Loss of exercise- and stress-induced increases in circulating 2-arachidonoylglycerol concentrations in adults with chronic PTSD

Kevin M. Crombie\textsuperscript{a,⁎}, Brianna N. Leitzela, Angelique G. Brellenthin\textsuperscript{b}, Cecilia J. Hillard\textsuperscript{c}, Kelli F. Koltyna

\textsuperscript{a}Department of Kinesiology, University of Wisconsin-Madison, 2000 Observatory Dr., Madison, WI, 53706, USA
\textsuperscript{b}Department of Kinesiology, Iowa State University, 534 Wallace Rd, Ames, IA, 50011, USA
\textsuperscript{c}Neuroscience Research Center and Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI, 53226, USA

\textsuperscript{⁎}Corresponding author at: UW-Natatorium, 2000 Observatory Drive, Room 1160, Madison, WI 53706, USA.
E-mail address: kmcrombie@wisc.edu (K.M. Crombie).

Received 7 January 2019; Received in revised form 30 March 2019; Accepted 8 April 2019
Available online 09 April 2019

1. Introduction

The endocannabinoid (eCB) system is a modulatory system that is both altered by stress and mediates the effects of acute stress, including contributing to restoration of homeostasis. Earlier studies suggest that circulating eCBs are dysregulated in adults with post-traumatic stress disorder (PTSD); however, it is not known whether circulating eCBs remain responsive to stress. The purpose of this study was to examine eCB and psychological responses to physical (exercise) and psychosocial (Trier Social Stress Test) stressors, using a randomized, counterbalanced procedure in adults with PTSD and healthy controls (\(N = 20\), mean age = 24, SD = 7 yrs). Results from mixed-design, repeated measures ANOVAs revealed significant increases (\(p < .05\)) in N-arachidonoyl ethanolamine (AEA) and oleoyl ethanolamide (OEA) following exercise and psychosocial stress in both groups. However, only the control group exhibited a significant increase (\(p < .05\)) in 2-arachidonoylglycerol (2-AG) following exercise and psychosocial stress exposure. These data extend our current understanding of circulating eCB responsiveness in PTSD, and provide preliminary evidence to suggest that the eCB system is hyperactive in PTSD following exposure to physical and psychosocial stressors.

ARTICLE INFO

Keywords:
Endocannabinoid
Stress
Exercise
Trier social stress test
Anandamide

ABSTRACT

The endocannabinoid (eCB) system is a modulatory system that is both altered by stress and mediates the effects of acute stress, including contributing to restoration of homeostasis. Earlier studies suggest that circulating eCBs are dysregulated in adults with post-traumatic stress disorder (PTSD); however, it is not known whether circulating eCBs remain responsive to stress. The purpose of this study was to examine eCB and psychological responses to physical (exercise) and psychosocial (Trier Social Stress Test) stressors, using a randomized, counterbalanced procedure in adults with PTSD and healthy controls (\(N = 20\), mean age = 24, SD = 7 yrs). Results from mixed-design, repeated measures ANOVAs revealed significant increases (\(p < .05\)) in N-arachidonoyl ethanolamine (AEA) and oleoyl ethanolamide (OEA) following exercise and psychosocial stress in both groups. However, only the control group exhibited a significant increase (\(p < .05\)) in 2-arachidonoylglycerol (2-AG) following exercise and psychosocial stress exposure. These data extend our current understanding of circulating eCB responsiveness in PTSD, and provide preliminary evidence to suggest that the eCB system is hyperactive in PTSD following exposure to physical and psychosocial stressors.

 PTSD is a stress-related psychiatric disorder resulting from exposure to one or more traumatic events. The core symptom clusters of PTSD are (Chrousos, 2009; McEwen & Stellar, 1993; McEwen, 2007).

An abundance of recent preclinical evidence indicates that the endocannabinoid (eCB) system (specifically CB1 receptor-endocannabinoid signaling) is both altered by stress and modulates the behavioral and endocrine effects of acute stress, including those processes that contribute to the restoration of homeostasis following stress exposure (Hill et al., 2010; Hillard, 2014, 2015; Lutz, Marsicano, Maldonado, & Hillard, 2015; Morena, Patel, Bains, & Hill, 2016). The eCB system is found in both the central and peripheral nervous systems, and consists of receptors (Cannabinoid-type 1, CB1R; Cannabinoid-type 2, CB2R), eCB ligands (N-arachidonylethanolamine or anandamide [AEA] and 2-arachidonoylglycerol [2-AG]), and enzymes involved in the synthesis and degradation of the eCBs (Di Marzo, De Petrocellis, & Bisogno, 2005). Preclinical and clinical evidence has demonstrated the important role of the eCB system in the regulation of several processes (e.g., activation and recovery of endocrine and nervous system responses to stress; fear processing) central to appropriate stress responding (Hill & Patel, 2013; Lutz et al., 2015).

PTSD is a stress-related psychiatric disorder resulting from exposure to one or more traumatic events. The core symptom clusters of PTSD are...
intrusive thoughts, avoidance of trauma-related stimuli, negative alterations in mood and cognitive processes, and marked alterations in arousal and reactivity, all of which must be associated with the traumatic event(s), and must have begun or worsened after the traumatic event(s) occurred (American Psychiatric Association, 2013). Individuals with PTSD often exhibit an inability to suppress stress, fear, and anxiety responses to trauma- or non-trauma related stimuli (Hill, Campolongo, Yehuda, & Patel, 2018; Rosen & Schulkin, 1998). Recent research suggests the hypothesis that hypoactivity of the CB1R/eCB signaling system could contribute to the symptoms of PTSD (Hill et al., 2018). As a result of these findings, strategies to increase eCB levels are thought to have therapeutic potential in chronic PTSD. In support of this notion, results from recent investigations have found modest effects, including enhanced fear extinction, reduced PTSD symptoms, and improved mental health outcomes (e.g., anxiety, depression, sleep quality, mood) of pharmacologic therapies targeting the eCB system (Jetly, Heber, Fraser, & Boisvert, 2015; Rabinak et al., 2014; Roitman, Mechoulam, Cooper-Kazaz, & Shalev, 2014).

Endocannabinoids can be measured in the circulation where their concentrations are dynamic (Hillard, 2018). Several previous studies demonstrated altered basal circulating concentrations of eCBs in adults with PTSD, although the observed alterations have not been consistent. For instance, individuals with PTSD stemming from the September 11 terrorist attacks were found to have reduced 2-AG concentrations compared to healthy controls (Hill et al., 2013), while individuals with non-combat related PTSD were found to have reduced AEA, but not 2-AG concentrations compared to both trauma-exposed individuals and healthy controls (Neumeister et al., 2013). On the other hand, another study found no difference in circulating 2-AG concentrations in individuals who developed PTSD following childhood sexual abuse (Schaef er et al., 2014), while yet another study found increased eCB concentrations in individuals traumatized by war compared to healthy controls (Jlauer et al., 2013). However, multiple studies have consistently demonstrated in healthy adults that eCB concentrations in the circulation are increased by psychosocial stress (Dlugos, Childs, Stuhr, Hillard, & de Wit, 2012; Hill, Miller, Carrier, Gorralka, & Hillard, 2009) and exercise (Brellenthin, Crombie, Hillard, & Koltyn, 2017; Raichlen, Foster, Seillier, Giuffrida, & Gerde man, 2013). The purpose of the present study was to compare the increases in eCB concentrations evoked by exercise and psychosocial stress (Trier Social Stress Test [TSST]) between healthy controls and individuals with PTSD. The results from this randomized, counterbalanced study demonstrate that baseline eCB concentrations are not significantly different between the groups, however, unlike in controls, 2-AG concentrations are not increased by exercise or psychosocial stress in those with PTSD.

2. Materials and methods

2.1. Participants

This study used two methods (posting recruitment flyers and local newspaper advertisement) to recruit participants (N = 20) between the ages of 18 and 45. All interested participants were screened by phone in order to determine if they met eligibility criteria based on their respective groups. Participants with PTSD (n = 10) had a current diagnosis of PTSD from their psychologist or psychiatrist. Control participants (not diagnosed with PTSD; n = 10) were matched with participants from the PTSD group on sex, age, and body mass index (BMI). Exclusion criteria for participants from both groups included: being pregnant or planning to become pregnant; a history of light headedness or fainting during blood draws or physical activity; current cigarette smokers; a history of chest pain during physical activity; bone, joint, cardiac, or other medical conditions that a physician has communicated may be worsened by physical activity; asthma; taking medications for any chronic diseases such as high blood pressure or diabetes; responding ‘yes’ to any of the seven questions on the Physical Activity Readiness Questionnaire (PAR-Q); a current diagnosis (from psychologist or psychiatrist) of severe major depressive disorder; a current or past diagnosis of any psychotic disorder; or any major medical, cognitive, or neurological disorders.

2.2. Procedures

All procedures were approved by the Health Sciences Institutional Review Board at the University of Wisconsin—Madison and the work described has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Participants came to the laboratory for two, 90-minute study visits. Each participant’s visits were scheduled so they occurred at the same time of day (±60 min) and occurred at least 48 h but no more than one week apart. Participants were instructed not to eat within 2 h or exercise within 24 h of testing in order to minimize eCB variations and any potential carryover effects from previous exercise sessions.

2.2.1. Study visits 1 and 2

Written informed consent was obtained upon arrival at the first study visit. In order to ensure participants met inclusion and exclusion criteria, participants completed a neuropsychiatric interview (MINI 6.0; Sheehan et al., 1998) with a trained interviewer in a private, sound-dampened chamber. Participants completed baseline demographic and mental health questionnaires (PTSD Checklist for DSM-5 [PCL-5], Blevins, Weathers, Davis, Witte, & Domino, 2015; Beck Depression Inventory-II [BDI-II], Beck, Steer, & Brown, 1996; Beck Anxiety Inventory [BAI], Beck & Steer, 1990), in addition to validated and reliable pre-task questionnaires designed to assess mood states (Profile of Mood States [POMS], McNair, Lorr, & Droppleman, 1971; state anxiety subscale of the State-Trait Anxiety Inventory [STAI], Spielberger, 1983; Positive and Negative Affect Schedule [PANAS], Watson, Clark, & Tellegen, 1988). Upon completing questionnaires, blood pressure (BP), weight, and height were measured. Participants were randomly assigned using a computer program to participate in either an acute bout of aerobic exercise or psychosocial stress task (i.e., TSST) during their first study visit. Specific details pertaining to each study visit condition can be found below. Blood (5 ml) was drawn from the antecubital vein into ethylenediaminetetraacetic acid (EDTA) containing tubes (BD Vacutainer, K3E EDTA K3) immediately prior to and after completing the exercise or psychosocial stress tasks. Following completion of the task, participants completed the same questionnaires designed to assess mood states (i.e., POMS, STAI, PANAS). Procedures were the same for the second visit, although participants engaged in the other condition (exercise or psychosocial stress). After completing the assigned tasks of the second study visit, participants were provided with a study overview and were compensated ($70.00) for participation.

2.2.2. Aerobic exercise session

The aerobic exercise condition consisted of a 5-minute warm-up at low to moderate intensity (40%–60% maximum heart rate; MHR) on a treadmill, followed by running or incline walking at a moderate intensity (70%–75% MHR; ratings of perceived exertion [RPE] of 12–15) for 30 min, and finished with a 5-minute walking cool down. The exercise duration and intensity was selected based on previous research in our lab and others (Brellenthin et al., 2017; Crombie, Bre llenthin, Hillard, & Koltyn, 2018; Raichlen et al., 2013), demonstrating a significant increase in eCBs. Heart rate and RPE (Borg, 1998) were assessed every five minutes during the exercise session.

2.2.3. Psychosocial stress session

The psychosocial stress condition involved administration of the Trier Social Stress Test (TSST, Kirschbaum, Pirke, & Hellhammer, 1993), which is an acute psychosocial stressor frequently used in experimental conditions. The TSST was selected as it has been shown to...
be a reliable and ecologically valid stressor for examining the neurobiological response to acute psychological stress in both clinical and non-clinical populations. The TSST began with the experimenter instructing participants to sit quietly in a sound-dampened chamber for 10 min. At the end of this period, the experimenter reads the standardized TSST instructions to the participant. Participants had 10 min to prepare before they were escorted to an adjacent sound-dampened chamber, where they engaged in a free speech (5 min) and performed a difficult mental arithmetic task (5 min) in front of two trained study team members wearing white lab coats. All procedures took place in a separate room from the pre- and post-session blood draws and involved study team members who had not previously interacted with participants. Participants wore a heart rate monitor throughout the task. The duration of both the exercise and TSST tasks were 30 min.

2.2.4. Sample collection, processing, and endocannabinoid assays
Blood draws were carried out while participants were seated and were then centrifuged (4 °C at 3500 RPM) within 2 min of collection, separated into aliquots, and frozen at −80 °C until eCB and related lipid extractions took place. Following preparation, the concentrations of eCBs (AEA and 2-AG), along with related biogenic lipids (PEA, OEA, 2-oleoylglycerol (2-OG)) were quantified in 5 µl of the methanol extract using stable isotope-dilution, electrospray ionization liquid chromatography/mass spectrometry (LC-ESI-MS-MS) as previously described (Crombie et al., 2018a). PEA and OEA are noncannabinoid fatty-acid ethanamides that share some biosynthetic and metabolic pathways with AEA and belong to the family of N-acyl ethanamides, but are agonists at peroxisome proliferator-activated receptors as opposed to CB1 and CB2 receptors. Similarly, 2-OG is a monoacylglycerol and is a structural analog of 2-AG as both have overlapping mechanisms of synthesis and degradation (Di Marzo et al., 2005; Hillard, 2000).

2.3. Data analyses
All analyses were conducted with SPSS Version 25.0 for Windows. A series of one-way ANOVAs were used to detect the presence of group differences in baseline variables. Circulating concentrations of AEA, 2-AG, PEA, OEA, and 2-OG and mood states were compared between and within groups using a series of 2 (group: PTSD and control) × 2 (time: pre- and post-task) mixed-design, repeated measures ANOVAs. In order to meet the normality assumption for parametric tests, lipid concentrations were logarithmically transformed (log10 for 2-AG, PEA, OEA, and 2-OG; log10 + 1 for AEA) prior to statistical analyses. The overall alpha family-wise was set at α = 0.05. Simple effects were calculated based on significant interaction effects.

3. Results
3.1. Participant characteristics
In total, twenty men and women with a mean age of 23.7 ± 7.2 years participated in this study. There were no significant differences (reported as M ± SD unless indicated otherwise) between groups for age (control (CON) = 22.2 ± 6.1; PTSD = 25.2 ± 8.1), sex (CON = 70% women; PTSD = 80% women), body mass index (BMI; kg/m²; CON = 24.0 ± 4.8; PTSD = 24.79 ± 5.1), race (CON = 70% Caucasian, 20% Asian, 10% multiracial; PTSD = 90% Caucasian, 10% Asian), ethnicity (CON = 90% non-Hispanic/Latino; PTSD = 100% non-Hispanic/Latino), education (CON = 100% completed high school; PTSD = 100% completed high school), self-reported overall health (CON = 80% very good or excellent, 20% good or fair; PTSD = 70% very good; 30% good or fair) or time of study visits (12.90 ± 4.3). However, the PTSD group had significantly higher baseline anxiety (CON = 5.2 ± 9.4; PTSD = 24.7 ± 11.6) and depression (CON = 5.7 ± 9.8; PTSD = 27.4 ± 11.0) compared to the control group. Based on their responses to the clinical interview (MINI 6.0), none of the participants from either group met criteria for cannabis abuse or dependence. Participants from the PTSD group (n = 10) had a current diagnosis of PTSD stemming from one or more of the following traumas: physical violence/abuse (n = 3), sexual assault/rape (n = 5) emotional/psychological abuse (n = 2), combat-war-related event (n = 1), and death of a family member (n = 1). The majority of adults with PTSD were diagnosed over 6 months ago (n = 8), although two individuals were diagnosed between 3 and 6 months ago. The mean number of PTSD symptoms (via PCL-5) endorsed by the PTSD group was 12.9 ± 3.2 with a mean symptom severity score of 43.1 ± 14.5.

3.2. Acute exercise and psychosocial stress session variables
There were no significant group differences in blood pressure and heart rate prior to and following both the exercise and psychosocial stress sessions. There were no significant group differences in average RPE (CON = 13.16 ± 0.9; PTSD = 13.17 ± 0.9), heart rate (CON = 160.7 ± 16.9; PTSD = 157.6 ± 17.9), treadmill speed (km/hr; CON = 8.5 ± 1.7; PTSD = 7.1 ± 1.3), and treadmill incline (% grade; CON = 1.2 ± 0.7; PTSD = 1.7 ± 1.2) during the exercise session, as well as heart rate (CON = 93.7 ± 16.8; PTSD = 107.0 ± 9.0) during the psychosocial stress session. Both groups exhibited a significant increase in heart rate (beats per minute) from baseline during the exercise (CON = 93.77 ± 20.21; PTSD = 85.20 ± 13.33) and psychosocial stress sessions (CON = 32.3 ± 21.9; PTSD = 37.2 ± 28.4).

3.3. eCB responses to exercise and psychosocial stress
The results (Fig. 1) indicated that there were significant time effects for AEA (F1,16 = 80.00, p < .001) and OEA (F1,16 = 75.78, p < .001), with the concentrations of lipids increasing significantly from pre- to post-exercise for both groups. There was a significant group x time interaction for 2-AG (F1,16 = 4.50, p = .05), as analysis of simple effects indicated a significant increase in 2-AG from pre- to post-exercise for the control group (F1,16 = 13.40, p = .002), but not the PTSD group (F1,16 = 0.43, p = .520). Endocannabinoid and related lipid responses to acute psychosocial stress were similar to the exercise session. The results (Fig. 2) indicated that there were significant time effects for AEA (F1,17 = 4.81, p = .042) and OEA (F1,16 = 10.24, p = .006), with the concentrations of lipids increasing significantly from pre- to post-stress. There was also a significant group x time interaction for 2-AG (F1,17 = 5.72, p = .029), as analysis of simple effects indicated a significant increase in 2-AG from pre- to post-stress for the control group (F1,17 = 4.45, p = .05) and a small decrease from pre- to post-stress for the PTSD group (F1,17 = 2.873, p = .10). There were no significant (p > .05) changes for PEA and 2-OG after exercise or psychosocial stress for either group, although there were significant group effects for PEA for the exercise (F1,16 = 5.84, p = .028) and psychosocial (F1,16 = 4.48, p = .05) sessions, with greater concentrations for the PTSD group.

3.4. Mood responses to exercise and psychosocial stress
In the exercise arm, results indicated that there were significant time effects for vigor (F1,16 = 6.32, p = .023) and positive affect (F1,18 = 5.06, p = .038), indicating a significant increase from pre- to post-exercise for both groups. There were significant group x time interactions for tension (F1,18 = 6.13, p = .025), depression (F1,18 = 4.99, p = .040), fatigue (F1,18 = 10.54, p = .005), confusion (F1,18 = 6.29, p = .023), total mood disturbance (TMD)(F1,18 = 4.45, p = .05), state anxiety (F1,18 = 4.46, p = .05), and negative affect (F1,18 = 4.48, p = .05). Simple effects indicated significant reductions from pre- to post-exercise for the PTSD group (ps = .001 to .005) compared to the control group (ps = .319 to .930).

For the psychosocial stress arm, the results indicated that there were significant time effects for tension (F1,18 = 14.74, p = .001), state...
4. Discussion

The major findings from this study are that acute psychosocial stress and aerobic exercise increase circulating 2-AG concentrations in healthy controls, but not in those with PTSD while both interventions increased circulating concentrations of AEA regardless of PTSD diagnosis. These findings extend our understanding of eCB dysregulation in PTSD and suggest that recruitment of 2-AG by both psychological and physical stress is blunted in individuals with PTSD. These results are in accord with an earlier study from our group in which circulating concentrations of AEA were significantly increased following aerobic exercise in adults with PTSD; however, that study also found a significant increase in 2-AG in adults with PTSD, although the magnitude of the effect was much smaller for adults with PTSD compared to the healthy controls (Crombie et al., 2018a).

Considerable evidence suggests that an important role for 2-AG in the brain is to regulate responses to acute stress (Gorzalka, Hill, & Hillard, 2008; Hill et al., 2010). In particular, eCB/CB1R signaling has been implicated in the regulation of numerous psychological (e.g., emotional behavior, mood) and physiological reactions to stress (Hill & Gorzalka, 2009; Hillard, 2015). Activation of eCB/CB1R signaling opposes or buffers many of the effects of stress, particularly effects on amygdalar information processing (Hill et al., 2018). Among the data supporting this mechanism of action include studies showing that mice unable to synthesize 2-AG in the amygdala or with amygdalar-specific CB1R blockade exhibit increased vulnerability to the effects of repeated stress (Bluett et al., 2017). Recent preclinical data from Patel and colleagues demonstrated that 2-AG concentrations were significantly, positively correlated with brain concentrations (Bedse et al., 2017), suggesting that the changes in circulating 2-AG are related to changes in brain eCB signaling. Thus, we hypothesize that a reduction in the ability of acute physical and psychosocial stress to evoke a measurable increase in circulating 2-AG reflects a loss of the 2-AG stress-opposition system and that this loss could contribute to the symptoms of PTSD.

Earlier studies in humans also demonstrate that circulating concentrations of eCBs are dynamically regulated by acute psychosocial stress. In one study, TSSST exposure significantly increased circulating concentrations of 2-AG and increased AEA concentrations by 10%, although this change was not statistically significant (Hill et al., 2009). In a second study, administration of the TSST resulted in significant increases in circulating AEA, OEA, and PEA in healthy adults compared to a control condition (speaking with a friendly research assistant about neutral topics); while 2-AG was found to increase similarly after both the TSST and control sessions (Dlugos et al., 2012). The results of the current study are in accord with both of these studies as circulating concentrations of AEA and 2-AG were both significantly elevated immediately after task completion in healthy adults. Another manipulation known to increase circulating eCB concentrations is exercise. Several studies demonstrated that a bout of moderate-intensity aerobic or isometric exercise increases circulating concentrations of AEA (Brellenthin et al., 2017; Heyman et al., 2012; Koltyn, Brellenthin, Cook, Sehgal, & Hillard, 2014; Sparling, Giuffrida, Piomelli, Rosskopf, & Dietrich, 2003; Raichlen, Foster, Gerdeman, Seillier, & Giuffrida, 2003).
and 2-AG (Brellenthin et al., 2017; Cedernaes et al., 2016; Crombie, Brellenthin, Hillard, & Koltyn, 2018; Koltyn et al., 2014) in healthy men and women. The current findings of significant increases in AEA, 2-AG, and OEA in healthy controls following aerobic exercise are in accord with these previous investigations.

In addition to evoking an eCB response, aerobic exercise elicited significant increases in positive mood states and significant reductions in negative mood states in both healthy adults and adults with chronic PTSD, which is consistent with previous research in clinical (O’Connor, 2005) and non-clinical populations (Raglin & Morgan, 1987). In contrast, the psychosocial stress task resulted in significant mood disruption, including increases in tension, state anxiety, and negative affect, in both groups. Although exercise can be considered a physical stressor due to its ability to activate the HPA-axis, there are several key distinctions between exercise (a physical form of eustress) and psychosocial stress that might explain the differential mood responses. In

**Fig. 2.** Circulating endocannabinoid and related lipids pre- and post-psychosocial stress in healthy controls (CON) and adults with posttraumatic stress disorder (PTSD). *significant time effect for AEA and OEA (p < .05); ‡significant group-time interaction for 2-AG (p < .05); **significant group effect for PEA (p < .05). AEA = N-arachidonoylethanolamine (a); 2-AG = 2-arachidonoylglycerol (b); PEA = palmitoylethanolamide (c); OEA = oleoylethanolamide (d); 2-OG = 2-oleoylglycerol (e).

**Table 1**
Mood responses pre- and post-psychosocial stress and exercise.

<table>
<thead>
<tr>
<th>Mood Outcomes</th>
<th>Exercise Session</th>
<th>Psychosocial Stress Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre M ± SD</td>
<td>Post M ± SD</td>
</tr>
<tr>
<td>TMD a,d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>106.7 ± 29.09</td>
<td>99.40 ± 2.92</td>
</tr>
<tr>
<td>PTSD</td>
<td>149.67 ± 34.90</td>
<td>109.89 ± 16.55</td>
</tr>
<tr>
<td>Negative Affect b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.78 ± 2.05</td>
<td>10.56 ± 1.01</td>
</tr>
<tr>
<td>PTSD</td>
<td>17.67 ± 4.85</td>
<td>12.99 ± 2.32</td>
</tr>
<tr>
<td>Positive Affect b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29.40 ± 9.83</td>
<td>32.10 ± 10.30</td>
</tr>
<tr>
<td>PTSD</td>
<td>21.00 ± 10.34</td>
<td>27.78 ± 9.67</td>
</tr>
<tr>
<td>State Anxiety c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34.00 ± 6.65</td>
<td>31.67 ± 8.25</td>
</tr>
<tr>
<td>PTSD</td>
<td>50.00 ± 9.77</td>
<td>38.67 ± 10.31</td>
</tr>
</tbody>
</table>

Note. PTSD = posttraumatic stress disorder; TMD = total mood disturbance.

a Scores were obtained from the Profile of Mood States (POMS) questionnaire.
b Scores were obtained from the Positive and Negative Affect Schedule (PANAS).
c Scores were obtained from the 20 state-anxiety items from the State-Trait Anxiety Inventory (STAI).
d Total mood disturbance (TMD) was derived by adding 100 to the sum of the negative mood state subscales (i.e., tension, depression, confusion, fatigue, anger) minus the positive mood state subscale (i.e., vigor).

2012, 2013) and 2-AG (Brellenthin et al., 2017; Cedernaes et al., 2016; Crombie, Brellenthin, Hillard, & Koltyn, 2018; Koltyn et al., 2014) in healthy men and women. The current findings of significant increases in AEA, 2-AG, and OEA in healthy controls following aerobic exercise are in accord with these previous investigations.

In addition to evoking an eCB response, aerobic exercise elicited significant increases in positive mood states and significant reductions in negative mood states in both healthy adults and adults with chronic PTSD, which is consistent with previous research in clinical (O’Connor, 2005) and non-clinical populations (Raglin & Morgan, 1987). In contrast, the psychosocial stress task resulted in significant mood disruption, including increases in tension, state anxiety, and negative affect, in both groups. Although exercise can be considered a physical stressor due to its ability to activate the HPA-axis, there are several key distinctions between exercise (a physical form of eustress) and psychosocial stress that might explain the differential mood responses. In
particular, acute exercise leads to activation of the body’s stress re-
sponse, yet results in a generally positive experience that is of limited
duration and involves a sense of mastery and accomplishment (McEwen, 2007). Moreover, as Heijnen, Hommel, Kibele, and Colzato (2015) previously discussed, acute physical stress (i.e., exercise) often
leads to improved cognitive functioning, increases in growth hormone,
and increased inactivation of cortisol into cortisone (the inert steroid),
whereas psychological stress typically has opposing effects (Heijnen
et al., 2015). Despite these differences and the observed differential
effects on mood, both stress conditions elevate eCBs in the circulation
in healthy controls.

There are several important considerations (e.g., potential sex dif-
fences, sample size, additional biological measures not collected) that
should be addressed in future research in order to better understand
the observed differential 2-AG response. For instance, 80% of participants
from the PTSD group in the current investigation were women, and
Dlugos et al. found men exhibited a greater increase in eCBs following
the TSST compared to women (Dlugos et al., 2012). Additionally, due to
the small sample size, we were underpowered to conduct correlational
analyses between changes in eCBs and mood states following exercise
and psychosocial stress exposure. However, even if such analyses were
conducted, it would be impossible to elucidate the mechanistic re-
lationships among the stress exposure, eCB mobilization, and mood
states. Future studies should examine the temporal relationship be-
 tween changes in circulating eCBs and mood following acute stress by
examining both outcomes at several time points following stress ex-
posure in larger samples in order to help elucidate these relationships.

Another limitation of this study was the lack of assessment of ad-
ditional biological variables known to be stress responsive (e.g., cor-
tisol), which would have served as a manipulation check and provided
additional information regarding differences in psychobiological re-
sponses to stress in healthy controls and adults with PTSD. Relatedly,
the current study did not assess early life stress exposure, which is
commonly reported in individuals with PTSD. Recent preclinical data
has demonstrated that following early life stress, the mobilization of 2-
AG in the hippocampus of adult rats is compromised following both
acute stress and glucocorticoid administration (Atsak et al., 2018).
Therefore, it is possible that a breakdown in the glucocorticoid-eCB
crosstalk could contribute to the documented effects, especially given
that exercise and psychosocial stress increased circulating 2-AG levels
in healthy controls but not those with PTSD. This hypothesis could have
been tested in humans had we measured cortisol in conjunction with
eCBs. As such, future studies with larger samples should assess prior
trauma exposure and early life stress, in addition to cortisol.

5. Conclusions

In conclusion, our preliminary data suggest that adults with PTSD
exhibit a blunted mobilization of 2-AG into the circulation in response
to two different forms of acute stress (psychosocial and aerobic ex-
ercise) compared to healthy controls. These findings extend our un-
derstanding of eCB dysregulation in PTSD beyond basal concentrations,
and are consistent with the hypothesis that 2-AG/CB1R signaling is
hypoaffective in adults with PTSD (Hill et al., 2018). Additional research
is needed, as there may be a complex relationship between mood re-
sponses (generally improved mood following exercise versus increases
in negative mood states following psychosocial stress) and circulating
eCBs following acute exposure to different types of stressors. Despite
this discrepancy, it appears that acute bouts of aerobic exercise elicit a
positive mood response (decreased negative and increased positive
mood states) in adults with PTSD, although it is unknown how long
these effects last. Collectively, these findings advance our under-
standing of eCB system functioning in PTSD, which has implications
for the design, development, and testing of treatments aimed at enhancing
eCB system dysregulation in order to improve mental health outcomes
(e.g., mood, PTSD symptoms, fear processing, stress resiliency) in PTSD.

Role of funding sources

This work was supported by the Dr. Raymond A. Weiss Research
Endowment from the American College of Sports Medicine Foundation;
the University of Wisconsin Virginia Horne Henry Fund; and the
Advancing a Healthier Wisconsin Endowment at the Medical College
of Wisconsin. The funding sources had no role in the collection, analysis,
and interpretation of data; in writing the report; or in the decision to
submit the article for publication.

Declarations of interest

None.

Acknowledgements

We would like to express many thanks to our undergraduate assis-
tants (Logan Kovacs and Alejandro Hernandez) for assisting with the
study visits, Garrett Sauber for technical assistance conducting the eCB
assays, and all of the participants.

References

American Psychiatric Association (2013). Diagnostic and statistical manual of mental dis-
Glucocorticoid-endocannabinoid uncoupling mediates fear suppression deficits after
pynen.2018.02.021.
The Psychological Corporation.
San Antonio, TX: The Psychological Corporation (BDI-II).
(2017). Functional redundancy between canonical endocannabinoid signaling sys-
org/10.1016/j.biopsycho.2017.03.002.
The posttraumatic stress disorder checklist for DSM-5 (PCL-5): Development and initial
org/10.1002/jts.22059.
Endocannabinoid signaling modulates susceptibility to traumatic stress exposure.
Nature Communications, 8, 14782. https://doi.org/10.1038/ncomms14782.
Kinetcs.
creases circulating anandamide and other N-acylethanolamines in healthy humans.
67.040903.120816.
responses to aerobic exercise in individuals with posttraumatic stress disorder.
and Opioid System Interactions in Exercise-Induced Hypoalgesia. Pain Medicine, 19(1),
Di Marzo, V., & Di Marzo, V. (2001). The biosynthesis, fate and phar-
creases circulating anandamide and other N-acylethanolamines in healthy humans.
Gonzalez, B. B., & Hillard, C. J. (2008). Regulation of endocannabinoid sig-
aling by stress: Implications for stress-related affective disorders. Neurosciences and
10.004.
(2013). Plasma concentrations of endocannabinoids and related primary fatty acid