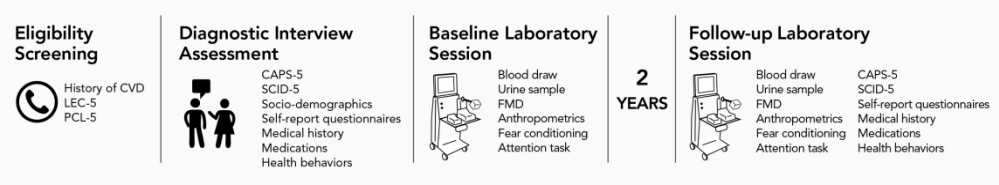


Figure 2. Study timeline. CAPS-5=Clinician-Administered PTSD Scale for DSM-5. CVD=cardiovascular disease. FMD=flow-mediated dilation. LEC-5=Life Events Checklist for DSM-5. PCL-5=PTSD Checklist for DSM-5. SCID-5=Structured Clinical Interview for DSM-5.

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# Key Dimensions of Posttraumatic Stress Disorder and Endothelial Dysfunction: A Protocol for a Mechanism-Focused Cohort Study



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|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2020-043060.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 24-Feb-2021 |
| Complete List of Authors: | Cleveland, Shiloh; University of California Los Angeles, Psychology<br>Reed, Kristina; University of California Los Angeles, Psychology<br>Thomas, Jordan; University of California Los Angeles, Psychology<br>Ajijola, Olujimi; University of California Los Angeles, Neurocardiology Research Center of Excellence, Cardiac Arrhythmia Center<br>Ebrahimi, Ramin; University of California Los Angeles, Medicine; Veterans Affairs Greater Los Angeles Healthcare System, Medicine, Cardiology Section<br>Hsiai, Tzung; University of California Los Angeles, Medicine; University of California Los Angeles, Bioengineering<br>Lazarov, Amit; Tel Aviv University, School of Psychological Sciences<br>Montoya, Amanda; University of California Los Angeles, Psychology<br>Neria, Yuval; Columbia University Irving Medical Center, Psychiatry; Columbia University Irving Medical Center, Epidemiology<br>Shimbo, Daichi; Columbia University Irving Medical Center, Medicine<br>Wolitzky-Taylor, Kate; University of California Los Angeles, Psychiatry and Biobehavioral Sciences<br>Sumner, Jennifer; University of California Los Angeles, Psychology |
| Primary Subject Heading: | Mental health |
| Secondary Subject Heading: | Cardiovascular medicine |
| Keywords: | Anxiety disorders < PSYCHIATRY, Adult psychiatry < PSYCHIATRY, Cardiology < INTERNAL MEDICINE |
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# Key Dimensions of Posttraumatic Stress Disorder and Endothelial Dysfunction: A Protocol for a Mechanism-Focused Cohort Study

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Word count: 4,000

## ABSTRACT

### Introduction

Both trauma exposure and posttraumatic stress disorder (PTSD) are associated with increased risk of cardiovascular disease (CVD), the leading cause of death in the United States. Endothelial dysfunction, a modifiable, early marker of CVD risk, may represent a physiological mechanism underlying this association. This mechanism-focused cohort study aims to investigate the relationship between PTSD (both in terms of diagnosis and underlying symptom dimensions) and endothelial dysfunction in a diverse, community-based sample of adult men and women.

### Methods and analysis

Using a cohort design, 160 trauma-exposed participants without a history of CVD are designated to the PTSD group (*n*=80) or trauma-exposed matched control group (*n*=80) after a baseline diagnostic interview assessment. Participants in the PTSD group have a current (past-month) diagnosis of PTSD, whereas those in the control group have a history of trauma but no current or past psychiatric diagnoses. Endothelial dysfunction is assessed via flow-mediated vasodilation (FMD) of the brachial artery and circulating levels of endothelial cell-derived microparticles (EMPs). Two higher-order symptom dimensions of PTSD—fear and dysphoria—are measured objectively with a fear conditioning paradigm and attention allocation task, respectively. Autonomic imbalance, inflammation, and oxidative stress are additionally assessed and will be examined as potential pathway variables linking PTSD and its dimensions with endothelial dysfunction. Participants are invited to return for a 2-year follow-up visit to re-assess PTSD and its dimensions and endothelial dysfunction in order to investigate longitudinal associations.

### Ethics and Dissemination plan

This study is conducted in compliance with the Helsinki Declaration and University of California, Los Angeles Institutional Review Board. The results of this study will be disseminated via articles in peer-reviewed journals and presentations at academic conferences and to community partners.

**Trial registration number**

NCT03778307; Pre-results

**Keywords:** Trauma; posttraumatic stress disorder; mental health; endothelial dysfunction; cardiology

## ARTICLE SUMMARY

### Strengths and limitations of this study

- This is the first study to assess endothelial dysfunction, an early marker of CVD risk, using both functional (FMD) and cellular (EMP) approaches in a sample of trauma-exposed individuals.
- This study is the first to investigate whether PTSD and its dimensions are related to change in endothelial dysfunction over time.
- This study comprehensively examines whether objective measures of underlying dimensions of PTSD—fear and dysphoria—are related to endothelial dysfunction.
- Whereas previous research on PTSD and endothelial dysfunction has been conducted in predominantly male samples of veterans and police officers, we have a diverse, community-based sample of men and women with and without current PTSD who have been exposed to a range of traumas.
- Although the approach of assessing key constructs in the research laboratory permits more controlled measures of these constructs, it limits the generalizability of findings to participants' experiences in real-world settings.

## INTRODUCTION

Despite advances in prevention and intervention, cardiovascular disease (CVD) remains the leading cause of death in the United States.[1] Growing research demonstrates longitudinal links between trauma exposure, posttraumatic stress disorder (PTSD), and CVD, suggesting trauma and PTSD as novel targets for reducing CVD risk. Trauma is common; the vast majority of individuals (50-90%) experience a trauma in their lifetime, including natural disasters, physical assault, and unwanted sexual contact.[2 3] PTSD is the quintessential trauma-related disorder, characterized by symptoms of re-experiencing of the trauma, avoidance of trauma reminders, negative alterations in cognition and mood, and hyperarousal.[4] PTSD has been associated with heightened risk of incident CVD, even after accounting for numerous risk factors.[5-11] Trauma exposure, independent of trauma-related psychopathology, has also been linked to elevated CVD risk.[5 12-14]

Given this evidence, experts in clinical psychology and cardiology have called for CVD risk surveillance after trauma.[15] To support such efforts, it is critical to identify intermediary mechanisms linking posttraumatic stress and CVD risk that can guide monitoring and intervention approaches. Endothelial dysfunction may be one such mechanism. Endothelial cells, which form the inner lining of blood vessels, are one of the first physiological indicators of reduced capacity to respond to cardiovascular demand, with endothelial dysfunction implicated in the pathophysiology of atherosclerosis and CVD.[16 17] For every 1% decrease in brachial artery flow-mediated vasodilation (FMD; a functional measure of endothelial dysfunction), CVD risk increases by 9-13%.[18-20] Endothelial dysfunction can also be assessed by quantifying circulating levels of endothelial cell-derived microparticles (EMPs), direct measures of endothelial cell injury involved in atherosclerosis risk.[21-24] As endothelial dysfunction can be detected in CVD-

free individuals[25] and is a malleable risk marker responsive to intervention,[26 27] it is an optimal target for prevention and risk mechanism research.

Initial studies in select samples (women aged 40-60 years, male veterans or police officers) suggest that trauma exposure and elevated PTSD symptoms are associated with lower FMD.[28-30] Additionally, work in trauma-exposed individuals reveals that the greater the PTSD symptom severity, the lower the FMD, even among individuals without PTSD diagnoses.[28] PTSD has also been associated with biomarkers of endothelial dysfunction,[31 32] although no studies have considered specific markers of cellular injury (EMPs).

Existing research has also been limited by treating PTSD as a homogeneous diagnosis. However, PTSD has great heterogeneity in how symptoms manifest.[33] Thus, understanding which aspects of PTSD particularly affect intermediary mechanisms will inform monitoring efforts and intervention development. Broadly, PTSD consists of two higher-order dimensions: fear and dysphoria.[34-36] Fear reflects an alarm response to perceived danger, whereas dysphoria represents low positive affect and anhedonia.[34] PTSD dimensions hold promise for distinguishing specific versus nonspecific aspects of the disorder.[34] Whereas dysphoria is considered an auxiliary component of PTSD, shared with depression, fear is considered a core component of PTSD.[37] Longitudinal network analyses indicate that fear responses are central aspects of acute and chronic posttraumatic stress responses, whereas dysphoria symptoms are secondary responses that develop over time.[35] Furthermore, impaired fear learning and inabilities to suppress fear in safe situations are key pathological processes in PTSD.[38] Experimentally, fear conditioning paradigms provide a translational framework for examining dysregulated fear responses,[39] which can be measured using objective psychophysiological measures, including skin conductance (SC) responses and fear-potentiated startle (FPS).[40-42] In fear conditioning

research, PTSD has been characterized by psychophysiological responses indicative of dysregulated fear responding, including difficulties with safety signal processing and fear inhibition.[43-45] Additionally, some trauma-exposed individuals without PTSD exhibit psychophysiological responses akin to individuals with PTSD,[46 47] reflecting similar trauma-related psychophysiological fear that could explain their lower FMD.[28]

Furthermore, psychophysiological fear responses are promising targets for understanding links between PTSD and CVD risk, as they may engage key biological mechanisms. For example, repeated bouts of psychophysiological reactivity may promote autonomic dysregulation[48-50] and elevated systemic inflammation and oxidative stress,[51-53] which can have deleterious effects on cardiovascular health.[54] However, research has yet to comprehensively examine mechanisms by which psychophysiological fear—or another PTSD dimension—may relate to endothelial dysfunction.

**Objectives**

This study examines links between PTSD and its dimensions with endothelial dysfunction in trauma-exposed men and women (see Figure 1 for conceptual model). First, we examine the association of PTSD diagnosis with endothelial dysfunction, assessed via functional (FMD) and cellular (EMP) measures. We hypothesize that participants with versus without PTSD will have lower FMD and, secondarily, higher EMP levels. Second, we investigate which PTSD dimensions are most strongly associated with endothelial dysfunction. We predict that stronger psychophysiological fear responses (measured by FPS and SC) will be associated with lower FMD and, secondarily, higher EMP levels. Although we focus on the core dimension of fear, we investigate the dysphoria-endothelial dysfunction relation to comprehensively assess potential intervention targets. Third, we explore autonomic imbalance, inflammation, and

oxidative stress as potential mechanisms. Finally, we examine whether PTSD and its dimensions predict change in endothelial dysfunction over 2 years.

## METHODS AND ANALYSIS

### Brief Study Overview

This mechanism-focused cohort study examines primarily cross-sectional and, secondarily longitudinal, associations. Enrollment began in 2019, and the study is projected to continue through 2023. Investigating the PTSD diagnosis-endothelial dysfunction relation utilizes a case-control approach, whereas analyses of PTSD dimensions are conducted in the full sample. The sample will include 160 men and women without CVD from the Los Angeles area (80 individuals with PTSD; 80 trauma-exposed controls). Group designations are determined via diagnostic interview assessment. No-trauma controls were not included, as this study focuses on identifying what aspects of PTSD symptoms are linked to endothelial dysfunction.

### Participants

Eligible participants are ≥18 years old, fluent English speakers, and trauma-exposed. Participants in the PTSD group have a current PTSD diagnosis based on the past-month Clinician-Administered PTSD Scale for *DSM-5* (CAPS-5),[55] whereas controls are trauma-exposed with no psychiatric diagnoses and a CAPS-5 score <25.[56] Exclusions include (a) individual CVD history (physician-diagnosed myocardial infarction, angina, heart failure, peripheral artery disease, stroke, transient ischemic attack, atrial fibrillation, ventricular arrhythmias); (b) current psychotropic medication use (except benzodiazepines taken as-needed), as this has been shown to influence key study variables including FMD, psychophysiological responses, and attentional allocation;[57-61] (c) current bipolar, psychotic, or moderate or severe substance use disorder; (d) mild or severe cognitive impairment (Mini-Mental State Exam

(MMSE) score ≤18);[62] (e) acute, unstable, or severe medical disorder or pregnancy; or (f) needing immediate psychiatric intervention (e.g., active suicidality).

**Recruitment and Enrollment**

The study timeline is depicted in Figure 2. Participants are recruited via online advertisements, University of California, Los Angeles (UCLA) staff listservs, flyers throughout the community, and word-of-mouth referrals. Interested individuals are phone-screened to assess initial eligibility. After obtaining verbal consent, research personnel assess inclusion and exclusion criteria: English fluency, CVD history, psychotropic medication use, lifetime exposure to 17 types of trauma (using the Life Events Checklist for *DSM-5* (LEC-5)),[63] and past-month PTSD symptoms with respect to the trauma participants identify as most distressing (using the PTSD Checklist for *DSM-5* (PCL-5)).[64] The most distressing trauma is categorized as combat, non-combat interpersonal violence (e.g., assault), or non-interpersonal violence (e.g., accident).[65] Time since trauma is also recorded. Eligible individuals are scheduled for a diagnostic interview assessment.

**Procedures**

***Diagnostic Interview Assessment***

Highly trained research personnel administer standardized clinical interviews. Interviewers have at least a Bachelor's degree and complete extensive training including self-guided study, didactics, diagnosing cases from recordings, and role-playing. After obtaining written informed consent, the interviewer conducts a brief clinical interview to build rapport and review information about the participant's history and current functioning.

The first diagnostic interview administered is the CAPS-5,[55] a gold standard structured interview querying past-month experiences of the 20 *DSM-5* PTSD criteria.[66] Symptoms are

anchored to an index trauma self-identified by participants as their most distressing event. Responses for each symptom are scored with respect to frequency and intensity and combined into a severity score ranging from 0 (Absent) to 4 (Extreme/incapacitating). Symptom endorsement is used to determine current PTSD diagnosis, and severity scores are summed for a total severity score. Presence and history of other psychopathology are evaluated using the Structured Clinical Interview for *DSM-5,* Research Version (SCID-5-RV),[67] the gold standard in psychopathology assessment. Mood disorder episodes and psychotic symptoms are assessed for all participants; anxiety, substance abuse, eating, and obsessive-compulsive disorders are assessed as needed based on screening questions. Interviewers then assess participants' medical history and health behaviors, including family history of CVD, personal history of medical conditions, pregnancy, medications, smoking, and physical activity. If cognitive impairment is suspected, the MMSE is administered.[62]

Participants conclude the visit by completing several valid and reliable questionnaires. After reporting on sociodemographics, participants complete measures of childhood adversity: the Childhood Trauma Questionnaire[68] (measuring child abuse and neglect); the Childhood Experiences of Care and Abuse[69] antipathy and neglect items (anchored to participants' primary caregiver); the Conflict Tactics Scale[70] (measuring family violence); and a food insecurity measure based on the U.S. Department of Agriculture's Food Security Scale.[71] Participants report on PTSD symptoms (anchored to the CAPS-5 index trauma) using the PCL-5[64 72] and depressive symptoms using the Patient Health Questionnaire-8.[73] Participants describe their sleep with the Pittsburgh Sleep Quality Index (PSQI),[74] PSQI Addendum for PTSD,[75] Insomnia Severity Index,[76] and RU-SATED.[77]

Propensity scores are used in recruitment to improve balance of sociodemographic and trauma-related characteristics across the PTSD and trauma-exposed control groups.[78] Scores reflecting propensity of PTSD group membership are calculated using age, gender, race/ethnicity, trauma type, time since trauma, and two-way interactions among these variables. Matching during recruitment is based on quintiles of the propensity score distribution, with scores re-estimated weekly throughout recruitment. Participants are recruited if there are <3 unmatched individuals from their group in the same quintile.

Diagnostic interview assessments are reviewed by interviewers and supervised by a licensed clinical psychologist. Participants are informed of their eligibility via phone, with eligible participants scheduled for a laboratory visit ~2-3 weeks later.

***Baseline Laboratory Visit***

Participants are instructed to avoid exercise, food and drink (except water), smoking, and use of other tobacco, marijuana, or cannabidiol-containing products at least eight hours before this visit. Medications and vitamins (except diabetes medication, blood thinners, statins, and birth control) are avoided for at least 48 hours.

Participants are asked to arrive at the research laboratory by 8am. They are escorted to a study room for initial measurements and specimen collection; assessments are conducted by a research nurse. Height, weight, waist and hip circumferences, and left arm circumference are measured, and a spot urine sample is collected. An appropriately sized blood pressure cuff is placed on the left arm. After a 5-minute rest, three seated blood pressure measures are taken 1 minute apart using a validated device (Omron HEM907XL). Blood is then drawn into serum-separating, citrate, and EDTA tubes. The citrate tube is centrifuged immediately and processed to measure EMPs. EDTA tubes are centrifuged within one hour of collection at 1500g at 4°C for 10

minutes to isolate plasma; aliquots are stored at -80°C for inflammatory marker assays. Serum and whole blood are extracted from serum-separating and EDTA tubes for comprehensive metabolic and lipid panels and HbA1c, respectively.

Participants are then escorted to a temperature-controlled room to measure brachial artery FMD using a high-resolution, semi-automatic ultrasonography system (UNEXEF38G).[79-81] Testing occurs between 8-10am to account for circadian effects on FMD and is conducted according to published guidelines.[82 83] Participants lie supine, and a blood pressure cuff is placed around the right forearm. After a 15-minute rest, a baseline measure of brachial artery diameter is obtained. The UNEXEF38G system has a high-resolution linear artery transducer, coupled to computer-assisted analysis software that uses an automated edge detection system for measurement. The cuff is then inflated to 200mmHg (or 50mmHg above systolic pressure, if >150mmHg) for 5 minutes. Images of the brachial artery are obtained continuously until 2 minutes after cuff deflation.

After FMD measurement, participants are provided a snack before completing tasks assessing posttraumatic fear and dysphoria. Task order is counterbalanced. Prior to completing the fear conditioning paradigm used to assess posttraumatic fear, participants undergo a heart rate response to deep breathing (HRDB) protocol; both tasks involve psychophysiological measurements. HRDB tests autonomic function, specifically parasympathetic tone.[84] Participants lie supine; respiration is measured with a respiration belt and ECG is measured with electrodes placed above the left and right collar bones and on the left lower forearm and recorded with the Biopac MP160 ECG wireless module. ECG and respiration data are collected during a 1-minute resting baseline and 10-second respiratory cycle (5-second inhale, 5-second exhale), repeated 6 times.[84]

Posttraumatic fear is assessed using an established fear conditioning protocol with two phases: Fear Acquisition and Extinction.[43 85] During Fear Acquisition, participants complete a 4-trial habituation phase in which two colored shape conditioned stimuli (CS) and a 108dB, 40-millisecond broadband noise startle probe alone (NA) are presented to familiarize participants. Next, participants undergo a 36-trial conditioning phase, with 3 blocks of 12 trials: 4 reinforced CS (CS+), 4 nonreinforced CS (CS-), and 4 NA trials. CS are presented on a computer monitor for 6 seconds; the startle probe is delivered binaurally via headphones on every trial after 6 seconds. During conditioning, only the CS+ is reinforced with an unconditioned stimulus (US) on every trial. The US is a 250-millisecond airblast of 140psi intensity directed at the larynx, which has consistently produced robust FPS.[43 85] On the CS+ trials, the US co-terminates with the CS+ 0.5 seconds following the startle probe; CS- trials terminate immediately after the startle probe. After a 10-minute break, participants undergo the 72-trial Fear Extinction phase: 6 blocks of 4 trials of each type (CS+, CS-, NA). During Extinction, the CS+ is presented without the US. Inter-trial intervals are randomized to be 9-22 seconds for Fear Acquisition and Extinction.

Psychophysiological fear responses are collected using Biopac MP160 electromyography (EMG) and SC wireless modules; ECG is also recorded. FPS is measured through corrugator and orbicularis EMG. Startle magnitude is assessed as the peak amplitude of EMG contraction 20-200ms following the startle probe. SC data are collected using electrodes on the hypothenar surface of the non-dominant hand to measure sympathetic arousal. Psychophysiological data are filtered, rectified, and smoothed using MindWare software. Several indicators of psychophysiological fear responses are calculated, including: 1) FPS to CS+ during early extinction (fear load[85]), 2) FPS to CS+ during late extinction, and 3) FPS to CS- during late fear acquisition. These measures reflect expression of conditioned fear, fear inhibition, and fear

discrimination.[85-87] FPS is calculated by subtracting startle magnitude to the NA from startle magnitude to the CS in each block to account for individual differences in startle magnitude and habituation. Comparable scores for SC response are secondary predictors. Although frequently used to index fear, SC response may be a more nonspecific measure of arousal that is not as closely tied to fear neurocircuitry as FPS.[87-89] SC response is calculated by subtracting SC prior to stimulus onset from maximum SC during CS presentation.

Posttraumatic dysphoria is assessed with an established and reliable eye-tracking paradigm adapted for dysphoria.[90] Attention allocation is measured using a remote high-speed eye-tracker (EyeLink Portable Duo). The task consists of free-viewing 4x4 matrices of NimStim[91] happy and sad faces. Trials begin with a fixation cross, shown until a 1000-millisecond fixation is recorded, to verify trials begin with gaze fixated at the matrix's center. Matrices are presented for 6000 milliseconds, followed by a 2000-millisecond inter-trial interval. Participants view 60 different matrices, presented in 2 blocks of 30 each, with a 60-second break between blocks. Eye-tracking data are used to define fixations as 100 milliseconds+ of stable fixation within 1-degree visual angle. Dwell time for two Areas of Interest (AOIs)—the eight sad faces and eight happy faces—are calculated for each matrix. Dwell time for each AOI is averaged across the 60 matrices.

***Follow-up Visit***

Approximately two years after the baseline visit, all participants are invited to return for a follow-up; we aim to re-evaluate at least 80 individuals (40+ in each group). At follow-up, participants repeat all procedures from the baseline laboratory visit.

**Outcome Measures**

***Main Outcomes***

**FMD.** Our primary outcome is brachial artery FMD. As described above, pulsed Doppler velocity signals are obtained at baseline and after cuff deflation using the UNEXEF38G ultrasonography system. FMD is the percent difference in brachial artery diameter, before and after occlusion.

**EMPs.** Circulating EMPs expressing CD62E and CD31 (endothelial cell activation and apoptosis, respectively)[24] assessed using flow cytometry are secondary outcome measures. EMP sample preparation is completed within two hours of blood draw, as previously described.[92-95] Citrated blood is centrifuged at 160g at 4℃ for 10 minutes to obtain platelet-rich plasma, which is further centrifuged at 1500g at 4℃ for 6 minutes to yield platelet-poor plasma. Fifty microliters of platelet-poor plasma are each incubated in two sets: 1) 4μL of brilliant violet 421 (BV421)-conjugated monoclonal antibody to CD62E (BD), 4μL of phycoerythrin (PE)-conjugated monoclonal antibody to CD31 (BD), 4μL of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody to CD42b (BD), and 2) 4μL of PE-conjugated monoclonal antibody to CD31, 4μL of FITC-conjugated Annexin V (BD). EMPs are the number of particles with size <1.5μm 1) positively labeled by CD62E, 2) positively labeled by CD31 and negatively labeled by CD42b, and 3) positively labeled by CD31 and Annexin V. Allophycocyanin (APC)-conjugated monoclonal antibody CD45 (a pan-leukocyte marker; BD) is used to exclude contamination by leukocyte microparticles.[93] Negative controls include the appropriate FITC-labeled, PE-labeled, BV421-labeled, and APC-labeled isotype-matched IgG. EMPs are quantified as the number of EMPs per μL (ThermoFisher Attune NxT Flow Cytometer).

**Potential Pathway Variables**

We are exploring autonomic imbalance, inflammation, and oxidative stress as potential mechanisms linking PTSD and its dimensions with endothelial dysfunction. Average respiratory

sinus arrhythmia (RSA) amplitude across the cycles of the HRDB task—defined as the difference between the end of expiration and end of inspiration in heart rate—reflects parasympathetic-mediated cardiac control.[84 96] Blood-based inflammatory markers are assayed from plasma, including high-sensitivity C-reactive protein measured by ELISA (R&D Systems). Interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-α, and interferon-gamma are measured in a multiplex assay utilizing a V-PLEX Custom Human Cytokine Proinflammatory Panel on the Meso Scale Discovery electrochemiluminesence platform. Oxidative stress is assessed by measuring urinary F2-isoprostanes using an ELISA (Oxford Biomedical Research).

**Statistical Analyses**

We will first conduct cross-sectional associations of PTSD and its dimensions with endothelial dysfunction; longitudinal associations will be considered in exploratory analyses. For cross-sectional analyses, two sample t-tests will compare participants with and without PTSD on 1) FMD and 2) EMPs expressing CD62E and CD31. We will extend these analyses using linear regression models adjusting for covariates (described below). We will consider total PTSD severity score as a continuous predictor. Second, objective measures of posttraumatic fear (e.g., fear load based on FPS) will be the exposures in cross-sectional linear regression analyses of FMD and EMP outcomes. Additionally, we will explore objective measures of posttraumatic dysphoria as predictors of endothelial dysfunction. The approach for these analyses will mirror that for posttraumatic fear, with exposures operationalized as total dwell times for the sad and happy AOIs.

For longitudinal analyses, we will first examine how measures of PTSD and its dimensions at baseline predict change in FMD using linear regression, and then examine how changes in PTSD and its dimensions over follow-up predict change in FMD using linear

regression. Analyses will consider EMPs as secondary outcomes. For longitudinal analyses, FMD at follow-up will be the outcome; FMD at baseline will be a covariate.

To examine if autonomic imbalance, inflammation, and oxidative stress explain associations of PTSD and its dimensions with endothelial dysfunction in cross-sectional and/or longitudinal analyses, we will estimate total and direct effects of measures of PTSD and its dimensions on endothelial dysfunction and test for significant indirect effects using bootstrapping methods.[97]

For all analyses, we will examine a series of adjusted models. The base adjusted model will include age, gender, race, and ethnicity; subsequent models will sequentially adjust for: 1) other sociodemographics (e.g., education, marital status); 2) CVD medical risk factors (e.g., diabetes, hypertension); 3) medications (e.g., medication for hypertension, hyperlipidemia); and 4) anthropometrics and health behaviors (e.g., body mass index, smoking). Even though posttraumatic fear has been found to be distinct from the core symptoms of depression,[98 99] we will also consider depression as a potential confounder given high comorbidity of PTSD and depression.[2] Data on depression diagnoses and symptoms will be examined as covariates, and we will explore how comorbidity of PTSD and other psychopathology (e.g., depression) relates to endothelial dysfunction. To avoid overfitting in adjusted models, we will fit group lasso regression, which performs variable selection and regularization of regression coefficients, to identify important predictors of endothelial dysfunction and improve model interpretability.[100]

**Sample Size Estimation and Power**

Analyses are powered to our primary outcome, FMD, and based on the smallest effect size from prior research to be conservative in our calculations. We estimated our effect size based on Cohen's $d$=0.48 for the difference in percent change in FMD in patients with versus

without PTSD.[28] Power analysis for a two sample *t*-test was conducted to determine a sufficient sample size using alpha=.05, power=.80, a medium effect size (Cohen's *d*=0.48), two tails, and equal allocations of participants to the PTSD and trauma control groups. Based on these assumptions, the desired sample size was 70 participants per group. We conservatively selected 80 participants per group.

Given our hypothesis that psychophysiological fear is the key PTSD dimension that will relate to endothelial dysfunction, we considered power for this analysis based on our primary exposure for this aim: FPS. We assumed a standard deviation (SD) of 70 for FPS scores[85] and a SD of 3.5% for FMD.[28] With *N*=160 and a two-sided significance level of .05, we have 80% power to detect a change of 0.011% in FMD for each unit of FPS score increase.[101] This is a small effect size,[28] so our sample is large enough to model psychophysiological fear and FMD in adjusted models.

**Ethics and dissemination**

This study is conducted in compliance with the UCLA Institutional Review Board and Helsinki Declaration. Participants sign informed consent and Health Insurance Portability and Accountability Act Authorization for Research forms at enrollment. Research personnel review the study in detail and answer questions. Individuals are informed that participation is optional and that they may withdraw at any time. After individuals are fully informed about study procedures, participants who elect to proceed with the study sign the consent document, as do research personnel. The findings of this study will be disseminated online (ClinicalTrials.gov, Open Science Framework), presented at conferences, and published in peer-reviewed journals.

**Patient and public involvement**

Patients and the public were not involved in the design or conduct of the study. We plan to share findings with the public through dissemination to community partners.

## DISCUSSION

This is the first study to comprehensively investigate the ways in which PTSD and dimensions of posttraumatic stress relate to endothelial dysfunction, one of the earliest modifiable precursors to CVD. To distill pathways by which posttraumatic stress may lead to CVD risk, we focus on psychophysiological fear—a key dimension of PTSD—although we consider objective measures of dysphoria, a more nonspecific dimension. This study incorporates state-of-the-art measures of endothelial dysfunction by investigating brachial artery FMD and EMPs. No investigation of trauma-exposed individuals has considered functional and cellular measures of endothelial dysfunction as we do here. Furthermore, unlike prior research with predominantly white, male samples,[27 28] this study will include men and women of diverse racial/ethnic backgrounds who have been exposed to a range of traumas, increasing generalizability of findings.

Despite these strengths, there are limitations. Assessments are conducted in the laboratory, which offers a controlled environment that enhances internal validity but limits generalizability to real-world settings. Additionally, although we use state-of-the-art measures of endothelial dysfunction, neither metric indicates endothelial function reactivity to trauma-related cues. Further research using provocation measures or assessing cardiovascular reactivity in naturalistic settings is needed to address this issue.

PTSD predicts incident CVD, but the field needs intervention targets and intermediary mechanisms to determine if PTSD interventions can offset CVD risk. This study will test whether endothelial dysfunction could be an early subclinical, modifiable mechanism by which

PTSD increases CVD risk, and whether posttraumatic fear or another dimension could be the target to offset that risk in vulnerable, trauma-exposed individuals in future intervention studies.

## AUTHOR CONTRIBUTIONS

**Contributors:**

JAS, DS, YN, AL, OAA, RE, TH, and KW-T contributed significantly to the planning, conception, design, and successful funding of this study. SC, KR, JT, JAS, and KW-T are contributing significantly to the acquisition of data. SC, KR, and JAS drafted the initial version of this manuscript. AKM, DS, YN, AL, OAA, RE, TH, and JAS will be involved in the analyses and interpretation of the data. All authors revised the draft critically for important intellectual content and gave final approval for this version of the manuscript to be submitted for publication.

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Shiloh Cleveland and Kristina Reed contributed equally to this work.

## FUNDING STATEMENT

This study is supported by National Heart, Lung, and Blood Institute (NHLBI) grant number R01HL139614.

**Disclaimer:**

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## COMPETING INTERESTS

There are no competing interests declared for this study.

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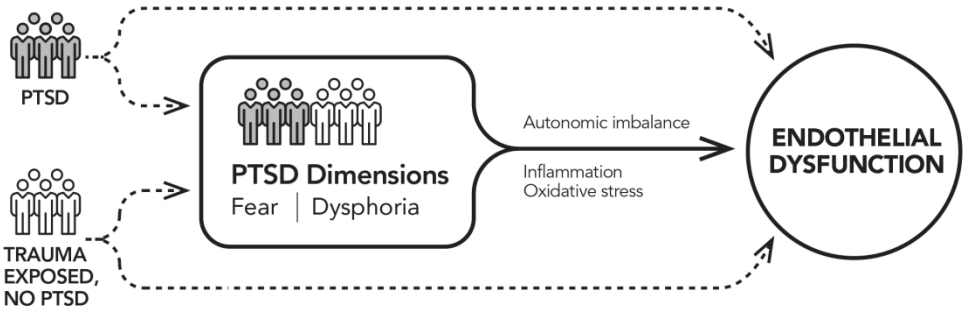
## FIGURE LEGENDS

Figure 1. Conceptual model for the study. The aim examining whether PTSD diagnosis is related to endothelial dysfunction is indicated by the dashed arrows. The aim examining associations of posttraumatic fear and dysphoria with endothelial dysfunction is indicated by the solid arrow. Mechanisms examined in exploratory analyses are printed above and below the solid arrow.

Figure 2. Study timeline. CAPS-5=Clinician-Administered PTSD Scale for *DSM-5*. CVD=cardiovascular disease. FMD=flow-mediated dilation. LEC-5=Life Events Checklist for *DSM-5*. PCL-5=PTSD Checklist for *DSM-5*. SCID-5=Structured Clinical Interview for *DSM-5*.

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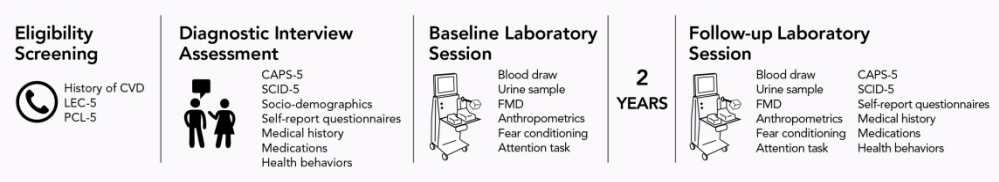


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