OPEN

Inflammatory and Cardiovascular Responses to Active and Passive Acute Psychological Stress

Victoria G. Linsley, MSc, Nicolette C. Bishop, PhD, Matthew J. Roberts, PhD, Malik Hamrouni, PhD, Mayada Demashkieh, MSc, and Nicola J. Paine, PhD

Objective: Acute psychological stress is a risk factor for cardiovascular disease (CVD), possibly through promoting a heightened inflammatory profile. Active stressors are commonly used to investigate cardiovascular and immune reactivity; however, this response may not translate to other stress modalities. We aimed to decipher potential differences in immune responses to passive and active stressors.
Methods: Eighty-eight participants completed this study. After a baseline period, a passive (International Affective Picture System [IAPS]) and active stress task (Paced Auditory Serial Addition Test [PASAT]) were completed in a randomized order, with 45-minute rest post-tasks. Cardiovascular measures (including SBP, DBP, HR) were collected continuously. Blood samples were collected after each time point determining inflammatory responses, including circulating and stimulated interleukin-6 (IL-6), systemic inflammation response index (SIRI), neutrophil/lymphocyte ratio (NLR), TNF-α, and P- and E-selectin.
Results: Cardiovascular measures were higher during the PASAT than IAPS ($p < .001$). Circulating IL-6 levels increased from baseline to 45-minutes after both tasks ($p \leq .001$), with no difference between 45-minute post-PASAT and 45-minute post-IAPS ($p > .05$). SIRI increased from baseline to post-IAPS ($p = .013$), 45-minute post-IAPS ($p = .004$), and 45-minute post-PASAT ($p < .001$). No difference in SIRI between 45-minute post-PASAT and 45-minute post-IAPS existed. NLR increased from baseline to 45-minute post-PASAT ($p = .008$). There were no significant time effects for TNF-α, P-selectin, or E-selectin (all $p > .05$).
Conclusion: Both stressors increased circulating IL-6 levels and SIRI. Cardiovascular measures were higher during the active task, but the magnitude of inflammatory responses did not significantly differ between tasks. Regardless of stress modality, an immune response ensues, potentially increasing the risk of CVD over time.

Key words: inflammatory markers, psychological stress, interleukin-6, active stressor, passive stressor, cardiovascular disease

From the School of Sport, Exercise and Health Sciences, National Centre for Sport and Exercise Medicine, Loughborough University (Linsley, Bishop, Roberts, Hamrouni, Demashkieh, Paine), Loughborough; and National Institute for Health Research (NIHR) Leicester Biomedical Research Centre, University Hospitals of Leicester NHS Trust and the University of Leicester (Bishop, Roberts, Paine), Leicester, United Kingdom.
Address correspondence to Nicola J. Paine, PhD, School of Sport, Exercise and Health Sciences, National Centre for Sport and Exercise Medicine, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK. E-mail: N.J.Paine@lboro.ac.uk
ORCID IDs: https://orcid.org/0000-0002-5036-7965 (V.G.L.); https://orcid.org/0000-0001-6221-3907 (N.C.B.); https://orcid.org/0000-0002-9550-752X (M.D.); https://orcid.org/0000-0001-9988-9310 (N.J.P.)
Article Editor: Suzanne C. Segerstrom
Received for publication August 8, 2024; revision received November 22, 2024.
Supplemental digital content is available for this article.

ISSN: 2998-8748
DOI: 10.1097/PSY.0000000000001367

Abbreviations: BMI = body mass index, **CVD** = cardiovascular disease, **DBP** = diastolic blood pressure, **ECG** = electrocardiogram, **GAD-7** = General Anxiety Disorder-7, **GEE** = generalized estimated equations, **GLM** = generalized linear models, **HR** = heart rate, **IAPS** = International affective picture system, **IL-6** = interleukin-6, **LPS** = lipopolysaccharide, **MDD** = major depressive disorder, **MVPA** = moderate-vigorous intensity physical activity, **NF-κB** = nuclear factor-kappa beta, **NLR** = neutrophil/lymphocyte ratio, **PASAT** = Paced Auditory Serial Addition Test, **PBMC** = peripheral blood mononuclear cells, ***Q*** = cardiac output, **RPE** = rating of perceived exertion, **SAM** = sympatho-adrenal medullary, **SB** = sedentary behavior, **SBP** = systolic blood pressure, **SIRI** = systemic inflammation response index, **TNF-α** = tumor necrosis factor-α, **TSST** = Trier Social Stress Task

(*Biopsychosoc Sci Med* 2025;87:107–117)

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide (1), with mortality rates reaching approximately 200 deaths per 100,000 people in Western European countries in 2022 (2). CVD encompasses many preventable diseases such as angina, stroke, and myocardial infarction (3). The common underlying disease process in CVD is atherosclerosis, a progressive, inflammatory disease of the arteries that begins early in life and manifests with age and exposure to risk factors (4,5). Psychological stress, an inevitable aspect of everyday life, is a risk factor for CVD and can present in different forms such as active (e.g., completing an exam) or passive (e.g., financial pressures) stressors (6). Chronic psychological stress, such as caring for an ill family member, is associated with a higher risk of developing CVD, compared to individuals without caregiving responsibilities (7). Acute bouts of psychological stress can also trigger cardiac events. For instance, a meta-analysis of 13 studies found that short stressful events, such as watching live football, were associated with a higher risk of cardiovascular events due to increased mental and emotional stress (8). Moreover, substantial increases in cardiac-related events or deaths have been observed in response to sudden, highly stressful events like natural disasters (e.g., earthquakes) (9–11).

Acute stress triggers many coordinated systems, including nervous, endocrine, and immune responses, to produce a physiological response (fight or flight), following a perceived or physical threat (12). Acute psychological stress immediately activates the sympathetic nervous system, leading to a systemic release of catecholamines (e.g., adrenaline and noradrenaline) via the sympatho-adrenal medullary (SAM) system activation. Simultaneous withdrawal of parasympathetic nervous system activity also occurs, resulting in vasoconstriction and decreased vagal tone. This leads to physiological responses such as increased heart rate (HR) and blood pressure (BP) (13,14) alongside an increase in proinflammatory cytokine release (e.g., interleukin [IL]-6, tumor necrosis factor-alpha [TNF-α], and IL-1β) from immune cells (15,16), following activation of nuclear factor-kappa beta (NF-κB: a key transcription factor). Peripheral blood mononuclear cells (PBMCs), e.g., monocytes, migrate to areas of inflammation in response to cytokine/chemokine signaling and secrete proinflammatory cytokines to sustain the inflammatory response (17).

This acute increase in inflammation is essential when tissue damage has occurred, or an invading pathogen is present, to repair damaged tissue and restore homeostasis. However, chronic inflammation or repeated, exaggerated stress reactivity, where the inflammatory response is not resolved, results in host tissue damage leading to significant health consequences such as atherosclerosis (18,19). These physiological responses are similar to those observed during exercise (20,21). However, in response to psychological stress, they appear metabolically unjustified because the body mobilizes fuel stores, increases blood flow to the extremities, and primes the immune system for a physical challenge that does not occur (22,23). Additionally, larger stress-evoked circulating IL-6 responses have been associated with higher CRP in males, indicating that exaggerated stress responses may have a deleterious impact on an individual's resting inflammatory profile (24). Hence, repeated exposure to acute bouts of psychological stress may increase CVD risk through promoting a heightened inflammatory milieu.

Numerous studies have explored the cardiovascular, cortisol, and immune responses to stress, mainly focusing on active stressors where the participant engages in a task, such as the Trier Social Stress Task (TSST) (25) or the Paced Auditory Serial Addition Test (PASAT) (13). However, the magnitude of inflammatory responses to an acute passive psychological stress paradigm has not been explored, but this may be important in determining mechanisms behind how different stressors impact CVD risk.

Typically, circulating IL-6, TNF-α, and lipopolysaccharide (LPS)-stimulated IL-6 are investigated as markers of inflammation in response to psychological stress (15). Circulating IL-6 is a reliable predictor of hypertension, atherosclerosis, and other CVDs, such as myocardial infarction (26,27). Higher circulating TNF-α levels have also been associated with greater CVD risk (28). Therefore, circulating levels of these markers may be used as prognostic indicators of future CVD development. LPS-stimulated IL-6 is a useful marker for assessing the functional response of immune cells to a stressor, possibly reflecting the primary immune response more accurately than circulating levels because a plethora of cell types can release IL-6 (e.g., adipocytes and endothelial cells) (29). The magnitude of the stimulated IL-6 response to psychological stress may be important because an insufficient response could leave the individual susceptible to infection, whereas an exaggerated response may increase the risk of inflammatory diseases (29,30).

Endothelial adhesion molecules E-selectin and P-selectin have yet to be investigated in the context of active or passive psychological stress reactivity. These markers may indicate acute stress–induced changes in leukocyte rolling, adhesion, and infiltration into inflamed tissue (31,32), which could be important as higher P-selectin concentrations have been correlated with greater incident cardiovascular events in a sample of females (33). Additionally, in a sample of young adults (25–35 years), higher circulating P- and E-selectin levels were associated with markers of atherogenic inflammatory arterial wall alterations (34). Because E- and P-selectins are important markers of cardiovascular health, we aimed to explore any potential differences in circulating levels, after exposure to acute psychological stress (both active and passive) to develop current theories surrounding mechanisms underpinning the relationship between psychological stress, inflammation, and potential CVD risk.

Novel leukocyte ratios such as the neutrophil/lymphocyte ratio (NLR) and systemic inflammation response index (SIRI) may predict future CVD, as they possibly reflect an individual's inflammatory profile more effectively than individual cell counts (35,36). Higher NLR values have been associated with cardiovascular events in both patients with CVD and healthy controls (pooled analysis of 38 studies) (37). Likewise, higher SIRI scores have been associated with increased all-cause and CVD mortality in a sample of adults with no known chronic conditions (n = 85,154) (36). Neither the NLR or SIRI has been used as a marker of the inflammatory response magnitude to acute psychological stress; hence, we aimed to decipher any changes in these markers due to acute passive and active psychological stress exposure.

Limited evidence exists surrounding differences in immune reactivity to an active and passive stressor in the same sample. These differences may be important to combat CVD risk related to the type of stress an individual is most susceptible to, e.g., active stress such as sitting an exam or passive stress when worrying about financial issues. Because different modalities of stress may perturb different pathways (38), leading to distinct inflammatory responses, this may help to identify mechanistic variations in how active or passive psychological stress can impact an individual's inflammatory profile. Furthermore, previous studies investigating reactivity to passive stress often used tasks such as the cold pressor, which involves submerging extremities into a container of cold water (~0°C–5°C) for a period of 3–4 minutes (39–41). This test involves elements of psychological stress but also includes a physical/pain component; therefore, identifying which physiological/immune responses correspond to which modality of stress is difficult. Hence, we aimed to explore the impact of passive stress on markers of inflammation and cardiovascular measures by using a bank of unpleasant images from the International Affective Picture System (IAPS), a robust instrument for replicating real-life emotions in a controlled setting, without the presence of a physical stressor (42). We also investigated differences in markers of inflammation and cardiovascular responses to an active stressor compared to a passive stressor to elucidate any differences between the modalities of stress encountered. We hypothesized that both tasks would initiate an increase in inflammatory and cardiovascular measures, but these would both be higher in response to the PASAT.

METHODS

Participants

Ninety-two healthy, young (aged 18–25 years) adults were recruited via noticeboards, word of mouth, and social media adverts in the period spanning March 2022 to June 2023. Participants were excluded if a) BMI was >40 kg/m^2 or body fat percentage was >40%, b) resting brachial systolic BP was >140 mmHg, c) they had a current or previous clinically diagnosed psychological condition, d) they had a clinically diagnosed disease/condition, e) they had a history of or currently smoked/used recreational drugs (including e-cigarettes and vapes), f) they were taking prescribed or over-the-counter medication that could affect the outcomes of interest (e.g., anti-inflammatory drugs). Prior to the main trial, participants refrained from vigorous exercise for 24 hours, alcohol for 12 hours, food and drink (other than water) for 4 hours, and over-the-counter medication for 72 hours. The visit was rearranged if the participant was ill/infectious or had been in the previous 7 days, as reported by the participant. This study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave informed consent, and ethical approval was given by the Loughborough University Research ethics committee (2021-5456-4541).

Protocol

This study consisted of two visits to the Loughborough University laboratory. Visit 1 was a screening session to assess eligibility, which consisted of obtaining written informed consent from the participant, before measurement of resting brachial BP (Omron M6 Comfort; Omron Healthcare, Milton Keynes, United Kingdom) and completion of a series of validated questionnaires to ensure the participant was eligible for the study and to collect sociodemographic information and information on potential confounders, e.g., anxiety traits (General Anxiety Disorder-7 [GAD-7]). Participants were instrumented with two devices to measure movement behaviors including time spent sedentary (ActivPAL3 inclinometer; PAL Technologies Ltd, Glasgow, United Kingdom) and daily physical activity levels (Actigraph GT3X BT+; ActiGraph, Florida, USA). Participants were asked to wear the devices for 8 full days and nights and fill out an activity log to identify periods of sleep and time where the devices were removed (e.g., for bathing).

After at least 8 days, the participant returned to the lab for visit 2. The main trial consisted of a baseline period of rest (20 minutes), two stress tasks, and two 45-minute recovery periods. The order in which the stress tasks took place was randomly assigned for each participant. Each main trial started at 10 AM to avoid any diurnal variation in outcome measures. Upon arrival to the laboratory, repeat consent was obtained before measurement of height (274 stadiometer; Seca GmbH, Hamburg, Germany), weight, body fat percentage (mBCA 515 bioimpedance scales; Seca GmbH), waist circumference (Seca 201 tape measure) and resting brachial BP (Omron M6 Comfort; Omron Healthcare) were completed. Participants were then instrumented for the outcome measures in the trial, before remaining seated in an upright position throughout the trial. A nature documentary (Frozen Planet, BBC, United Kingdom) was played during the rest periods in line with previous work (13,43,44). The Borg 6–20 rating of perceived exertion scale (45) and a 7-point Likert scale (rating of 0–6; 0 being “not at all,” 6 being “extremely”) were used after completion of each period in the stress protocol to assess how difficult, stressful, arousing, and engaging and how well the participant thought they were doing.

Active Stress Task: Paced Auditory Serial Addition Task

An 8-minute version of the Paced Auditory Serial Addition Task (PASAT) (46) was used due to its good test-retest reliability (47), and it reliably perturbates the cardiovascular system (48–50) as well as inflammatory modifications (13,51). The task required the participant to add two single digit numbers together read out by the audio file, remember the second number and add it to the next number read out. This pattern continued until the end of the test, with 1 point being awarded for every correct answer. The time between number presentations decreased as the test proceeded (2.4, 2.0, 1.6, 1.2 seconds, respectively (52)). An initial instruction audio file was played through a tablet device (iPad; Apple Inc., Cupertino, California) followed by a practice test. Final instructions regarding task manipulation (as outlined below) were then played, before the main test commenced. To enhance the stressfulness of the task, elements of social evaluation and competition were included. A leader board was visually displayed on a wall, in line of sight of the participant, to elicit pressure in the form of inter-individual competition. The task was video recorded and streamed through a television screen to which the participants were asked to look at throughout. The participants were told that the video would be sent to body language experts to analyze their performance; if they looked away from the screen a loud, aversive noise was delivered. A researcher stood near the participant with a clipboard to mark the test. An aversive buzzer was sounded every 10 numbers, in response to an incorrect answer or hesitation. If no error was made, the buzzer was pressed on the 10th number to maintain consistency on the number of aversive sounds given to each participant (44).

Passive Stress Task: IAPS

During the passive stress task, participants were instructed to look at a screen showing a bank of images selected from the “negative valence” IAPS group to elicit an emotional response (53) for a total of 6 minutes. Each image was displayed on a TV screen for a total of 20 seconds. If at any point participants looked away from the screen, they were asked to look back at the screen.

Cardiovascular Measures

Continuous cardiovascular measures were collected throughout the testing protocol (last 10 minutes of the baseline period, throughout each stress task and for 8 minutes immediately after the termination of each stress task). A Human Non-Invasive Blood Pressure (NIBP) system (ADInstruments, Oxford, United Kingdom) was used to measure the systolic BP (SBP), diastolic BP (DBP), and cardiac output (Q) over each time period, by placing an oscillometric blood pressure cuff on the middle phalanx of the middle finger to detect changes in arterial diameter. Lab Chart 8 (ADInstruments) with the Non-Invasive Cardiac Output package was used for data collection and analysis. A three-lead electrocardiogram (ECG), with electrodes placed below each clavicle and the lower left rib, was used to measure heart rate (HR). The ECG signal was amplified (PowerLab;

ADInstruments) and analyzed using the Lab Chart 8 software program (ADInstruments). Mean SBP, DBP, *Q*, and HR were calculated for each time point and used in the analyses.

Blood Sampling and Analysis

A 20-gauge cannula (BD Nexiva; BD, Franklin Lakes, New Jersey) was inserted into a suitable antecubital vein to allow blood draws to be made at baseline, immediately after each stress task and 45 minutes after each stress task. The first 2 ml of blood was collected in a syringe and disposed of. Then 4.9 ml of blood was collected in a potassium ethylene diamine tetra acetic acid (K3 EDTA) S-monovette tube (Sarstedt AG & Co. KG, Nümbrecht, Germany), and 15 ml of blood was collected in two 7.5-ml S-monovette tubes treated with sodium heparin (Sarstedt AG & Co. KG). After each blood collection, the cannula was flushed with a 0.9% NaCl solution to maintain patency.

For each time point, 20 µl of EDTA was used to measure total and differential leukocyte counts (neutrophils, lymphocytes, and monocytes) on a benchtop hematology analyzer (Yumizen H500; Horiba Medical, Montpellier, France). SIRI was determined ([neutrophil count × monocyte count]/lymphocyte count) as it has been closely associated with cardiovascular and all-cause mortality risk (35). NLR was calculated as this has also been associated with all-cause and cardiovascular mortality risk (54). The remaining blood was centrifuged (3500 rpm, 10 minutes, 4°C) before the plasma was aliquoted (~500 µl) into microtubes and stored at −80°C for future analysis. IL-6 and TNF-α were measured using high-sensitivity ELISA kits (R&D systems, Minneapolis, Minnesota). P-selectin and E-selectin were measured in duplicate using ELISA kits (R&D systems), according to the manufacturer's instructions. All samples were measured in duplicate. The intra-assay coefficients of variance were as follows: IL-6, 2.0%; TNF-α, 1.0%; P-selectin, 0.6%; and E-selectin, 2.0%, respectively.

PBMCs were separated from heparanized blood by density-graded centrifugation for each timepoint. After isolating PBMCs, cells were counted using a hemocytometer (BRAND GMBH + CO KG, Wertheim, Germany). A total of 170,000 cells were added to 500 µl of media into a well on a 24-well plate. The LPS-stimulated well consisted of 170,000 cells in 475 µl RPMI and 25 µl LPS (100 ng/ml). The control well consisted of 170,000 cells in 500 µl in RPMI. After a 4-hour incubation period (CO_2: 5%, temperature: 37°C), the supernatant from each well was centrifuged (300*g*, 5 minutes), and the resultant supernatant was aliquoted (~200 µl) into two microtubes and stored at −80°C for future analysis. LPS-stimulated IL-6 production from PBMCs was assessed in duplicate using ELISA kits (R&D systems, Minneapolis, Minnesota). The intra-assay coefficient of variance was 4.8%.

Data Reduction and Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 29 (IBM, Chicago, Illinois), and statistical significance was set at $p < .05$. Missing Actigraph, ActivPAL, and immune marker data were imputed using a 5× multiple imputation method (55). Normality tests indicated non-normal distribution for most dependent variables, leading to the use of generalized estimated equations (GEE) with an autoregressive (AR (1)) correlation matrix and gamma with log-link models for analysis. Linear models were used for normally distributed variables (HR, SBP, DBP). Analyses were adjusted for age, biological sex (male = 0 or female = 1), body fat percentage, ethnicity, moderate-vigorous physical activity (MVPA) volume, sedentary behavior (SB) volume, and anxiety traits (GAD-7 score), which are known to alter inflammatory status (unadjusted GEE results are presented in Table S1, Supplemental Digital Content, http://links.lww.com/PSYMED/B65). Cramer's phi (φ_c) was used to determine effect sizes, interpreted as small = 0.1, medium = 0.3, and large = 0.5 effects. GEE post-hoc analyses with Bonferroni correction were conducted for significant main effects of time. Generalized linear models (GLMs) were used to determine correlations between changes in inflammatory markers and cardiovascular reactivity variables, adjusting for relevant covariates (age, biological sex [male = 0 or female = 1], body fat percentage, ethnicity, task order, MVPA volume, SB volume and anxiety traits [GAD-7 score]) (13). Pearson's bivariate (continuous covariates)/point biserial (dichotomous covariates) correlations between markers of inflammation (circulating IL-6, stimulated IL-6, TNF-α, P-selectin, E-selectin, total and differential leukocytes, NLR, and SIRI) and the relevant covariates are presented in Table S2, Supplemental Digital Content, http://links.lww.com/PSYMED/B65.

Sample Size Determination

Sample size estimates were calculated using a smaller, more conservative effect size than has been reported for the changes in circulating IL-6 seen in response to acute psychological stress (15). Using RStudio (pwrss package) with an effect size of $eta^2 = 0.02$, power of 0.9, and alpha of .05, the minimum sample size was determined to be 77.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request and in line with ethical and consent considerations.

RESULTS

Sample Characteristics

A sample of 88 participants completed both sessions of the two-visit protocol. Table 1 provides a summary of participant characteristics. The sample consisted of young adults (mean [SD] age = 21.5 [2.2] years) with 41% females, predominantly of White ethnicity (51%).

Cardiovascular Responses and Self-Reported Perception to the Stress Tasks

Table 2 outlines the adjusted cardiovascular responses to both stress tasks, Table S3 (Supplemental Digital Content, http://links.lww.com/PSYMED/B65) outlines the unadjusted models. There was a significant main effect of time for HR, SBP, DBP, and *Q*, where all increased significantly in response to the PASAT ($p < .001$). HR, SBP, DBP, and *Q* significantly decreased 8 minutes after the PASAT ($p < .001$).

SBP ($p = .022$) and DBP ($p < .001$) significantly increased in response to the IAPS, whereas *Q* ($p < .001$) significantly decreased. *Q* increased significantly during the 8-minute recovery period post-IAPS ($p < .001$). There was no significant

TABLE 1. Participant Characteristics at Baseline (n = 88)

Variable	Mean (SD)/N (%)
Age (y)	21.5 (2.2)
Sex (female)	37 (41)
BMI (kg/m²)	23.6 (3.2)
Body fat %	21.7 (7.6)
Resting brachial SBP (mm Hg)	110 (12)
Resting brachial DBP (mm Hg)	69 (8)
Ethnicity	
Asian	27 (30)
Black	13 (14)
White	46 (51)
Other	5 (5)
GAD-7 Score (0–21)	3.9 (3.3)
Daily SB (h/d)	9.4 (1.5)
Daily MVPA (min/d)	97.3 (29.6)

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; GAD-7 = Generalized Anxiety Disorder-7; SB = sedentary behavior; MVPA = moderate-vigorous intensity physical activity.

difference in SBP or DBP between post-IAPS and post 8-minute IAPS measures ($p > .05$). There was no significant change in HR during the IAPS ($p > .05$). HR, SBP, DBP, and Q were significantly higher during the PASAT than the IAPS (all $p < .001$).

Table 2 outlines self-reported responses to both tasks; unadjusted models are reported in Table S3 (Supplemental Digital Content, http://links.lww.com/PSYMED/B65). Perceived stress and difficulty significantly increased from baseline to post-PASAT and IAPS, respectively (both $p < .001$). Both returned to baseline levels after 45 minute of recovery ($p > .05$ from baseline to 45 minute after both stress tasks, respectively). Participants self-reported lower excitement and engagement during the IAPS (both $p < .001$). Subjective excitement returned to baseline levels after 45 minutes of rest ($p > .05$ from baseline to 45-minute post-IAPS). Perceived engagement remained significantly lower than baseline levels after a 45-minute period of rest ($p = .006$). There was a significant reduction in how well individuals thought they were performing during the PASAT compared to baseline ($p < .001$). Perceived intensity (RPE) significantly increased in response to both the PASAT and IAPS (both $p < .001$).

Inflammatory Responses to the Stress Tasks

Table 2 outlines fully adjusted models assessing inflammatory responses to both stressors, respectively, with a series of GEEs used to assess the effect of time for each inflammatory outcome. Unadjusted models are reported in Table S3 (Supplemental Digital Content, http://links.lww.com/PSYMED/B65). There was an effect of time for circulating IL-6 concentration (Wald $\chi^2 = 82.871$; $p < .001$; Figure 1A). Post-hoc analyses revealed a significant increase in circulating IL-6 levels from baseline to 45 minutes after both stress tasks, respectively ($p \leq .001$). Post-hoc analyses showed a significant increase in circulating IL-6 levels from post-PASAT to 45-minute post-PASAT ($p < .001$) and from post-IAPS to 45-minute post-IAPS ($p = .001$). There was no significant difference between post-PASAT and post-IAPS circulating IL-6 levels ($p > .05$) or 45-minute post-PASAT and 45-minute post-IAPS circulating IL-6 levels ($p > .05$).

There was a significant time effect (Wald $\chi^2 = 13.696$; $p = .008$) for LPS-stimulated IL-6 concentration (Figure 1B). However, there were no significant post-hoc differences between any time points (all $p > .05$).

There was no significant effect of time for circulating E-selectin (Wald $\chi^2 = 5.919$; $p = .205$), P-selectin (Wald $\chi^2 = 6.166$; $p = .187$), or TNF-α (Wald $\chi^2 = 4.713$; $p = .318$).

Total and Differential Leukocyte Counts

There was an effect of time on total leukocyte counts (Wald $\chi^2 = 68.063$; $p < .001$). Post-hoc analyses revealed a significant increase in total leukocyte counts from baseline to all other time points (all $p < .001$). Additionally, there was a significant increase from the IAPS to 45-minute post-IAPS ($p < .001$). There was no significant difference in leukocyte counts between the PASAT and 45-minute post-PASAT values ($p > .05$).

There was a significant main effect of time on monocyte counts (Wald $\chi^2 = 41.632$; $p < .001$). Post-hoc analyses revealed a significant increase in monocyte counts from baseline to all other time points (all $p \leq .003$) in addition to an increase from post-IAPS to 45-minute post-IAPS ($p = .051$). There was no significant difference for monocyte counts between the PASAT and 45-minute post-PASAT values ($p > .05$).

A main effect of time for lymphocyte counts existed (Wald $\chi^2 = 46.523$; $p < .001$). Post-hoc analyses revealed a significant increase in lymphocyte counts from baseline to post-PASAT ($p \leq .001$) and from post-IAPS to 45-minute post-IAPS ($p \leq .001$). A significant decrease in lymphocyte counts was evident from post-PASAT to 45-minute post-PASAT ($p = .002$). Lymphocyte counts were significantly higher post-PASAT compared to post-IAPS ($p < .001$).

There was a significant main effect of time for neutrophil counts (Wald $\chi^2 = 50.700$; $p < .001$). Post-hoc analyses revealed a significant increase in neutrophil counts from baseline to all other time points (all p values $\leq .001$). There was a significant increase in neutrophil counts from after both stress tasks to 45 minutes after tasks (both $p \leq .001$), respectively.

Systemic Inflammation Response Index

There was a significant effect of time on SIRI (Wald $\chi^2 = 26.455$; $p < .001$; Figure 1C). Post-hoc analyses revealed a significant increase in SIRI from baseline to post-IAPS ($p = .013$), from baseline to 45-minute post-IAPS ($p = .004$), and from post-IAPS to 45-minute post-IAPS ($p = .048$). There was a significant increase in SIRI from baseline to 45-minute post-PASAT ($p < .001$) and from post-PASAT to 45-minute post-PASAT ($p = .005$). There was no significant difference between 45-minute post-PASAT and 45-minute post-IAPS SIRI scores ($p > .05$).

Neutrophil/Lymphocyte Ratio

Figure 1D depicts the effect of time on NLR (Wald $\chi^2 = 32.711$; $p < .001$). Post-hoc analyses revealed a significant increase in NLR from baseline to 45-minute post-PASAT ($p = .008$) and from post-PASAT to 45-minute post-PASAT ($p < .001$).

TABLE 2. Cardiovascular, Inflammatory, and Self-reported Responses to the Paced Auditory Serial Addition Test (PASAT) and International Affective Picture System (IAPS) (n = 88)

	Baseline	PASAT	Post-PASAT	IAPS	Post-IAPS	Wald χ^2	Effect Size (φ_c)	p
Cardiovascular								
Heart rate (bpm)	61.45 ± 2.12	75.15 ± 2.26*	63.12 ± 2.19*,**	61.52 ± 2.05***	63.68 ± 2.10*,**	348.44	0.99	<.001
Systolic blood pressure (mm Hg)	111.38 ± 2.45	136.5 ± 2.64*	124.33 ± 2.69*,**	115.89 ± 2.78**,***	117.38 ± 2.62*	338.58	0.98	<.001
Diastolic blood pressure (mm Hg)	60.46 ± 1.99	81.96 ± 2.13*	70.89 ± 2.13*,**	66.5 ± 2.07*,***	67.11 ± 2.03*	579.43	1.28	<.001
Cardiac output (L/min)	4.74 ± 0.15	5.48 ± 0.22*	4.86 ± 0.17*	4.42 ± 0.16*,**,***	4.62 ± 0.16,**	87.95	0.50	<.001
Inflammatory								
Circulating IL-6 (pg/ml)	0.70 ± 0.08	0.76 ± 0.08	1.11 ± 0.13*,**	0.79 ± 0.09	1.01 ± 0.12*,**	82.87	0.49	<.001
Total leukocyte counts ($\times 10^9$/L)	4.98 ± 0.21	5.41 ± 0.22*	5.57 ± 0.24*	5.30 ± 0.24*	5.71 ± 0.27*,**	68.06	0.44	<.001
Monocyte counts ($\times 10^9$/L)	0.38 ± 0.02	0.41 ± 0.02*	0.42 ± 0.02*	0.4 ± 0.02*	0.42 ± 0.02*,**	41.63	0.34	<.001
Neutrophil counts ($\times 10^9$/L)	2.56 ± 0.13	2.81 ± 0.15*	3.05 ± 0.18*,**	2.82 ± 0.17*	3.11 ± 0.20*,**	50.70	0.38	<.001
Lymphocyte counts ($\times 10^9$/L)	1.71 ± 0.05	1.86 ± 0.06*	1.77 ± 0.05**	1.72 ± 0.05***	1.80 ± 0.05**	46.52	0.36	<.001
System inflammation response index (SIRI)	0.59 ± 0.04	0.63 ± 0.05	0.75 ± 0.07*,**	0.68 ± 0.06*	0.76 ± 0.07*,**	26.46	0.27	<.001
Neutrophil lymphocyte ratio (NLR)	1.52 ± 0.06	1.52 ± 0.07	1.75 ± 0.10*,**	1.65 ± 0.09	1.74 ± 0.11	32.71	0.30	<.001
Stimulated IL-6 (pg/ml)	176.94 ± 35.84	125.49 ± 29.68	93.76 ± 16.51	157.26 ± 30.28	107.82 ± 23.99	13.70	0.20	<.001
E-selectin (ng/ml)	29.02 ± 1.44	29.89 ± 1.50	29.61 ± 1.46	29.41 ± 1.43	28.98 ± 1.45	5.92	0.13	.205
P-selectin (ng/ml)	46.69 ± 3.91	46.40 ± 3.85	42.56 ± 3.39	46.48 ± 3.74	45.49 ± 3.81	6.17	0.13	.187
Circulating TNF-α (pg/ml)	0.59 ± 0.03	0.58 ± 0.03	0.57 ± 0.03	0.58 ± 0.03	0.57 ± 0.02	4.71	0.12	.318
Self-reported measures								
Perceived stress	1.47 ± 0.09	5.29 ± 0.15*	1.30 ± 0.06**	2.96 ± 0.15*,***	1.25 ± 0.06**	1038.14	1.72	<.001
Perceived difficulty	1.44 ± 0.10	6.00 ± 0.17*	1.26 ± 0.06**	2.59 ± 0.13*,***	1.28 ± 0.07**	1042.92	1.72	<.001
Perceived excitement	3.23 ± 0.18	3.70 ± 0.19	3.42 ± 0.20	2.26 ± 0.15*,***	3.56 ± 0.22**	57.85	0.41	<.001
Perceived engagement	4.54 ± 0.23	4.85 ± 0.22	4.12 ± 0.21*,**	3.69 ± 0.20*,***	4.11 ± 0.21*	73.80	0.46	<.001
Perceived accomplishment (rating of how well participants were doing)	4.92 ± 0.13	3.03 ± 0.12*	5.14 ± 0.14**	4.92 ± 0.15***	5.18 ± 0.15	153.03	0.66	<.001
Perceived intensity (RPE)	7.71 ± 0.25	13.30 ± 0.46*	7.25 ± 0.23*,**	9.31 ± 0.34*,***	7.10 ± 0.20*,**	389.29	1.05	<.001

φ_c = Cramer's phi; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α; RPE = rating of perceived exertion.

Data are presented as mean ± SE.

* Significant difference from baseline (p < .05).

** Significant difference from stress (e.g., PASAT or IAPS).

*** Significantly different from PASAT. Post PASAT/IAPS measures were taken 8 minutes post-stress for the cardiovascular measures and 45 minutes post-stress for the inflammatory measures.

Cardiovascular and Inflammatory Marker Relationships

Tables S3 and S4 (Supplemental Digital Content, http://links.lww.com/PSYMED/B65) outline associations between stress-induced changes in circulating IL-6, stimulated IL-6, SIRI, and NLR with changes in cardiovascular measures, respectively. There was a significant inverse relationship between changes in circulating IL-6 concentration from baseline to post-IAPS with changes in SBP (p = .006) and changes in DBP (p = .025), respectively. Changes in stimulated IL-6 concentration from baseline to post-PASAT were positively associated with ΔDBP (p = .008). Changes in stimulated IL-6 levels from baseline to 45-minute post-PASAT were positively associated with changes in SBP (p = .018) and changes in DBP (p = .008), respectively. Changes in SIRI from baseline to post-IAPS were positively related to the changes in HR (p < .001) during the IAPS. There was a positive relationship between changes in SIRI from baseline to 45-minute post-IAPS and changes in HR (p = .016), during the IAPS.

Changes in NLR from baseline to post-IAPS were positively associated with changes in HR (p = .005) during the IAPS. A positive relationship existed between changes in NLR from baseline to 45-minute post-PASAT (p = .034) and 45-minute post-IAPS (p = .030) with changes in HR during the PASAT/IAPS.

DISCUSSION

In this study, we aimed to reveal any differences in immune responses to an active and passive stressor. Both tasks

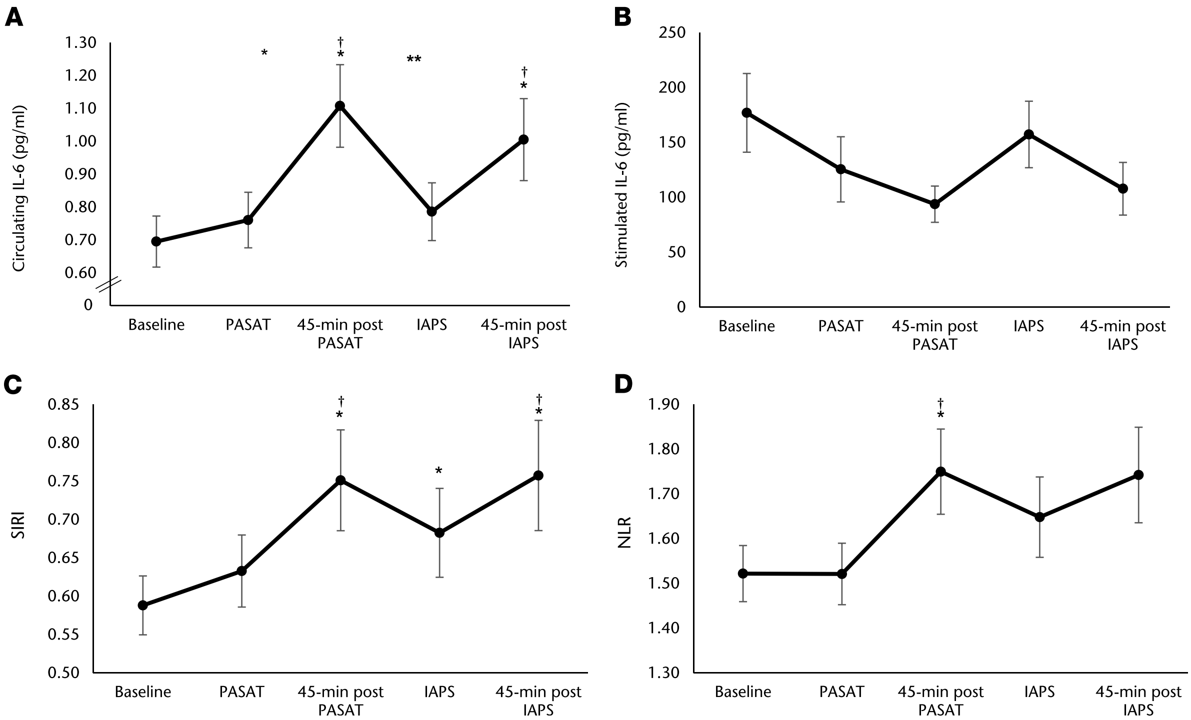

FIGURE 1. The effect of time on (A) circulating IL-6 concentration, (B) stimulated IL-6 concentration, (C) SIRI, (D) NLR. PASAT = Paced Auditory Serial Addition Test; IAPS = International Affective Picture System; IL-6 = interleukin-6; SIRI = system inflammation response index; NLR = neutrophil/lymphocyte ratio. *Significant difference from baseline. †Significant difference from respective stress task. Data are presented as mean ± SE.

lead to an increase in total and differential leukocyte counts, SIRI, and circulating IL-6, to a similar magnitude, suggesting both modalities of stress promote a heightened immune response. Conversely, we found no significant differences at any time point for TNF-α, P-selectin, or E-selectin levels. We observed a significant effect of time for LPS-stimulated IL-6 but no post-hoc differences. Our study represents an important advancement in the literature by investigating multiple cytokine and immune responses to passive stress, given meta-analytic analysis exploring the impact of acute psychological stress on circulating and stimulated immune markers included 33 studies but all used an active stressor (15).

Circulating IL-6 and TNF-α Responses to Acute Psychological Stress

Circulating IL-6 levels increased in response to both psychological stressors, reaching significance 45 minutes after the stressor terminated. This is consistent with the findings of a meta-analysis (29 studies), which reported no significant increase in circulating IL-6 levels immediately after an acute psychological stressor, but a marked increase in measures 40 minutes after the stressor had subsided (15). The studies included in this review only used active stressors, such as mental arithmetic and Stroop-Colour Word test (15). Our study is the first to report circulating IL-6 responses to a passive stress task and demonstrates a similar response irrelevant of the stress modality. Because SBP and DBP increased in response to both stressors, this suggests that the SAM system was mobilized, which stimulates the immediate release of catecholamines (e.g., adrenaline and noradrenaline), promoting increased cardiac inotropy and vascular tone, possibly explaining this BP reactivity (56). Nonetheless, we observed a significant difference in cardiovascular variables during the PASAT compared to the IAPS, with HR, SBP, DBP, and Q, all increasing to a greater extent during the PASAT than the IAPS (all $p < .001$). For example, Q is modified by changes in HR and/or stroke volume and is regulated by autonomic nervous system, endocrine, and paracrine signaling pathways (57), which could lead to inflammatory modifications. This may suggest that cardiovascular parameters play a more dominant role in the mechanisms behind increased IL-6 release in response to an active stressor with other pathways potentially being responsible for the inflammatory response to the IAPS. However, because we did not measure cortisol levels, we also cannot rule out the role of HPA-axis activity on the stress-related inflammatory responses. Despite these cardiovascular differences, we observed no differences in the magnitude of circulating IL-6 reactivity between the tasks; hence, the modality of stress and subsequent cardiovascular reactivity is not a determining factor for the immune response. Because the IAPS was reported to be significantly "less stressful" than the PASAT through our subjective task ratings, this highlights the potential "under the radar," deleterious impact of passive psychological stress on an individual's inflammatory profile. This should be considered in noncommunicable disease prevention, as what may be deemed a slightly stressful experience could lead to the same inflammatory response as a highly stressful event and predispose an individual to inflammatory diseases, if repeated exposures occur.

TNF-α is released in response to NF-κB activation and has been reported to induce IL-6 production, so it is surprising that we found no difference in circulating TNF-α levels at any time point (58). However, this may be due to the timing of when samples were taken as peak TNF-α levels appear to occur 2 hours after a challenge, followed by a rapid decline, owing to its short half-life (~18.2 minutes; (59)). Additionally, TNF-α reactivity to acute active psychological stressors, averaged over samples taken 20–120 minutes after stress (from 11 studies), only revealed a "small" increase, compared to the "large" increases reported for circulating IL-6 levels (15). Although, in the 10 samples consisting of healthy participants, 8 reported no significant change in TNF-α levels in response to an active stressor, which is consistent with our findings. Furthermore, higher TNF-α levels have been related to symptoms of depression in a healthy population, independent of anxiety symptoms; however, anxiety symptoms were not significantly related to TNF-α levels (60). Because the stress tasks used in our study may evoke more anxiety than depressive symptoms, this could explain the lack of changes in TNF-α levels.

Circulating E-selectin and P-selectin Responses to Acute Psychological Stress

Our study is the first to examine potential changes in E-selectin and P-selectin in the context of psychological stress reactivity; however, we observed no differences at any time point. P- and E-selectin may be important mediators of the inflammatory response, due to being vital in the recruitment of leukocytes to areas of inflammation. Dysregulated expression of P-selectin has been shown to contribute to pathological inflammation in models of atherosclerosis, stroke, and other cardiovascular disorders (61). We measured circulating E- and P-selectin, respectively, meaning they must have undergone protease-dependent cleavage from activated or damaged endothelial cells (or platelets), and although these are often used as biomarkers of systemic endothelial activation (62), they may not reflect an acute inflammatory response. In vitro, E-selectin expression on endothelial cells has been shown to peak between 3 and 6 hours after cytokine (TNF-α) exposure, with soluble levels peaking 12–24 hours after the initial exposure (63). Hence, future studies could look at the expression of cellular adhesion molecules on endothelial cells or the time course of circulating E- and P-selectin levels in response to an acute psychological stressor to determine if these levels change later than 45 minutes after stress task. Elevated plasma P-selectin levels have been reported as a consequence, rather than a cause, of CVD (64), and increasing soluble P-selectin levels have been shown to predict atherosclerotic progression (65). This may indicate that circulating selectin levels could be more indicative of CVD progression rather than predicting future CVD risk, and as we recruited a healthy sample, this could explain the lack of changes in E- and P-selectin levels, respectively.

SIRI and NLR Responses to Acute Psychological Stress

To our knowledge, this is the first study to explore the impact of a passive and active stressor on SIRI and NLR respectively. Using leukocyte ratios is a relatively inexpensive measure compared to cytokine analysis and can easily be obtained from routine full blood counts, including those from clinical populations. SIRI significantly increased in response to the IAPS, with higher levels than baseline evident post-IAPS and 45 minutes after the task. SIRI also increased in response to the PASAT but like the circulating IL-6 response, the difference was only significant 45 minutes after stress exposure. SIRI encompasses neutrophil, monocyte, and lymphocyte counts and may provide a more robust and well-rounded indicator of one's inflammatory profile than using individual leukocyte counts (36). Increased SIRI levels have been associated with a higher risk of all-cause and cardiovascular related deaths, with one cohort study of 42,875 participants, revealing that those with SIRI levels >1.43 were at a 126% greater risk of cardiovascular mortality than those with a SIRI score of <0.68 after a 20-year follow-up period (35). At the 45-minute post-IAPS timepoint, SIRI scores reached 0.76 (a 0.17 increase from baseline; $p = .004$), similar to the increase recorded 45 minutes post-PASAT (0.16 increase from baseline; $p < .001$), highlighting the heightened inflammatory response to both tasks, which could possibly be a mechanism behind psychological stress triggering cardiovascular events or how repeated exposures to acute psychological stress could lead to future CVD. SIRI has also been explored in the context of chronic stress, with one study reporting higher SIRI levels in those with a greater risk of developing clinical depression (mean SIRI: nondepressed = 1.24; depressed = 1.32) (66). However, a recent study found no difference in SIRI between healthy controls and patients with major depressive disorder (MDD) (67), suggesting that further research is required, consisting of various psychological disorders to elucidate the effectiveness of SIRI as a marker of future disease risk. Interestingly, in our study, the SIRI reactivity scores did not significantly differ between the active and passive stress tasks, possibly showing that the modality of stress is irrelevant in terms of the consequential immune response magnitude.

Our study is the first to examine changes in NLR under acute psychological stress, showing that NLR significantly increased from baseline to 45 minutes post-PASAT, which appeared to be driven by a significant increase in the number of circulating neutrophils. In contrast, we found no difference in NLR levels in response to the passive stress task (both immediately and 45 minutes post-IAPS) despite a significant elevation in neutrophil counts. The change in NLR from baseline to 45-minute post-PASAT was positively associated with ΔHR during the PASAT. Similarly, ΔHR during the IAPS was positively associated with ΔNLR from baseline to 45-minute post-IAPS. Therefore, the magnitude of HR response to a psychological stressor seems to impact on the magnitude of NLR response, given that the HR response to the IAPS was significantly lower than during the PASAT. Higher NLR levels have been observed in patients presenting with acute stroke (68), myocardial infarction (69), and atherosclerosis (70), and elevated NLR levels have also been reported to relate to higher all-cause mortality risk (71,72). Therefore, repeated exposure to acute active psychological stressors, leading to an increase in NLR, may result in adverse health profiles, but more evidence is required to support this notion. The evidence surrounding relationships between NLR levels and psychological conditions is inconsistent. One study reported higher NLR levels in those with MDD compared to healthy controls (73), whereas another study reported no significant difference between NLR levels between

MDD patients and healthy controls (67). Therefore, more research is required for both chronic and acute stress relationships with NLR.

LPS-Stimulated IL-6 Responses to Acute Psychological Stress

Although changes in leukocyte counts may indicate immune system perturbation, they do not reflect the functionality of cells; therefore, we explored differences in IL-6 production from PBMCs stimulated with LPS in response to both an active and passive stressor. We found a significant effect of time on stimulated IL-6 levels, but post-hoc tests did not reveal any differences between specific time points. Previous research has been conducted exploring the impact of acute active psychological stress on stimulated IL-6 levels, and there is significant heterogeneity in the reported outcomes (15). A meta-analysis reported no significant differences in stimulated IL-6 production in response to an active stressor in the first 10 minutes post-task, but a tendency for levels to increase when findings were averaged from 15 to 120 minutes after active stressor (15). Hence, the time points we examined may not have been sufficient to detect changes in LPS-stimulated IL-6 production in response to either task (74,75). As mentioned, typically other studies that have investigated stimulated IL-6 responses have utilized an active stress task, and future research is needed to further explore the differences in stimulated IL-6 production from PBMCs especially in response to a passive stressor, as this had not been explored prior to this study.

Methodological Considerations and Future Directions

Although we found a stress-induced inflammatory response to both tasks, the mechanisms behind these responses, especially for passive stressors, remain unclear. Future research should explore changes in hormone levels such as adrenaline, noradrenaline, and cortisol to elucidate which systems are activated in response to different stressors, especially passive stress as this has not been explored in comparison to acute active stressors prior to this study. We recruited a young, healthy population who were free of disease; therefore, our findings may not be translatable to different population groups, including clinical or at-risk populations. Also, we did not see stress-induced changes in TNF-α levels (as seen in previous work with acute active stress tasks (15)), or E- or P-selectin levels, which may have been due to the time points at which we took blood samples (45 minutes after stress task). Alternatively, due to logistical issues, plasma was not separated and stored in the freezer until the end of the testing protocol, which may have led to the demise of TNF-α, given its short half-life (59). Therefore, future research could explore longer recovery periods of 90 minutes after both passive and active stress tasks to elucidate any changes that occur after 45 minutes of rest. This may be useful in determining temporal patterns of immune reactivity to both passive and active stress tasks and provide insights to possibly help prevent stress-induced cardiovascular events. However, due to participant burden such as time commitment to the study and a prolonged fasting period, extending the rest times may be challenging from a recruitment perspective. Additionally, we did not include a nonstress control condition, so it is unclear as to the magnitude of change in inflammatory markers due to diurnal variation compared to stress reactivity alone.

CONCLUSION

Our study is the first to demonstrate that both passive and active stress tasks induced an increase in circulating IL-6 and SIRI. Although cardiovascular changes were greater during the active stress task, there were no significant differences in the magnitude of the inflammatory response between the tasks. This suggests that regardless of the stress modality, an augmented inflammatory profile seems to ensue, which, over time, could predispose an individual to a heightened CVD risk. However, future research is required to confirm these findings and determine mechanisms behind these responses, especially in the context of passive stress as limited evidence is currently available.

The authors report no conflict of interest in relation to this article.

Source of Funding and Conflicts of Interest: This project was supported by the Academy of Medical Sciences/the Welcome Trust/the Government Department of Business, Energy and Industrial Strategy/the British Heart Foundation/Diabetes UK Springboard Award (SBF006\1166).

This is a summary of independent research funded by the Academy of Medical Sciences and carried out at the National Institute of Health and Care Research (NIHR) Leicester Biomedical Research Centre (BRC). The views expressed are those of the author(s) and not necessarily those of the Academy of Medical Sciences, the NIHR, or the Department of Health and Social Care.

REFERENCES

1. WHO. Factsheet. Cardiovascular diseases (CVDs). 2021 [cited 2024 Jul 4]. Available at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Accessed April 7, 2024.
2. Mensah GA, Habtegiorgis Abate Y, Abbasian M, Abd-Allah F, Abdollahi A, Abdollahi M, et al. Global Burden of Cardiovascular Diseases and Risks, 1990–2022. J Am Coll Cardiol 2023;82:2350–473.
3. Olvera Lopez E, Ballard BD, Jan A. Cardiovascular Disease. Treasure Island, FL: StatPearls; 2023.
4. Pahwa R, Jialal I. Atherosclerosis. Treasure Island, FL: StatPearls; 2021.
5. Björkegren JLM, Lusis AJ. Atherosclerosis: recent developments. Cell 2022;185:1630–45.
6. Kivimäki M, Steptoe A. Effects of stress on the development and progression of cardiovascular disease. Nat Rev Cardiol 2018;15:215–29.
7. Capistrant BD, Moon JR, Berkman LF, Glymour MM. Current and long-term spousal caregiving and onset of cardiovascular disease. J Epidemiol Community Health 2012;66: 951–6.
8. Lin L-L, Gu H-U, Yao Y-Y, Zhu J, Niu Y-M, Luo J, et al. The association between watching football matches and the risk of cardiovascular events: a meta-analysis. J Sports Sci 2019;37: 2826–34.
9. Gold LS, Kane LB, Sotoodehnia N, Rea T. Disaster events and the risk of sudden cardiac death: a Washington State investigation. Prehosp Disaster Med 2007;22:313–7.
10. Suzuki S, Sakamoto S, Koide M, Fujita H, Sakuramoto H, Kuroda T, et al. Hanshin-Awaji earthquake as a trigger for acute myocardial infarction. Am Heart J 1997;134(5 Pt 1): 974–7.
11. Trichopoulos D, Katsouyanni K, Zavitsanos X, Tzonou A, Dalla-vorgia P. Psychological stress and fatal heart attack: the Athens (1981) earthquake natural experiment. Lancet 1983;1: 441–4.
12. Russell G, Lightman S. The human stress response. Nat Rev Endocrinol 2019;15:525–34.
13. Chauntry AJ, Bishop NC, Hamer M, Kingsnorth AP, Chen Y-L, Paine NJ. Sedentary behaviour is associated with heightened cardiovascular, inflammatory and cortisol reactivity to acute psychological stress. Psychoneuroendocrinology 2022;141:105756.

14. Turner AI, Smyth N, Hall SJ, Torres SJ, Hussein M, Jayasinghe SU, et al. Psychological stress reactivity and future health and disease outcomes: a systematic review of prospective evidence. Psychoneuroendocrinology 2020;114:104599.
15. Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis. Brain Behav Immun 2017;64:208–19.
16. Hinterdobler J, Schott S, Jin H, Meesmann A, Steinsiek A-L, Zimmermann AS, et al. Acute mental stress drives vascular inflammation and promotes plaque destabilization in mouse atherosclerosis. Eur Heart J 2021;42:4077–88.
17. Xiong H, Pamer EG. Monocytes and infection: modulator, messenger and effector. Immunobiology 2015;220:210–4.
18. Fleit HB. Chronic inflammation. In: McManus LM, Mitchell RN, eds. Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms. Amsterdam, Netherlands: Elsevier Inc.; 2014. p. 300–14.
19. Marchio P, Guerra-Ojeda S, Vila JM, Aldasoro M, Victor VM, Mauricio MD. Targeting early atherosclerosis: A focus on oxidative stress and inflammation. Oxid Med Cell Longev 2019; 2019:8563845.
20. Kop WJ, Weissman NJ, Zhu J, Bonsall RW, Doyle M, Stretch MR, et al. Effects of acute mental stress and exercise on inflammatory markers in patients with coronary artery disease and healthy controls. Am J Cardiol 2008;101:767–73.
21. Ponce P, Del Arco A, Loprinzi P. Physical activity versus psychological stress: effects on salivary cortisol and working memory performance. Medicina (Kaunas) 2019;55:119.
22. Obrist PA. Presidential Address, 1975. The cardiovascular-behavioral interaction—as it appears today. Psychophysiology 1976;13:95–107.
23. Turner JR, Carroll D. Heart rate and oxygen consumption during mental arithmetic, a video game, and graded exercise: further evidence of metabolically-exaggerated cardiac adjustments? Psychophysiology 1985;22:261–7.
24. Lockwood KG, Marsland AL, Cohen S, Gianaros PJ. Sex differences in the association between stressor-evoked interleukin-6 reactivity and C-reactive protein. Brain Behav Immun 2016;58:173–80.
25. Man ISC, Shao R, Hou WK, Xin S, Yan F, Lee M, et al. Multi-systemic evaluation of biological and emotional responses to the Trier Social Stress Test: a meta-analysis and systematic review. Front Neuroendocrinol 2023;68:101050.
26. Zhang B, Li X-L, Zhao C-R, Pan C-L, Zhang Z. Interleukin-6 as a predictor of the risk of cardiovascular disease: a meta-analysis of prospective epidemiological studies. Immunol Invest 2018;47:689–99.
27. Su J-H, Luo M-Y, Liang N, Gong S-X, Chen W, Huang W-Q, et al. Interleukin-6: a novel target for cardio-cerebrovascular diseases. Front Pharmacol 2021;12:745061.
28. Yuan S, Carter P, Bruzelius M, Vithayathil M, Kar S, Mason AM, et al. Effects of tumour necrosis factor on cardiovascular disease and cancer: a two-sample Mendelian randomization study. EBioMedicine 2020;59: 102956.
29. Prather AA, Carroll JE, Fury JM, Mcdade KK, Ross D, Marsland AL. Gender differences in stimulated cytokine production following acute psychological stress. Brain Behav Immun 2009;23:622–8.
30. Nathan C. Points of control in inflammation. Nature 2002;420:846–52.
31. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol 2007;27:2292–301.
32. Zinellu A, Mangoni AA. Systematic review and meta-analysis of the effect of statins on circulating E-selectin, L-selectin, and P-selectin. Biomedicines 2021;9:1707.
33. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391–7.
34. Eikendal ALM, Bots ML, Gohar A, Lutgens E, Hoefer IE, den Ruijter HM, et al. Circulating levels of P-selectin and E-selectin relate to cardiovascular magnetic resonance–derived aortic characteristics in young adults from the general population, a cross-sectional study. J Cardiovasc Magn Reson 2018;20:54.
35. Xia Y, Xia C, Wu L, Li Z, Li H, Zhang J. Systemic Immune Inflammation Index (SII), System Inflammation Response Index (SIRI) and risk of all-cause mortality and cardiovascular mortality: a 20-year follow-up cohort study of 42,875 US adults. J Clin Med 2023;12:1128.
36. Jin Z, Wu Q, Chen S, Gao J, Li X, Zhang X, et al. The associations of two novel inflammation indexes, SII and SIRI with the risks for cardiovascular diseases and all-cause mortality: a ten-year follow-up study in 85,154 individuals. J Inflamm Res 2021;14:131–40.
37. Angkananard T, Anothaisintawee T, McEvoy M, Attia J, Thakkinstian A. Neutrophil lymphocyte ratio and cardiovascular disease risk: a systematic review and meta-analysis. Biomed Res Int 2018;2018:2703518.
38. Plourde A, Lavoie KL, Raddatz C, Bacon SL. Effects of acute psychological stress induced in laboratory on physiological responses in asthma populations: a systematic review. Respir Med 2017;127:21–32.
39. Larra MF, Capellino S, Schwendich E, von Haugwitz L. Immediate and delayed salivary cytokine responses during repeated exposures to cold pressor stress. Neuroimmunomodulation 2023;81–92.
40. Jarczewski J, Furgała A, Winiarska A, Kaczmarczyk M, Poniatowski A. Cardiovascular response to different types of acute stress stimulations. Folia Med Cracov 2019;59: 95–110.
41. Chauntry AJ, Bishop NC, Hamer M, Paine NJ. Sedentary behaviour, but not moderate-to-vigorous physical activity, is associated with respiratory responses to acute psychological stress. Biol Psychol 2023;177:108510.
42. Branco D, Gonçalves ÓF, Badia SBI. A systematic review of International Affective Picture System (IAPS) around the world. Sensors (Basel) 2023;23:3866.
43. Paine NJ, Ring C, Bosch JA, Mcintyre D, Veldhuijzen van Zanten JJCS. The effect of acute mental stress on limb vasodilation is unrelated to total peripheral resistance. Psychophysiology 2013;50:680–90.
44. Paine NJ, Ring C, Bosch JA, Drayson MT, Aldred S, Veldhuijzen van Zanten JJCS. Vaccine-induced inflammation attenuates the vascular responses to mental stress. Int J Psychophysiol 2014;93:340–8.
45. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 1982;14:377–81.
46. Gronwall DMA. Paced auditory serial-addition task: a measure of recovery from concussion. Percept Mot Skills 1977;44:367–73.
47. Willemsen G, Ring C, Carroll D, Evans P, Clow A, Hucklebridge F. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic and cold pressor. Psychophysiology 1998;35:252–9.
48. Whittaker AC, Chauntry AJ. Blunted cardiovascular reactivity to acute psychological stress predicts low behavioral but not self-reported perseverance: a replication study. Psychophysiology 2021;58:e13707.
49. Heaney JLJ, Ginty AT, Carroll D, Phillips AC. Preliminary evidence that exercise dependence is associated with blunted cardiac and cortisol reactions to acute psychological stress. Int J Psychophysiol 2011;79:323–9.
50. Phillips AC, Der G, Hunt K, Carroll D. Haemodynamic reactions to acute psychological stress and smoking status in a large community sample. Int J Psychophysiol 2009;73:273–8.
51. Veldhuijzen Van Zanten JJ, Ring C, Carroll D, Kitas GD. Increased C reactive protein in response to acute stress in patients with rheumatoid arthritis. Ann Rheum Dis 2005;64: 1299–304.
52. Tombaugh TN. A comprehensive review of the Paced Auditory Serial Addition Test (PASAT). Arch Clin Neuropsychol 2006;21:53–76.
53. Lang PJ, Bradley MM, Cuthbert BN. International Affective Picture System (IAPS): Technical Manual and Affective Ratings. NIMH Center for the Study of Emotion and Attention. Gainesville, 1997: 39–58. Available at: https://acordo.net/acordo/wp-content/uploads/2020/08/instructions.pdf. Accessed April 7, 2024.
54. Zhou E, Wu J, Zhou X, Yin Y. The neutrophil-lymphocyte ratio predicts all-cause and cardiovascular mortality among U.S. adults with rheumatoid arthritis: results from NHANES 1999–2020. Front Immunol 2023;14:1309835.
55. Little R, Rubin D. Statistical Analysis With Missing Data. John Wiley Sons [Internet]. 2002; 793. doi:10.1002/9781119013563
56. Shah K, Kumari R, Jain M. Unveiling stress markers: a systematic review investigating psychological stress biomarkers. Dev Psychobiol 2024;66:e22490.
57. Kobe J, Mishra N, Arya VK, Al-Moustadi W, Nates W, Kumar B. Cardiac output monitoring: technology and choice. Ann Card Anaesth 2019;22:6–17.
58. Pantsulaia I, Trofimov S, Kobyliansky E, Livshits G. Genetic and environmental influences on iL-6 and TNF-alpha plasma levels in apparently healthy general population. Cytokine 2002; 19:138–46.
59. Oliver JC, Bland LA, Oettinger CW, Arduino MJ, McAllister SK, Aguero SM, et al. Cytokine kinetics in an in vitro whole blood model following an endotoxin challenge. Lymphokine Cytokine Res 1993;12:115–20.
60. Martino PL, Pulopulos MM, Canto CD, Dupanlou ML, Rubio SM, Bonet JL. High levels of TNF-α are associated with symptoms of depression in health professionals at a hospital. Span J Psychiatry Ment Health. doi.org/10.1016/j.rpsm.2021.10.002.
61. Mcever RP. Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. Cardiovasc Res 2015;107:331–9.
62. Simons N, Bijnen M, Wouters KAM, Rensen SS, Beulens JWJ, van Greevenbroek MMJ, et al. The endothelial function biomarker soluble E-selectin is associated with nonalcoholic fatty liver disease. Liver Int 2020;40:1079–88.
63. Wyble CW, Hynes KL, Kuchibhotla J, Marcus BC, Hallahan D, Gewertz BL. TNF-alpha and IL-1 upregulate membrane-bound and soluble E-selectin through a common pathway. J Surg Res 1997;73:107–12.
64. Panicker SR, Mehta-D'souza P, Zhang N, Klopocki AG, Shao B, Mcever RP. Circulating soluble P-selectin must dimerize to promote in flammation and coagulation in mice. Blood 2017;130:181–91.
65. Sommer P, Schreinlechner M, Noflatscher M, Lener D, Mair F, Theurl M, et al. Increasing soluble P-selectin levels predict higher peripheral atherosclerotic plaque progression. J Clin Med 2023;12:6430.
66. Li X, Huan J, Lin L, Hu Y. Association of systemic inflammatory biomarkers with depression risk: results from National Health and Nutrition Examination Survey 2005–2018 analyses. Front Psych 2023;14:1097196.
67. Liu Y, Li C, Ren H, Han K, Wang X, Zang S, et al. The relationship of peripheral blood cell inflammatory biomarkers and psychological stress in unmedicated major depressive disorder. J Psychiatr Res 2024;176:155–62.
68. Li W, Hou M, Ding Z, Liu X, Shao Y, Li X. Prognostic value of neutrophil-to-lymphocyte ratio in stroke: a systematic review and meta-analysis. Front Neurol 2021; 12:686983.
69. Lee M-J, Park S-D, Kwon SW, Woo S-I, Lee M-D, Shin S-H, et al. Relation between neutrophil-to-lymphocyte ratio and index of microcirculatory resistance in patients with

ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. Am J Cardiol 2016;118:1323–8.
70. Adamstein NH, MacFadyen JG, Rose LM, Glynn RJ, Dey AK, Libby P, et al. The neutrophil-lymphocyte ratio and incident atherosclerotic events: analyses from five contemporary randomized trials. Eur Heart J 2021;42:896–903.
71. Song M, Graubard BI, Rabkin CS, Engels EA. Neutrophil-to-lymphocyte ratio and mortality in the United States general population. Sci Rep 2021;11:464.
72. Josse JM, Cleghorn MC, Ramji KM, Jiang H, Elnahas A, Jackson TD, et al. The neutrophil-to-lymphocyte ratio predicts major perioperative complications in patients undergoing colorectal surgery Colorectal Dis 2016;18:236–42.
73. Puangsri P, Ninla-aesong P. Potential usefulness of complete blood count parameters and inflammatory ratios as simple biomarkers of depression and suicide risk in drug-naive, adolescents with major depressive disorder. Psychiatry Res 2021;305: 114216.
74. Lawlor N, Nehar-belaid D, Grassmann JDS, Stoeckius M, Smibert P, Stitzel ML, et al. Single cell analysis of blood mononuclear cells stimulated through either LPS or anti-CD3 and anti-CD28. Front Immunol 2021;12:636720.
75. Miquelestorena-Standley E, da Silva AVV, Monnier M, Chadet S, Piollet M, Héraud A, et al. Human peripheral blood mononuclear cells display a temporal evolving inflammatory profile after myocardial infarction and modify myocardial fibroblasts phenotype. Sci Rep 2023;13:16745.