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The effect of acute physical and mental stress on soluble cellular adhesion molecule concentration

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ABSTRACT

Aims: This study investigated the impact of acute physical and mental stress on serum concentrations of vascular cell adhesion molecule (VCAM)-1 and CX_3CL1/fractalkine.

Materials and methods: Male volunteers ($n = 20$; 21.3 ± 0.55 years of age) completed a graded treadmill test to exhaustion and a 20-minute mental stress task (Stroop Color-Word Test, mental arithmetic) on separate, non-consecutive days. Heart rate (HR) was measured at baseline and throughout exercise and mental stress. Blood was collected at baseline (PRE), immediately following (POST) and 30 min after (POST30) exercise and mental stress. Soluble VCAM-1 and fractalkine were quantified in participant serum via enzyme-linked immunosorbent assays.

Key findings: Both treadmill exercise and the mental stress task significantly increased participant HR; although, exercise resulted in a substantially greater increase in participant HR compared to mental stress (197.82 ± 11.99 vs. 38.67 ± 3.10% [$p < 0.001$]). VCAM-1 (815.74 ± 139.55 vs. 738.67 ± 131.59 ng/mL [$p = 0.002$]) and fractalkine (1.032 ± 0.33 vs. 0.59 ± 0.20 ng/mL [$p < 0.001$]) were significantly elevated in participant serum POST maximal exercise before returning to values similar to baseline at POST30. The acute mental stress task did not significantly alter serum VCAM-1 or fractalkine at any time point.

Significance: In conclusion, maximal aerobic exercise results in a significant elevation of the soluble adhesion molecules VCAM-1 and fractalkine in the serum of adult males that does not occur following laboratory-induced mental stress. The findings of the current investigation may suggest a novel protective role for acute aerobic exercise in vascular health via exercise-induced CAM proteolysis.

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1. Introduction

Vascular inflammation and its molecular mediators (e.g., cytokines, adhesion molecules) play a critical role in the development of atherosclerosis [23]. Acute stress, both physical (exercise) and psychological, have been shown to augment vascular inflammation by increasing levels of circulating pro-inflammatory cytokines, such as interleukin (IL)-1β and IL-6 [31,33]. Furthermore, both forms of acute stress activate the sympathetic nervous system, initiating a neuroendocrine response resulting in the release of stress hormones (e.g., catecholamines, glucocorticoids) into circulation. Despite the observed similarities following acute trials, prolonged exposure to the different stressors results in diverse cardiovascular disease (CVD) outcomes. Specifically, exercise training improves cardiorespiratory fitness and is associated with a reduction in the risk of all-cause and CVD mortality, whereas chronic mental stress is implicated in the pathophysiology of atherosclerosis and increases CVD risk and mortality [3,10].

Cellular adhesion molecules (CAM) are transmembrane glycoproteins that are expressed by vascular endothelial cells (EC). These molecules facilitate the adherence of circulating leukocytes to the vessel wall and regulate the transendothelial migration of adhered leukocytes into extravascular tissues [25]. CAM have been implicated in several vascular pathologies, including atherosclerosis, a disease whose initiation and progression is directed by focal leukocyte adhesion [4,8,18]. Furthermore, CAM undergo protease-dependent cleavage, during which the extracellular domain is released and circulates in soluble form [16,22]. Thus, dissociation of CAM from vascular EC would reduce the adhesive properties of the vessel wall and potentially delay the progression of atherosclerotic disease. Interestingly, Sultan et al. [35]) have reported that high levels of fluid shear stress, recognized as the frictional force created when blood flows against the endothelial surface, initiate CAM proteolysis from human EC in vitro. These findings support the notion that elevated blood flow velocity, which is proportional to vascular shear stress, may reduce the expression of CAM on the vascular wall.

One major distinction between acute bouts of aerobic exercise and mental stress is the ability of exercise to elicit considerable

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cardiovascular strain. Compared to models of mental stress, aerobic exercise produces significantly higher in-task heart rate (HR) peaks, translating to greater cardiac output and shear stress throughout the coronary and peripheral arteries [32,37,39]. Thus, it is plausible that aerobic exercise may cause greater dissociation of CAM from the vascular wall into peripheral circulation compared to models of mental stress. In fact, several investigations have demonstrated that acute bouts of maximal and sub-maximal aerobic exercise increase the circulating concentrations of soluble CAM (sCAM) including intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [19,21,26–28]. However, the influence of acute mental stress on peripheral sCAM levels remains poorly understood. Therefore, to further investigate the role of hemodynamic force on the dissociation of endothelial CAM, our lab utilized an in vivo research design, during which participants underwent two separate acute stress tasks including (i) maximal treadmill exercise and (ii) laboratory-induced mental stress. The purpose of the current research study was to compare the effect of maximal aerobic exercise and acute mental stress on serum levels of soluble VCAM-1 and CX_3CL1/fractalkine in healthy adult males. We hypothesized that maximal treadmill exercise would elicit a significantly greater increase in peripheral sCAM levels compared to the mental stress task.

2. Methods

2.1. Participants

Twenty healthy males between the ages of 18–28 were recruited from the general population and volunteered to participate in this study. Participants were excluded from the study if they had known or suspected cardiovascular, metabolic, rheumatologic, or other inflammatory diseases/conditions or if they were taking medications that would potentially affect inflammatory conditions, using tobacco products (cigarettes, cigars, and chewing tobacco), or consuming an average of more than ten alcoholic beverages per week. Additionally, participants were excluded if they had a history of psychological disorder(s) and/or had experienced any major life event (e.g. death in family, divorce, or wedding) within 30 days of participation. To limit the effect of fitness on the physiological response to mental stress, those males who reported participating in more than 150 min per week of at least moderate-intensity physical activity were excluded from the study. Prior to each laboratory visit, participants were asked to fast overnight for at least 9 h, abstain from alcohol and caffeine intake for at least 24 h, and to abstain from physical activity/exercise for at least 48 h. Written informed consent was obtained from each participant before beginning the study. All experimental procedures were approved by the Virginia Commonwealth University's Institutional Review Board.

2.2. Laboratory procedures

Participants were initially screened by completing a medical history questionnaire and the International Physical Activity Questionnaire to determine participation in moderate and vigorous physical activity [7]. Upon inclusion, body fat percentage (BF%) was measured via a dual-X-ray absorptiometry scan (DXA; Lunar iDXA, GE Healthcare, Madison, WI) and participants were instructed to report to the Exercise Physiology Research Laboratory on two different occasions.

The first day, participants arrived at 7:00 AM. Following 30 min of seated rest, blood was collected (PRE) from an antecubital vein by a certified phlebotomy technician. Each participant was fitted with a Polar heart rate monitor (Polar Co., Port Washington, NY) and completed a graded exercise test on a treadmill (Trackmaster TMX425C, Full Vision, Inc., Newton, Kansas) to volitional exhaustion [12]. Briefly, each participant was fitted with respiratory gas analysis equipment (VMAX Spectra, Sensormedics Corp., Yorba Linda, CA). Following a 3-minute warm-up at 3 mph with 0% grade, participants began running at a speed that elicited ±5 bpm of 80% of their age-predicted maximal HR. After 4-minutes, workload was increased by 2% grade every 2 min until voluntary exhaustion. HR was obtained every minute, rating of perceived exertion (RPE) was obtained once every exercise stage, and a capillary blood lactate sample was obtained 1-minute post-exercise. Breath-by-breath carbon dioxide exhaled and oxygen inhaled were averaged every 10 s to calculate respiratory exchange ratio (RER: VCO_2/VO_2). The highest 10-second averaged breath-by-breath oxygen consumption value was identified as the peak oxygen consumption (VO_{2peak}). Maximal HR, RPE, lactate and RER were used to ensure that the participants gave maximal effort. All exercise testing was performed by trained technicians who were recognized as Certified Exercise Physiologists (ACSM/EP-C) through the American College of Sports Medicine. Additional antecubital vein blood samples were collected immediately following exercise (POST) and after 30 min (POST30) of seated recovery. The lowest HR observed at rest (baseline) and the maximal HR achieved during exercise (HR_{peak}) were used for statistical analysis. Following the seated recovery period, participants were familiarized with the mental stress challenge by participating in a 4-minute computer-based mental stress task consisting of 2 min of the Stroop Color Word task (SCW) followed by 2 min of Mental Arithmetic (MA).

Participants were instructed to return to the laboratory two days following the initial visit at 7:00 AM. While in the seated position, an intravenous catheter was inserted into an antecubital vein on the left arm by a certified phlebotomy technician, and a positive pressure adapter (CLC2000, ICU Medical, San Clemente, CA) was attached. Participants rested for 60 min and blood was drawn immediately before the initiation of the acute mental stress task (PRE). A detailed protocol for the 20-minute computer-based mental task, which has previously been shown to increase the State Anxiety Index, HR, and norepinephrine, has been described elsewhere [1,15]. Briefly, the mental stress task consisted of a 2-minute bout of SCW immediately followed by a 2-minute bout of MA (4-minute cycle) repeated for 5 cycles. The SCW task presented a single color-word (e.g., "red") every second that was written in a contrasting color font (e.g., "red" written in blue font) on screen. Furthermore, the participants heard a conflicting color, incongruous with the written word and font, from the computer speakers (e.g., "yellow"). Participants were instructed to identify the font color in which the word was written by selecting the appropriately color-matched key on the keyboard (#2 = red, #4 = green, #6 = blue, #8 = yellow) as accurately and quickly as possible. During the MA task, participants were given a three-digit number and were required to randomly subtract either 3, 7, 8, or 13 in a continuous manner and auditory feedback was given during each task when participants entered an incorrect answer. In addition, an investigator stayed in the room, inducing a socio-evaluative component by providing bogus critical feedback to the subject regarding the number of incorrect answers, the speed of their reactions, and their performance compared to their peers. Additional blood samples were collected immediately after (POST) and 30 min (POST30) following the acute mental stress task. HR was measured using a Polar HR monitor prior to the stress (baseline) and each minute during the mental stress task. The maximal HR achieved during the mental stress task (HR_{peak}) was used for statistical analysis.

2.3. Quantification of serum adhesion molecules

Blood samples were collected for analysis of VCAM-1 and fractalkine into serum separator tubes, which were gently inverted 5 times, allowed to clot at room temperature for 30 min and centrifuged for 15 min at 1000 × *g*. Serum was immediately aliquoted into microtubes and stored at −80 °C until further analysis. Concentrations of VCAM-1 and fractalkine were determined through enzyme-linked immunoassays according to the manufacturer's specifications (R&D Systems, Minneapolis, MN). All samples were analyzed in duplicate and the mean concentration of each sample was used during the statistical analysis.

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