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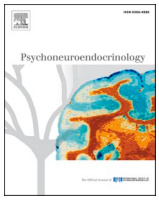

Virtual reality based active shooter training: Added physical stress increases anxiety but not stress biomarkers

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ABSTRACT

Tactical first responders such as firefighters and law enforcement officers are often exposed to concurrent or consecutive stressors. Exposure to concurrent stress challenges (i.e., combined mental and physical stress) has been shown to exacerbate stress biomarker responses. However, this has not been shown with short duration stressors (e.g., < 3 min). Therefore, this study compared markers of stress and perceived anxiety in response to a physical stressor [exercise alone; (EA)] to that of a dual stress challenge (DSC) which included a virtual reality based active shooter drill in addition to the exercise task. Fifty-four subjects (n = 54) completed either EA (n = 27) or DSC (n = 27). Measurements included salivary α−amylase (sAA), secretory immunoglobulin A (SIgA), heart rate (HR), and state anxiety inventory (SAI) which were collected four times: 1) 30 min prior to exercise 2) immediately prior to exercise 3) 5 min post exercise 4) 30 min post exercise. Both DSC and EA resulted in significant increases in salivary stress biomarkers ($p < 0.05$). The DSC resulted in significantly greater SAI values 5 min post exercise compared to the EA treatment. A secondary analysis demonstrated significantly lower sAA concentrations overall in females (n = 25) compared to males (n = 29), as well as significantly lower SIgA at five and 30 min post stress compared to males. These findings demonstrated that added mental stress causes significant increases in perceived anxiety compared to physical stress alone, and females demonstrate reduced stress biomarker responses to acute stress.

1. Introduction

Tactical first responders (e.g., military personnel, law-enforcement officers, and firefighters) are frequently exposed to intense physical, psychological, metabolic, and environmental stress (Soteriades, 2022; Papazoglou, 2023; Maglione, 2022). These stressors activate the *fight or flight* response, leading to increased heart rate, respiratory rate, blood flow, and substrate utilization (Chu, 2024). Acutely, this response supports physiological function and performance (Degroote, 2020; Pavlíčková, 2024); however, chronic exposure to high stress scenarios can contribute to the development of physiological and neurological diseases and/or disorders (Agorastos and Chrousos, 2022; Liu et al., 2017). Moreover, prior research indicates that exposure to combined/concurrent stressors exacerbates the fight or flight response (Huang, 2010; Huang, 2010; McAllister, 2019), which may further contribute to disease risk, especially among tactical operators (Hendricks, 2023; Huang, 2013).

Acute exposure to dual stress challenges (DSCs) results in an exacerbated physiological stress response (Huang, 2010; Huang, 2013; Huang, 2010; Webb, 2011). For example, the addition of psychological stress during exercise has been shown to significantly increase plasma concentrations of catecholamines (Huang, 2010; Webb, 2017; Webb, 2013), as well as markers of oxidative stress and immune function (Huang, 2010; Huang, 2010), compared to exercise alone. Some studies have examined the potential effects of nutritional interventions on stress biomarker responses to DSCs (McAllister, 2016; McAllister et al., 2021; Waldman, 2020; McAllister, 2020) demonstrating that carbohydrate ingestion can acutely reduce cortisol responses (McAllister, 2016). However, DSC exposure has also been associated with significantly greater anxiety, even when biomarkers of stress are unaffected

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(Degroote, 2020; McAllister, 2019).

Importantly, this response appears to be impacted by the cardiorespiratory fitness level of the individual (Webb, 2017; Webb, 2013; McAllister, 2020). For instance, individuals with high cardiorespiratory fitness have been shown to have higher catecholamine responses (Webb, 2017) whereas low fit individuals have been shown to have higher cortisol responses to DSCs (Webb, 2013). Notably, most studies on DSCs have involved 20 min of mental stress tasks performed during steady state exercise lasting approximately 35–37 min (Huang, 2010; Webb, 2017; Webb, 2013; McAllister, 2019). While these scenarios have implications for tactical first responders (Huang, 2013), additional research is needed to evaluate the impact of combined mental and physical stress during more ecologically valid, high stress tactical scenarios.

For tactical first responders, virtual reality (VR) based training interventions may serve as effective stress inoculation training methods to reduce stress and improve performance (Gamito, 2024). To that end, multiple studies have examined physiological responses to high stress VR scenarios, including active shooter (McAllister, 2024; McAllister, 2022; McAllister, 2024; Dillard, 2023), and hostage scenarios (Kleygrewe et al., 2023). Recent work from our lab has demonstrated that the stress response elicited by VR based scenarios is comparable to that observed in real life scenarios involving professional actors (Martaindale, 2024).

Acute exposure to virtual or video based tactical high stress scenarios has been shown to significantly elevate stress biomarkers such as salivary α-amylase (sAA), secretory immunoglobulin A (SIgA) (McAllister, 2024; McAllister, 2022; Martaindale, 2024), cortisol, and interleukin-6 (Groer, 2010). However, acute high intensity exercise also increases these biomarkers (Koibuchi and Suzuki, 2014; Hill, 2008; Nash, 2023). Military and law enforcement personnel often encounter scenarios involving intense physical exertion coupled with mental stress, underscoring the need for further research on DSC responses in this population. Accordingly, the primary aim of this study was to examine the stress response to exercise alone (EA) compared to a DSC involving exercise combined with a virtual reality based active shooter drill (VR-ASD). Additionally, prior work suggests that women demonstrate attenuated biomarker responses to acute exposure (McAllister, 2022; McAllister and Martaindale, 2021). Thus, a secondary aim of this study was to examine sex differences in stress responses. We hypothesized that

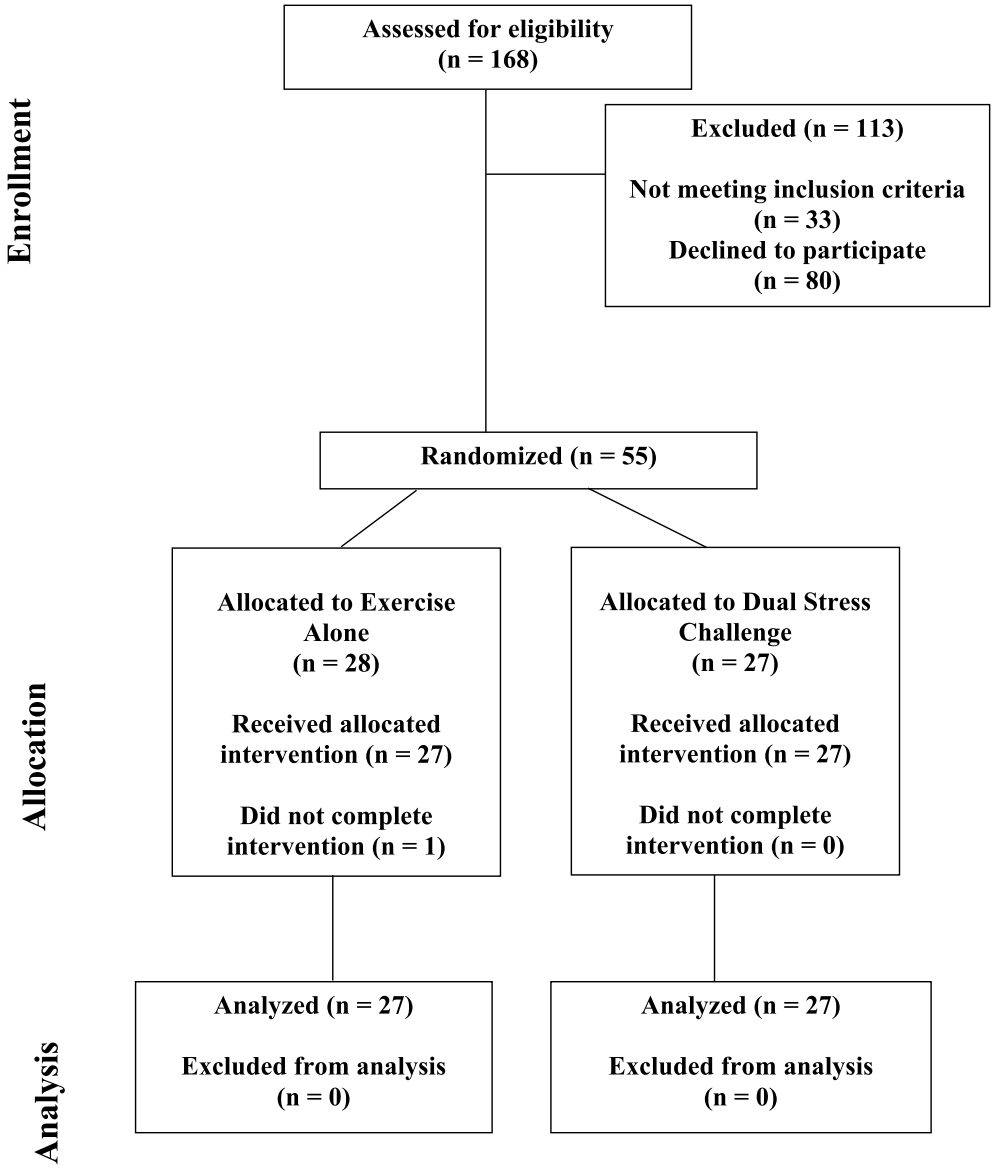


Fig. 1. Consort diagram of subject recruitment, screening, assignment, and analysis.

the DSC would result in significantly higher concentrations of sAA and SIgA post stress compared to EA, and that females would have reduced concentrations of these biomarkers compared to males.

2. Materials & methods

2.1. Subjects and experimental design

This study employed a randomized, between subjects, parallel design to examine the effect of mental and physical stress on stress related biomarkers. Subjects were recruited via flyers and word of mouth on the ((blinded for peer review)) University campus. Fig. 1 provides an overview of participant recruitment, screening, exclusion and randomization. Fifty-five (n = 55) subjects who met inclusion criteria were randomly assigned to either the EA or DSC group. The DSC condition involved exercise followed immediately by a VR-ASD.

The exercise protocol included a 3 min Ruffier squat test (Alahmari, 2020) followed by 1.5 min of jump rope. The VR-ASD consisted of a ~2 min VR based active shooter scenario. Subjects were randomized using a random number generator (random.org). The first subject in each set was assigned based on the number generated (even = EA: odd = DSC), and the subsequent two subjects were placed in the opposite group. This pattern was repeated until all participants were assigned.

All subjects provided written informed consent and completed an electronic health history questionnaire prior to participation. Inclusion criteria required subjects to be apparently healthy university students aged between 18 and 39 with no known history of vertigo, severe motion sickness, epilepsy, brain injury, cardiovascular or metabolic diseases, or currently taking any medication for psychological disorders (e.g., depression, anxiety, bipolar disorder). Subjects were also screened for recent major life stressors (e.g., childbirth, abortion, miscarriage, divorce) and any known adverse reactions to VR. Subjects were also required to be able to perform body weight squats and jump rope. Subjects were instructed to arrive in a $\geq$ 4-hour fasted state. All testing occurred between 11:00 and 18:00. The study protocol was approved by the ((blinded for peer review)) Institutional Review Board and registered as a clinical trial (NCT06680908). No subjects had been previously exposed to research involving a VR-ASD.

2.2. Experimental procedures

Primary testing was conducted in the (blinded for peer review)) at (blinded for peer review)), while the VR-ASD and exercise testing was administered in a nearby gymnasium (~20 m from the lab). Upon arrival, subjects underwent body composition assessment using the InBody 270 body composition analyzer (Seoul, South Korea). Immediately afterwards, subjects rinsed their mouths with bottled water and were escorted to the gymnasium to rest for ~10 min. Following the rest period, baseline measurements were collected: first saliva sample, heart rate (HR), and the State-Anxiety Inventory (SAI). These measurements were collected at four time points in total:

1. 30 min prior to exercise
2. Immediately prior to exercise
3. 5 min post exercise/stress
4. 30 min post exercise

Note, additional HR measurements were recorded two times during the Ruffier squat test (described below) but those data were used solely for the VO_2max prediction and excluded from statistical analysis.

2.2.1. Exercise testing

Subjects in both conditions completed the Ruffier squat test followed by two rounds of 20 jump rope repetitions. The DSC group then completed a cognitive stressor which included participation in a ~2 min VR-ASD which has been previously shown to elicit a significant increase in HR, SAI, sAA and SIgA (McAllister, 2024; McAllister, 2022; Martaindale, 2024). The Ruffier test, a validated measure of cardiorespiratory fitness (Alahmari, 2020; Guo, 2018; Trovato, 2023), involved performing 30 body weight squats in 45 s at a metronome cadence of 80 bpm. Subjects were required to follow the metronome tempo while maintaining proper form, including 90° knee flexion, a straight back, and arms extended forward. After the test, subjects sat and rested for one minute post-test. Subjects' HR was recorded at three timepoints (pre test, post test, and one minute post test; (Alahmari, 2020; Guo, 2018; Trovato, 2023) to estimate VO_2max using ACSM guidelines (see Table 1; (Pescatello, 2014). Immediately following the final HR measure, subjects completed two rounds of 20 jump rope repetitions in 90 s.

2.2.2. Virtual reality study procedure and active shooter drill

Subjects participated in a VR-ASD that has been previously used by our team and demonstrated to elicit an increase in stress biomarkers (McAllister, 2024; McAllister, 2022; Martaindale, 2024). Prior to beginning the familiarization session, subjects were fitted with the VR equipment and training pistol, and subsequently instructed to practice handling the weapon in a firing range for ~ 1 min. Immediately following the familiarization range, subjects were then briefed on the VR-ASD, with their role and objective. The VR-ASD scenario included multiple stimuli for subjects to process over the course of ~3 min, including: multiple gunshot victims, a panicked and wounded civilian running from the shooter, and the hostile shooter. If subjects took too long to stop the shooter, the shooter would then turn attention to the subject and begin firing. The VR testing took place inside of an open gymnasium, allowing for free movement within the VR environment.

2.2.3. State-anxiety inventory assessment and heart rate

The six-item short-form State Anxiety Inventory (SAI) was used to assess perceived anxiety. Items include statements such as, "I feel relaxed" and "I am worried", scored on a 4-point scale from "very much" to "not at all". Higher scores indicated greater perceived anxiety. The SAI was administered four times immediately following the saliva samples. The short-form SAI has established reliability and validity (Marteau and Bekker, 1992; Tluczek et al., 2009). The range for the SAI form was 6–24.

Heart rate was measured using Polar heart rate monitors located on the subjects left forearm (Polar Electro, Kempele, Finland). Heart rate was collection in conjunction with the SAI at each time point.

2.3. Saliva collection and analysis

Saliva samples were collected using a passive drool method using a saliva collection aid and cryovial (Salimetrics, PA, USA). Approximately 1.0 mL of saliva was collected at each time point. Subjects performed a mouth rinse ~10 min before providing each sample.

Samples were immediately stored at −80°C in the MAP laboratory. Samples were shipped overnight on dry ice via FedEx to a laboratory (Salimetrics, PA, USA). Salimetrics analyzed all samples in duplicate for concentrations of salivary α-amylase (sAA) and secretory immunoglobin-A (SIgA) using commercially available analysis kits (Salimetrics, PA, USA). The inter- and intra-assay coefficients of variation (%CV) was 4.7 % and 5.4 % for sAA and 8.7 % and 5.6 % for SIgA, respectively.

2.4. Statistical analysis

All statistical analysis was conducted using SAS v 9.4 (Cary, NC, USA). To examine potential treatment × timepoint interactions or between group and/or timepoint differences, data for sAA, SIgA, HR and SAI were analyzed via 3 × 4 (treatment × timepoint) factorial analysis of variance (ANOVA). Secondary analyses included three-way factorial ANOVAs (sex × treatment × timepoint) to assess potential sex differences for all primary variables (HR, SAI, sAA, and SIgA concentrations).

Significant main effects or interactions were followed up using Fisher's Least Significant Difference tests.

3. Results

3.1. Subjects

Fifty-four subjects (n = 54: 29 males; 25 females) completed experimental testing. An overview of the enrollment, screening and allocation is shown in Fig. 1. In the health history questionnaire, subjects were asked to self-report activity level more than three times per week (Yes = 51; No = 3), if they take any oral contraceptives (Yes = 5; No = 49), and if they regularly used nicotine (Yes = 6; No = 48). Descriptive characteristics of subjects for both groups can be found in Table 1.

3.2. Salivary biomarkers

No significant treatment × time interaction was noted for sAA or SIgA ($p > 0.05$). For sAA, significant main effects were found for treatment ($F = 8.16$, $p = 0.004$) and time ($F = 13.62$, $p < 0.001$). Post hoc analysis revealed that mean sAA concentrations were significantly higher in the EA group compared to the DSC group ($p = 0.004$). Additionally, sAA concentrations were significantly higher 5 min post exercise relative to all other time points ($p < 0.05$). For SIgA, no significant treatment x time interaction or main effect for treatment was observed ($p > 0.05$); however, a main effect for time was detected, with significantly higher concentrations 5 min post exercise compared to all other time points ($p < 0.01$). Mean sAA and SIgA data are presented in Fig. 2.

3.3. State anxiety inventory and heart rate

No significant treatment x time interaction or main effect for treatment was observed for HR ($p > 0.05$); however, a significant main effect for time was detected ($F = 6.97$; $p < 0.001$), with HR significantly elevated 5 min post exercise compared to all other time points ($p < 0.05$).

For the SAI, a significant treatment x time interaction was found ($F = 2.90$; $p = 0.03$). Post hoc analysis revealed no significant change in SAI scores from pre to post stress in the EA treatment ($p = 0.05$); however, the DSC treatment exhibited a significant increase from immediately pre exercise to 5 min post exercise ($p < 0.001$). Moreover, the two groups did not differ significantly with respect to SAI at the 30 min pre exercise and pre exercise timepoints ($p > 0.05$). However, the DSC treatment group demonstrated significantly higher SAI 5 min post exercise compared to the EA ($p = 0.01$). SAI values were not different between groups at 30 min post exercise. Mean HR and SAI values are shown in Fig. 3.

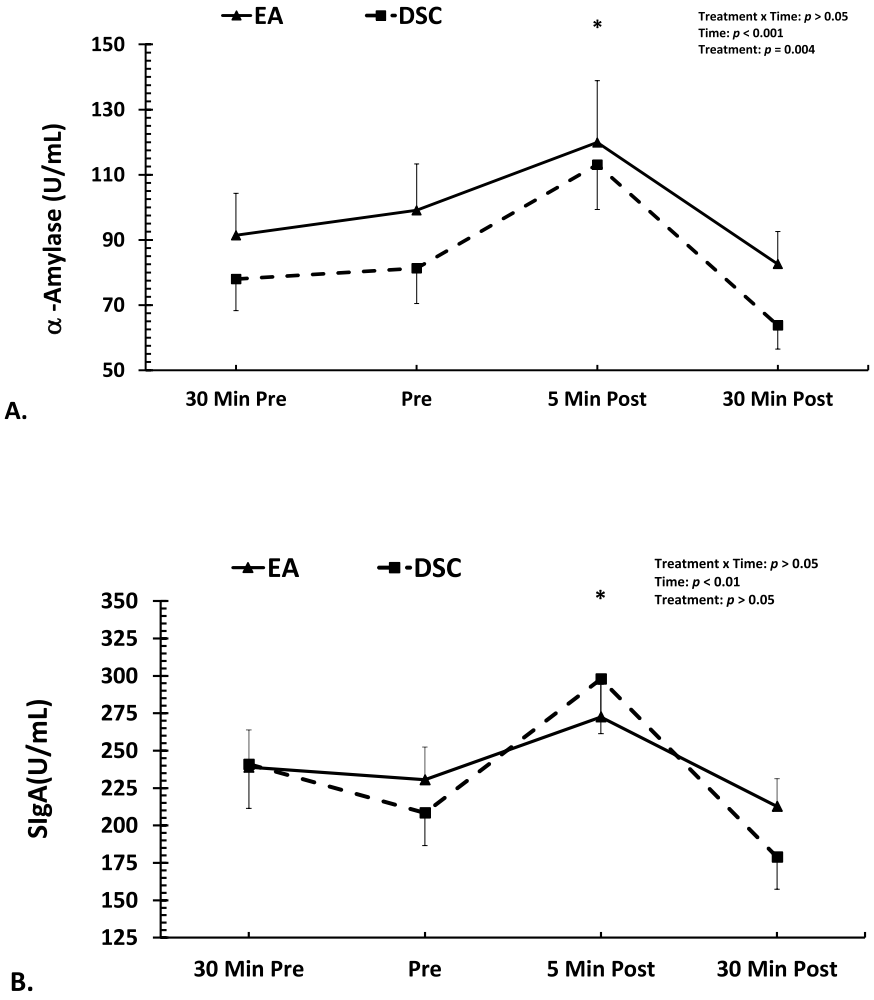


Fig. 2. Changes in salivary α-amylase (sAA) (A.) and Secretory immunoglobulin A (SIgA) (B.) concentrations are shown across time, as well as between treatments. EA = exercise alone, DSC = dual stress challenge, SIgA = Secretory Immunoglobulin A. * indicates higher concentrations of sAA and SIgA in both groups 5 min post exercise compared to all other time points. Data are shown as mean ± SE.

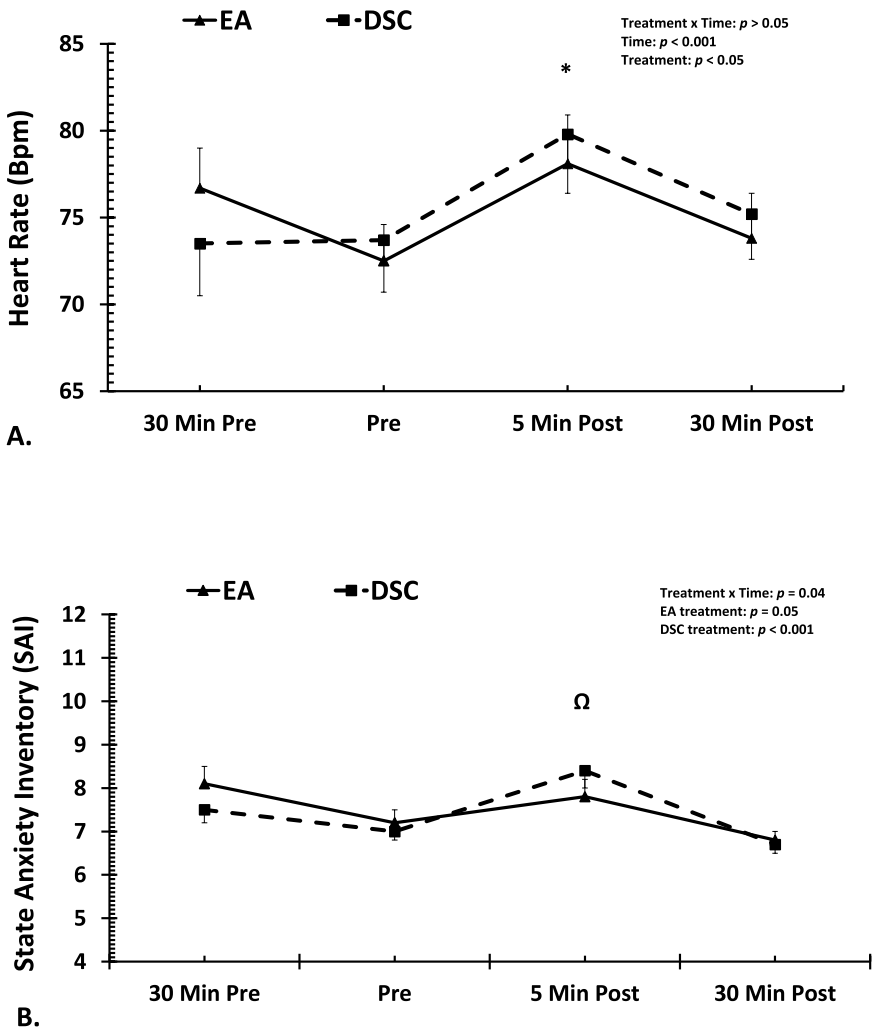


Fig. 3. Changes in heart rate (A) and State Anxiety Inventory (SAI) (B.) are shown across time, as well as between treatments. EA = exercise alone, DSC = dual stress challenge. * indicates higher ($p < 0.05$) HR in both groups 5 min post exercise compared to all other time points. Ω indicates significantly ($p < 0.05$) higher SAI values 5 min post exercise in DSC group compared to EA. Data are shown as mean ± SE.

3.4. Male/female comparisons: salivary biomarkers

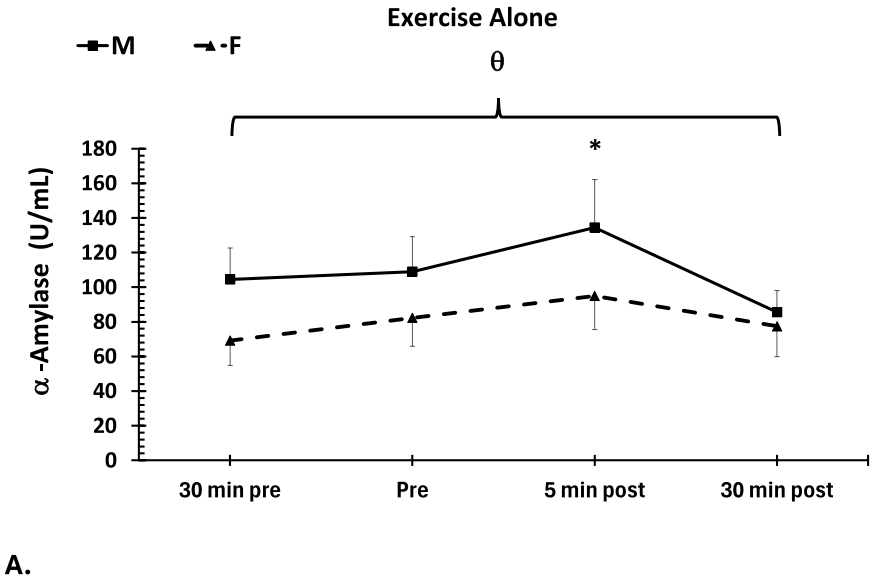
For sAA, no treatment × sex × timepoint or timepoint × sex interactions were observed ($p > 0.05$). However, a significant treatment × sex interaction was found ($F = 7.46$, $p = 0.007$) along with a significant main effect for time ($F = 12.22$, $p < 0.001$). Post hoc analysis showed that females in the EA condition had significantly lower sAA concentrations than males ($p < 0.001$; Fig. 4a), while no differences were observed between sexes in the DSC condition ($p = 0.966$; Fig. 4b). Across time, sAA concentrations were significantly elevated 5 min post exercise compared to all other time points ($p < 0.001$). Additionally, sAA concentrations 30 min post exercise were significantly lower than immediately prior to exercise ($p = 0.03$) and 5 min post exercise ($p < 0.001$). Mean sAA concentrations by sex are shown in Fig. 4.

For SIgA concentrations, no treatment × sex × timepoint interaction was found ($p > 0.05$): however, significant timepoint × sex ($F = 3.66$, $p = 0.01$) and treatment × sex interactions ($F = 28.05$, $p < 0.001$) were found. Post hoc analysis for the timepoint × sex interaction revealed no significant sex differences at baseline (30 min prior to exercise; $p = 0.54$) or immediately prior to exercise ($p = 0.43$). However, females demonstrated significantly lower SIgA concentrations 5 min post exercise ($p < 0.001$) and 30 min post exercise ($p = 0.03$) compared to males. Both males and females demonstrated a significant increase in SIgA from pre to 5 min post stress ($p < 0.05$). Mean SIgA concentrations by sex are shown in Fig. 5.

3.5. Male/female comparisons: heart rate and state anxiety inventory

No treatment × sex × timepoint or sex × timepoint interactions were found for HR ($p > 0.05$). However, a significant treatment × sex interaction was observed ($F = 33.3$, $p < 0.001$) along with a main effect for time ($F = 6.57$, $p < 0.001$). Post hoc analysis indicated no HR differences between males and females in the DSC condition ($p > 0.05$); however, in the EA condition, males demonstrated significantly lower HRs overall ($p < 0.001$). HR was significantly higher 5 min post exercise compared to 30 min prior to exercise ($p = 0.005$), immediately prior to exercise ($p < 0.001$), and 30 min post exercise ($p = 0.002$). Mean HR data by sex are shown in Fig. 6A & B.

Regarding SAI, no treatment × sex × timepoint or sex × timepoint interactions were detected ($p > 0.05$). However, a significant treatment × sex interaction was found ($F = 8.44$, $p = 0.004$), as well as a main effect for time ($F = 16.28$, $p < 0.001$). Post hoc analysis revealed significantly higher SAI overall in males compared to females in the EA condition ($p = 0.02$), with no differences between sexes for the DSC condition ($p > 0.05$). Across time, SAI scores significantly decreased from 30 min prior to exercise to immediately prior to exercise ($p < 0.001$) and were significantly higher 5 min post exercise compared to immediately prior to exercise ($p < 0.001$) and 30 min exercise



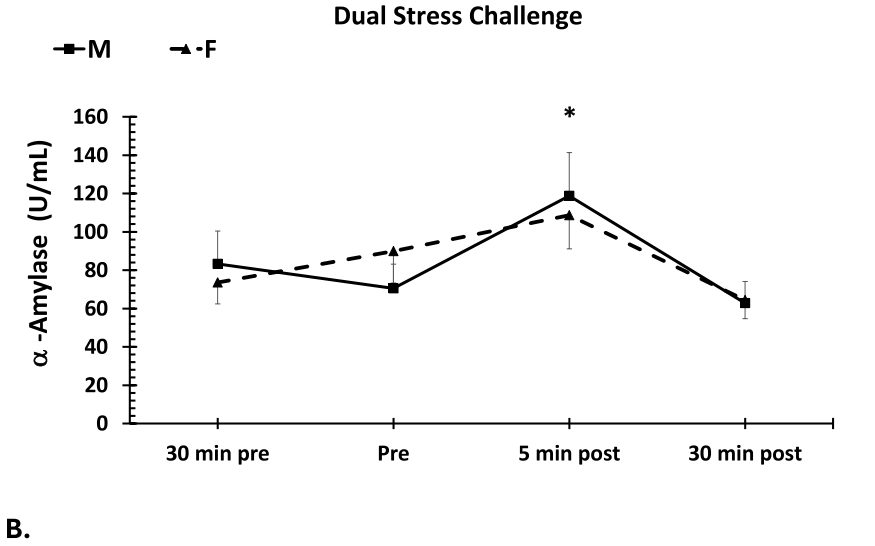


Fig. 4. Changes in salivary α-amylase concentrations (sAA) in the exercise alone (A.) and dual stress challenge (B.) groups. *indicates significantly ($p < 0.05$) higher than all other timepoints. θ indicates significantly lower sAA concentrations overall across all timepoints in females compared to males in the exercise alone group. Data are shown as mean ± SE.

($p < 0.001$). Mean SAI data by sex are shown in Fig. 6 C & D.

4. Discussion

The main findings from this study indicate that both EA and DSC conditions elicited significant increases in physiological and psychological stress and anxiety markers, including salivary sAA, SIgA, HR, and SAI. Notably, the DSC condition resulted in significantly higher 5 min post exercise SAI scores compared to EA, suggesting greater subjective anxiety in response to combined physical and mental stress. Secondary analyses revealed that females had lower sAA concentrations than males in the EA condition and lower SIgA concentrations 5 and 30 min post exercise across conditions. These findings suggest that while both males and females exhibit elevated physiological stress responses to acute stressors, females may have an attenuated stress biomarker response relative to males. Additionally, the addition of mental stress in the DSC increased subjective anxiety, regardless of sex.

These results are consistent with previous work reporting significant increases in HR, SAI, sAA, and SIgA in response to VR-ASDs; (McAllister, 2024; McAllister, 2022; Dillard, 2023; Martaindale, 2024). However, the current study is novel in its examination of added physical stress (i.e., exercise) prior to participation in the VR-ASD. To our knowledge, this is the first investigation to examine stress biomarker responses to a short duration physical stress (< 3 min) compared to a DSC incorporating an additional ~2 min of mental stress induced by the VR-ASD. While the addition of mental stress in the DSC did not significantly affect salivary stress biomarker concentrations compared to EA, it did significantly elevate anxiety which is consistent with previous findings (McAllister, 2019).

While past work has demonstrated that added mental stress from a DSC causes significant increases in stress biomarkers as compared to EA (Huang, 2010; Huang, 2010) it should be noted that this response likely depends on a variety of factors such as the duration of the stressor(s), the nature of the cognitive load, and the participants' individual training status (Webb, 2017; Webb, 2013; McAllister, 2019; Acevedo, 2006). In terms of the implications from the increased anxiety, longitudinal

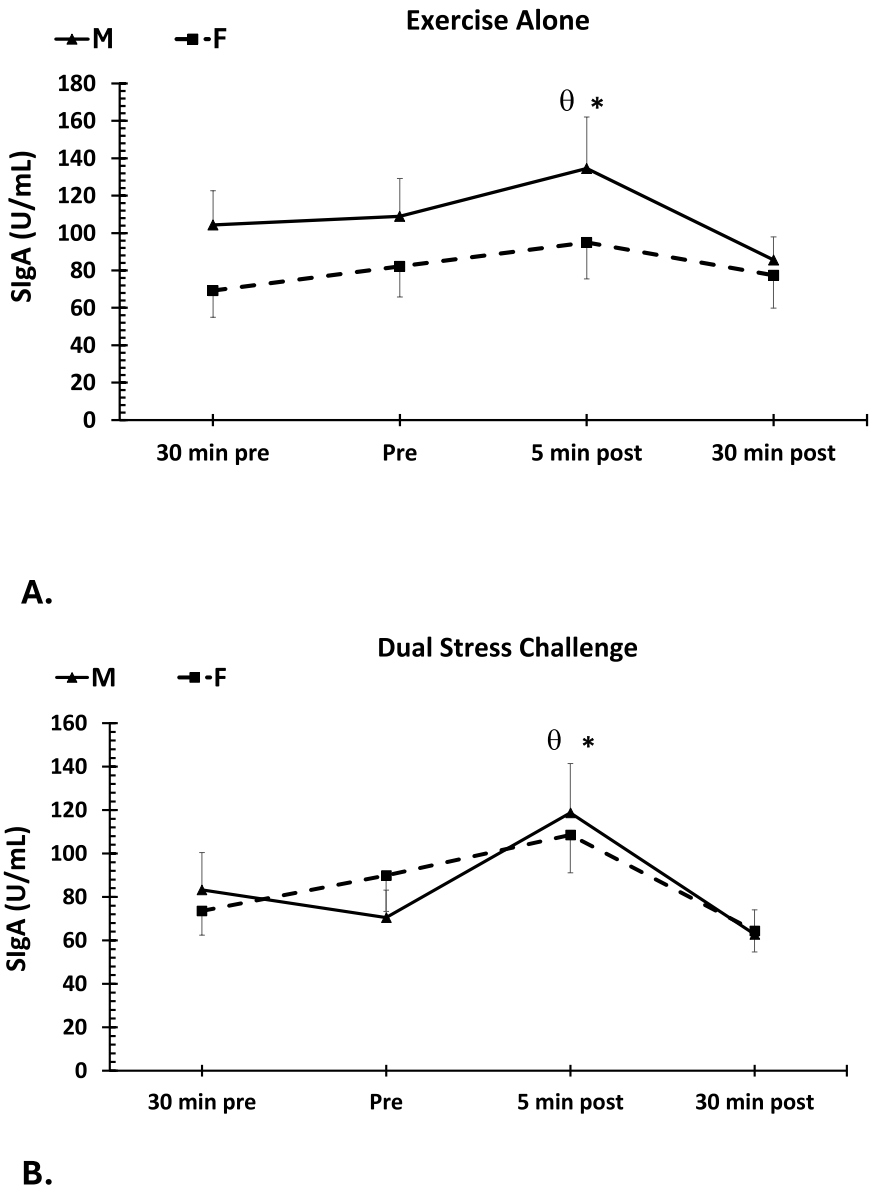


Fig. 5. Changes in salivary secretory immunoglobulin A concentrations in the exercise alone (A.) and dual stress challenge (B.) groups. SIgA = Secretory Immunoglobulin A. * indicates a significantly higher SIgA concentrations 5 min post stress compared to all other timepoints. θ indicates significantly ($p < 0.05$) lower SIgA concentrations in females compared to males at 5 min post as well as 30 min post exercise (as indicated by the significant ($p < 0.05$) timepoint x sex interaction). Note that for consistency and visual purposes, exercise alone and dual stress challenge data are presented in separate graphs for the readers. However the statistical analysis did not reveal a significant treatment x sex x time interaction. Data are shown as mean ± SE.

research is needed to evaluate whether repeated exposure to DSCs results in cumulative physiological or psychological strain. However, it is worth noting that acute stress induced increases in anxiety, even without concurrent biomarker changes, may enhance cognitive performance in some contexts (Degroote, 2020); however, further research is required to explore this relationship. It is worth noting that the SAI values in the DSC were 8 % higher post exercise compared to EA. While this was statistically significant, the absence of a significant difference in saliva biomarkers between the DSC and EA conditions in the present study suggests a potential dissociation between physiological stress and perceived anxiety markers, such that the increase in anxiety from the DSC did not result in a measurable impact on concentrations of sAA or SIgA compared to EA. Since the duration and intensity of the stressors can impact biomarker responses, it is possible that a longer duration of exposure to the DSC would results in different findings. Moreover, the present study included analysis of data from 5- and 30-min post exercise. Due to the transient nature of concentrations of these biomarkers in saliva, it is also possible that more frequent saliva sampling post exercise/stress may yield different results. However, these suggestions are only speculative and need additional data to confirm.

Sex based differences were also observed. Females demonstrated lower overall sAA concentrations and reduced SIgA responses following acute stress compared to males. These findings align with a growing body of literature documenting sex differences in stress biomarkers in both human and rodent trials (Verma et al., 2011; Critchlow, 1963; Bangasser and Valentino, 2014; Heck and Handa, 2019). In human trials, females typically show attenuated biomarker responses to acute stress compared to males (McAllister and Martaindale, 2021; Collins and Frankenhaeuser, 1978; Kudielka, 1998). These differences are potentially due to sex differences in the sensitivity of the hypothalamic pituitary adrenal axis (Bangasser and Valentino, 2014). Other contributing factors may include differences in age (Seeman, 2001) and cardiorespiratory fitness levels (Webb, 2017; Webb, 2013; McAllister, 2019; Acevedo, 2006). Since over half of the subjects were categorized as poor

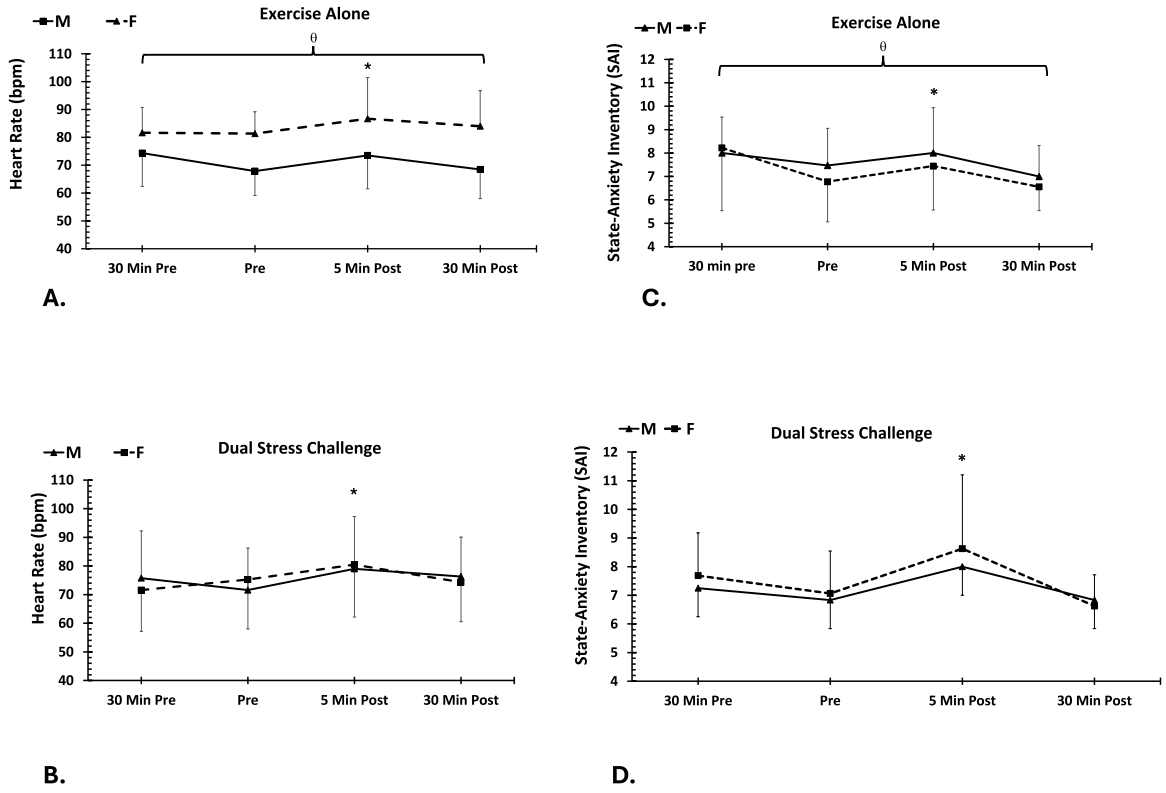


Fig. 6. Changes in heart rate between males and females in the exercise alone (EA) (A.) and dual stress challenge (DSC) (B.) groups. Also shown are changes in State Anxiety Inventory (SAI) in the EA (C.) and DSC groups (D.) between males and females. *indicates a significant ($p < 0.05$) increase 5 min post exercise. θ indicates a significant ($p < 0.05$) difference between males and females overall in the EA conditions for both HR and SAI. Data are shown as mean ± SE.

or very poor cardiorespiratory fitness (Pescatello, 2014), the lack of significant treatment x sex x timepoint interactions may be at least partially attributed to this confounding factor. While the menstrual cycle has been suggested to impact these biomarkers in females (Kirschbaum, 1999), recent findings from our team have shown that the menstrual cycle does not impact sAA and SIgA (Walker, 2025). Despite these differences, both sexes demonstrated significant increases in sAA, SIgA, HR, and SAI in response to both conditions. Additional studies are needed to examine potential long term or performance related effects of the reduced SIgA responses to stress between sexes.

Several limitations should be considered when interpreting the present findings. First, time of day has been shown to impact sAA and SIgA concentrations (Walker, 2025). To minimize this effect, all testing was conducted between 11:00 and 18:00. Additionally, subjects varied in terms of body fat percentage and cardiorespiratory fitness category (Table 1). In fact, many subjects were classified as having below average or poor cardiorespiratory fitness levels according to ACSM criteria (Pescatello, 2014), which may have influenced stress response. Moreover, the Ruffier test used to estimate VO_2max relied on HR data collected by an iPad and a wrist HR monitor (Polar verity sense; Polar Electro Ltd, Kempele, Finland) rather than the traditional 15 s pulse count method originally recommended for the Ruffier test VO_2max calculation (Alahmari, 2020; Guo, 2018; Trovato, 2023). Regardless, body fat percentages and cardiorespiratory fitness can impact physiological responses to stressors (Webb, 2017; Webb, 2013; McAllister, 2019; Acevedo, 2006). Future studies should seek to recruit a more homogenous sample in terms of fitness level and body composition. Additionally, the male and female groups were unbalanced (males n = 29; females n = 25), which limits the statistical power of the sex comparison. Although the observed sex differences are consistent with previous research (McAllister and Martaindale, 2021; Collins and Frankenhaeuser, 1978; Kudielka, 1998), this imbalance should be considered a limitation.

4.1. Conclusions

This study demonstrated that added mental stress in the DSC did not significantly increase concentrations in sAA or SIgA compared to EA; however, subjective anxiety was significantly impacted. To our knowledge, this was the first study to demonstrate that short duration physical stress (< 3 min) causes significant increases in stress biomarkers. Importantly, females exhibited attenuated biomarker responses compared to males, supporting previous findings of sex differences in acute stress reactivity. These findings underscore the need for future investigations to determine the potential impact of sex differences on longitudinal health outcomes and factors related to physical and cognitive performance. From an applicational standpoint, since the VR-ASD used in this study induced stress by involving a violent and realistic scenario, careful attention should be given to participant debriefing and/or potential psychological support protocols if considering using similar scenarios in training for law enforcement or military personnel.

Author contribution

Funding acquisition: MHM, MJM; conceptualization and research design: MJM, MHM, & CCD; performed data collection: NS and SU; analyzed data: MJM; interpreted experiment results: MJM; prepared figures: MJM, NS and SU; all authors drafted, revised and approved the final manuscript.

CRediT authorship contribution statement

Stephanie Uriegas: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Courtney C. Dillard:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **McAllister Matthew:** Writing – review & editing, Writing – original draft,

Visualization, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nate Sutton:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Data curation. **M. Hunter Martaindale:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Institution and ethics approval and informed consent

All experimental produces subsequently described were approved by the Institutional Review Board of Texas State University in line with the Declaration of Helsinki.

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Declaration of Competing Interest

None.

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References

Acevedo, E.O., et al., 2006. Cardiorespiratory responses of hi fit and low fit subjects to mental challenge during exercise. Int. J. Sports Med. 27 (12), 1013–1022.
Agorastos, A., Chrousos, G.P., 2022. The neuroendocrinology of stress: the stress-related continuum of chronic disease development. Mol. Psychiatry 27 (1), 502–513.
Alahmari, K.A., et al., 2020. Cardiorespiratory fitness as a correlate of cardiovascular, anthropometric, and physical risk factors: using the ruffier test as a template. Can. Respir. J. 2020, 3407345.
Bangasser, D.A., Valentino, R.J., 2014. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Front. Neuroendocr. 35 (3), 303–319.
Chu, B., et al., 2024. In Statpearls [internet]. Physiology, Stress Reaction. StatPearls Publishing.
Collins, A., Frankenhaeuser, M., 1978. Stress responses in Male and female engineering students. J. Hum. Stress 4 (2), 43–48.
Critchlow, V., et al., 1963. Sex difference in resting pituitary-adrenal function in the rat. Am. J. Physiol. 205 (5), 807–815.
Degroote, C., et al., 2020. Acute stress improves concentration performance. Exp. Psychol. 67 (2), 88–98.
Dillard, C.C., et al., 2023. Slow breathing reduces biomarkers of stress in response to a virtual reality active shooter training drill. Healthcare 11 (16).
Gamito, P., et al., 2024. Stress inoculation in police officers using virtual reality: a controlled study. Cyber Behav. Soc. Netw. 27 (4), 253–260.
Groer, M., et al., 2010. Salivary measures of stress and immunity in police officers engaged in simulated critical incident scenarios. J. Occup. Environ. Med. 52 (6), 595–602.
Guo, Y., et al., 2018. A 3-minute test of cardiorespiratory fitness for use in primary care clinics. PLoS One 13 (7), e0201598.
Heck, A.L., Handa, R.J., 2019. Sex differences in the hypothalamic-pituitary-adrenal axis' response to stress: an important role for gonadal hormones. Neuropsychopharmacology 44 (1), 45–58.
Hendricks, B., et al., 2023. Impact of stress and stress mindset on prevalence of cardiovascular disease risk factors among first responders. BMC Public Health 23 (1), 1929.
Hill, E.E., et al., 2008. Exercise and circulating cortisol levels: the intensity threshold effect. J. Endocrinol. Invest 31 (7), 587–591.
Huang, C.J., et al., 2010. Stress hormones and immunological responses to a dual challenge in professional firefighters. Int. J. Psychophysiol. 75 (3), 312–318.
Huang, C.J., et al., 2010. Psychological stress during exercise: immunoendocrine and oxidative responses. Exp. Biol. Med. 235 (12), 1498–1504.
Huang, C.J., et al., 2010. Psychological stress during exercise: lymphocyte subset redistribution in firefighters. Physiol. Behav. 101 (3), 320–326.
Huang, C.J., et al., 2013. Cardiovascular reactivity, stress, and physical activity. Front. Physiol. 4, 314.
Kirschbaum, C., et al., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom. Med. 61 (2), 154–162.
Kleygrewe, L., Hutter, R.I.V., Oudejans, R.R.D., 2023. No pain, no gain? The effects of adding a pain stimulus in virtual training for police officers. Ergonomics 66 (10), 1608–1621.
Koibuchi, E., Suzuki, Y., 2014. Exercise upregulates salivary amylase in humans (Review). Exp. Ther. Med 7 (4), 773–777.
Kudielka, B.M., et al., 1998. Sex differences in endocrine and psychological responses to psychosocial stress in healthy elderly subjects and the impact of a 2-week dehydroepiandrosterone treatment. J. Clin. Endocrinol. Metab. 83 (5), 1756–1761.
Liu, Y.Z., Wang, Y.X., Jiang, C.L., 2017. Inflammation: the common pathway of stress-related diseases. Front. Hum. Neurosci. 11, 316.
Maglione, M.A., et al., 2022. Stress control for military, law enforcement, and first responders: a systematic review. Rand Health Q 9 (3), 20.
Martaindale, M.H., et al., 2024. Can a virtual reality training scenario elicit similar stress response as a realistic Scenario-Based training scenario? Police Q. 27 (1), 109–129.
Marteau, T.M., Bekker, H., 1992. The development of a six-item short-form of the state scale of the spielberger State—Trait anxiety inventory (STAI). Br. J. Clin. Psychol. 31 (3), 301–306.
McAllister, M.J., et al., 2016. Exogenous carbohydrate reduces cortisol response from combined mental and physical stress. Int. J. Sports Med. 37 (14), 1159–1165.
McAllister, M.J., et al., 2019. Effects of psychological stress during exercise on markers of oxidative stress in young healthy, trained men. Physiol. Behav. 198, 90–95.
McAllister, M.J., et al., 2019. Effects of psychological stress during exercise on markers of oxidative stress in young healthy, trained men. Physiol. Behav. 198, 90–95.
McAllister, M.J., et al., 2020. Effects of curcumin on the oxidative stress response to a dual stress challenge in trained men. J. Diet. Suppl. 17 (3), 261–272.
McAllister, M.J., et al., 2022. Virtual reality based active shooter training drill increases salivary and subjective markers of stress. Yale J. Biol. Med. 95 (1), 105–113.
McAllister, M.J., et al., 2024. Stress response to virtual reality based active shooter training: impact of caffeine consumption. Psychoneuroendocrinology 161, 106923.
McAllister, M.J., et al., 2024. Impact of L-theanine and L-tyrosine on markers of stress and cognitive performance in response to a virtual reality based active shooter training drill. Stress 27 (1), 2375588.
McAllister, M.J., Martaindale, M.H., 2021. Women demonstrate lower markers of stress and oxidative stress during active shooter training drill. Compr. Psychoneuroendocrinol. 6, 100046.
McAllister, M.J., Gonzalez, A.E., Waldman, H.S., 2021. Time restricted feeding reduces inflammation and cortisol response to a firegrounds test in professional firefighters. J. Occup. Environ. Med. 63 (5), 441–447.
Nash, D., et al., 2023. IL-6 signaling in acute exercise and chronic training: potential consequences for health and athletic performance. Scand. J. Med. Sci. Sports 33 (1), 4–19.
Papazoglou, K., 2023. Stress, prevention, and resilience among first responders. Int. J. Environ. Res Public Health 20 (24).
Pavlíčková, K., et al., 2024. Acute stress promotes effort mobilization for safety-related goals. Commun. Psychol. 2 (1), 50.
Pescatello, L.S., et al., 2014. ACSM's Guidelines for Exercise Testing and Prescription, 9th ed.. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia.
Seeman, T.E., et al., 2001. Gender differences in age-related changes in HPA axis reactivity. Psychoneuroendocrinology 26 (3), 225–240.
Soteriades, E.S., et al., 2022. Exercise and occupational stress among firefighters. Int. J. Environ. Res Public Health 19 (9).
Tluczek, A., Henriques, J.B., Brown, R.L., 2009. Support for the reliability and validity of a six-item state anxiety scale derived from the State-Trait anxiety inventory. J. Nurs. Meas. 17 (1), 19–28.
Trovato, B., et al., 2023. Modified Isoinertial-Based ruffier test in healthy individuals: a feasibility study. J. Funct. Morphol. Kinesiol 8 (2).
Verma, R., Balhara, Y.P., Gupta, C.S., 2011. Gender differences in stress response: role of developmental and biological determinants. Ind. Psychiatry J. 20 (1), 4–10.
Waldman, H.S., et al., 2020. Exogenous ketone salts do not improve cognitive performance during a Dual-Stress challenge. Int. J. Sport Nutr. Exerc Metab. 30 (2), 120–127.
Walker, J.K., et al., 2025. Impact of the menstrual cycle phases and time of day on markers of stress: salivary α-amylase and secretory immunoglobulin a. Stress 28 (1), 2449098.
Webb, H.E., et al., 2011. Stress hormones and vascular function in firefighters during concurrent challenges. Biol. Psychol. 87 (1), 152–160.
Webb, H.E., et al., 2013. Aerobic fitness affects cortisol responses to concurrent challenges. Med. Sci. Sports Exerc. 45 (2), 379–386.
Webb, H.E., et al., 2017. Aerobic fitness impacts sympathoadrenal axis responses to concurrent challenges. Eur. J. Appl. Physiol. 117 (2), 301–313.