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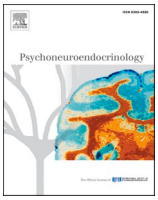

Stress response to virtual reality based active shooter training: Impact of caffeine consumption

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ABSTRACT

Participation in a virtual reality based active shooter training drill (VR-ASD) has been shown to increase biomarkers of stress; however, the impact of caffeine consumption on this response has not been studied. Caffeine ingestion has been shown to have favorable effects on physical and cognitive performance among athletic and tactical occupations alike. This study examined the impact of caffeine ingestion on subjective and physiological markers of stress in response to a mental stress task (MST) which involved participation in a VR-ASD and cognitive challenge consisting of mental arithmetic and a Stroop challenge. Fifty-three subjects were randomly assigned either caffeine (n = 26) or placebo (n = 27) prior to being exposed to the MST. Saliva samples, heart rate (HR), and state-anxiety inventory (SAI) scales, were collected before and after exposure to the MST. Saliva was analyzed for α-amylase (sAA), secretory IgA (SIgA), and cortisol (sCORT) concentrations. The MST resulted in significant increases in sAA, SIgA, HR, and SAI. Immediately post MST, sAA concentrations were significantly higher following the caffeine treatment compared to placebo. These data demonstrate that caffeine consumption results in significantly greater sAA concentrations post MST. This study was pre-registered as a clinical trial ("Impact of supplements on stress markers": NCT05592561).

1. Introduction

Law enforcement and military personnel are often exposed to a variety of high stress and potentially life-threatening scenarios. The high stress nature of the occupation can negatively impact physical and occupational performance, sleep quality, and various aspects of physical and psychological health (Huang et al., 2013). Acute exposure to high stress scenarios activates the hypothalamic pituitary adrenal (HPA) and sympathoadrenal (SA) axes resulting in increased blood concentrations of catecholamines (i.e., epinephrine and norepinephrine) and cortisol, as well as markers of oxidative stress and inflammation (Huang et al., 2013). In terms of law enforcement-specific stressors, exposure to simulated critical incident scenarios and virtual reality (VR) based active shooter drills have been shown to decrease cardiovascular vagal activity and increase markers of SA and HPA activity, such as salivary α-amylase (sAA) (Giessing et al., 2019; Groer et al., 2010; McAllister et al., 2022; McAllister et al., 2020) and salivary cortisol (sCORT) (Meyerhoff et al., 2004). Stress exposure in law enforcement personnel can also adversely impact occupational performance and decision making (Meyerhoff et al., 2004; Shane, 2010). It is important to note, while exposure to a *moderate* and controlled amount of stress is beneficial for performance (Henderson et al., 2012), chronic stress exposure results in strain on the HPA and SA axes, leading to an increase in oxidative stress, inflammation, and increased risk for developing cardiometabolic and neurological diseases (Huang et al., 2013).

Caffeine is arguably one of the most widely consumed and popular ergogenic aids worldwide. Ingestion of caffeine can increase central nervous system activity and increase adrenalin-induced enhancements in free fatty acid oxidation, which can result in improvements in endurance exercise performance (Costill et al., 1978). Caffeine has also been shown to reduce fatigue, and enhance cognitive and executive function (Gonzalez et al., 2022; Guest et al., 2022; Kerksick et al., 2022), as well as favorably impact mood, mental focus, and alertness (Black et al., 2015; Meeusen et al., 2013). Among tactical populations, ingestion of 150 – 300 mg may improve vigilance during sleep deprivation, as well as enhance marksmanship and reaction time (Cintineo et al., 2022; Gillingham et al., 2003; Gillingham et al., 2004; Johnson and Merullo, 1999; Kamimori et al., 2015; Kamimori et al., 2000; McLellan et al.,

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2007; Tharion et al., 2003). Caffeine consumption practices among law enforcement personnel may mimic that of the general population with an average daily consumption of approximately 180–200 mg (Fulgoni et al., 2015; Ogeil et al., 2018). Given the potentially favorable effects of caffeine ingestion on wakefulness, arousal, cognitive performance, and reaction time, (Doty et al., 2017; Kamimori et al., 2015; McLellan et al., 2016), it is likely law enforcement personnel can benefit from moderate caffeine doses when responding to stressful conditions, such as during active shooter scenarios.

With respect to human trials, findings from Klein et al. (2010) demonstrated that sAA activity increases in a dose dependent manner with respect to caffeine intake (i.e., 0, 200 mg, 400 mg) when exposed to a mental stressor. However, to our knowledge, no study has observed the physiological effects caffeine might have on salivary stress markers in response to a VR-ASD. Based on previous work showing that caffeine ingestion increases various aspects of cognitive performance and physiological arousal (McLellan et al., 2016), we hypothesized that ingestion of caffeine would increase salivary stress markers and attenuate a decline in mental performance in response to a VR-ASD. Therefore, the purpose of this study was to examine the impact of caffeine ingestion on subjective and physiological markers of stress in response to a mental stress task (MST) involving a VR-ASD and cognitive challenge.

2. Materials and methods

2.1. Subjects and experimental design

Using a randomized, single-blinded, between subjects, parallel experimental design, subjects were randomly assigned to ingest either caffeine (n = 26) or a color, odor, and flavor matched placebo (n = 27) prior to being exposed to a MST, which involved a VR-ASD and cognitive challenge protocol. Each subject provided electronic informed consent prior completing a health history questionnaire. Subjects were current university students and were recruited from flyers, emails, and word of mouth on university campus. Subjects were required to be free from any known cardiovascular or metabolic diseases, as well as any major stressors within the last 30 days, such as birth of a child, abortion, or divorce. Additionally, subjects were required to not have a history of motion sickness or vertigo, nor have been diagnosed with a brain injury or epilepsy. Subjects were asked to arrive at least 4-hours fasted, as well as to avoid nicotine exposure, intense exercise, and alcohol within a 24-hour window of testing. All testing occurred between early and late afternoon (~12:00–17:30). All procedures were reviewed and approved by the University Institutional Review Board. This study has been pre-registered as a clinical trial (NCT05592561).

2.2. Experimental procedures

Upon arrival to the testing site, body mass was measured using a digital scale (Rice Lake Weighing Systems, Rice Lake, WI, USA), and height was measured using a portable stadiometer (SECA, CA, USA). Subjects were then instructed to rinse their mouth with bottled water and rest in a quiet room for ~10 min. During this time, subjects were equipped with a heart rate (HR) monitor (Polar verity sense; Polar Electro Ltd, Kempele, Finland). Immediately after the 10-minute rest period, an initial saliva sample was collected in addition to HR and state-anxiety inventory (SAI) measures being collected (45 min prior to the MST). Five minutes after this (40 min pre-MST), subjects consumed their assigned treatment: either the placebo (crystal light + 8 oz of water) or caffeine supplement (200 mg caffeine + crystal light + 8 oz of water). Throughout the experimental session saliva samples, HR, and SAI measures were collected a total of four times: 1) 45 min prior to the start of the MST, 2) immediately before the MST, 3) immediately after the MST, and 4) 30 min after the MST. Primary dependent variables included salivary stress markers: sAA, secretory IgA (SIgA), and sCORT. These salivary markers are commonly use indicators of physiological stress (Boyanov et al., 2021; Soo-Quee Koh and Choon-Huat Koh, 2007; Takai et al., 2004; Tsujita and Morimoto, 1999). Additional dependent variables included HR and SAI. An overview of experimental procedures is provided in Fig. 1.

2.2.1. Mental stress task: cognitive challenge & VR-ASD

The mental stress protocol used for this experiment consisted of a cognitive challenge and a previously studied VR-ASD (McAllister et al., 2022). The cognitive challenge (~4 min) was performed both before and after participating in a ~2-minute VR-ASD for a total exposure of ~10 min. The VR-ASD consisted of a ~1-minute familiarization period immediately followed by a ~1-minute active shooter scenario. The cognitive challenge included a modified version of the Stroop challenge (~2 min) and mental arithmetic calculations (2 min) presented to the subject via e-Prime 2 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Immediately upon completion of the cognitive challenge, the subjects completed the VR-ASD scenario (~2 min) followed by the same cognitive challenge with different questions (another 4 min). The total duration of the MST was ~10 min.

2.2.2. Stroop challenge

For the Stroop challenge, the subjects were presented with a 0.5 second display of a word on the computer screen (i.e., yellow, green, red, or blue). However, the word was written in conflicting-color font presenting a mental conflict. Subjects were required to identify the text font color as quickly as possible using a color-coded keypad. Note, 0.5 ss was allowed for the response. Feedback included total attempts, total correct and incorrect responses, missed responses, and response time.

2.2.3. Arithmetic calculations

The arithmetic calculations consisted of addition and subtraction with single, double-, and triple-digit numbers (e.g., 736 minus 58). The subject was given 10 s to respond to each mathematical question by entering the answer into the numeric keypad. Participants that responded with an incorrect answer triggered an automated buzzer sound. The subjects were presented with 2-minutes of the Stroop Challenge followed by 2-minutes of mental arithmetic. This occurred both before and after the VR-ASD.

2.3. Virtual reality active shooter drill

The VR-ASD was the same scenario as previously described (McAllister et al., 2022). Briefly, the subject advanced down a ~3-meter-long hallway where two wounded victims were encountered. A white female victim with traumatic injuries was laying on the ground while an African American female victim "ran" out of a classroom with a gunshot wound to the left arm and leg. Upon arriving to the classroom, the subject observed a white female on the ground with a traumatic head injury as well as the shooter (white male) firing a handgun at an African American male. The subject was required to fire his/her weapon to prevent the shooter from turning toward the subject and eliciting a response. Once the shooter fell after being shot by the subject, the VR-ASD scenario ended. The VR-ASD was presented using HTC VIVE Pro VR equipment (HTC Corp, New Taipei, Taiwan). The VR laboratory environment was larger than the virtual environment (~35 ft x 20 ft); therefore, subjects were able to physical maneuver throughout the ASD without disruption.

2.4. State-Anxiety Inventory Assessment

In terms of assessing subjective stress, a 6-item SAI was used at four timepoints, which were concurrent with each saliva sample and HR measurement (previously described in the *Experimental Procedures* section). The SAI included six short statements such as "I feel calm" and "I am tense" and was scored on a scale of 1–4. Subsequently, composite scores for each scale were used in analysis. The SAI has been established

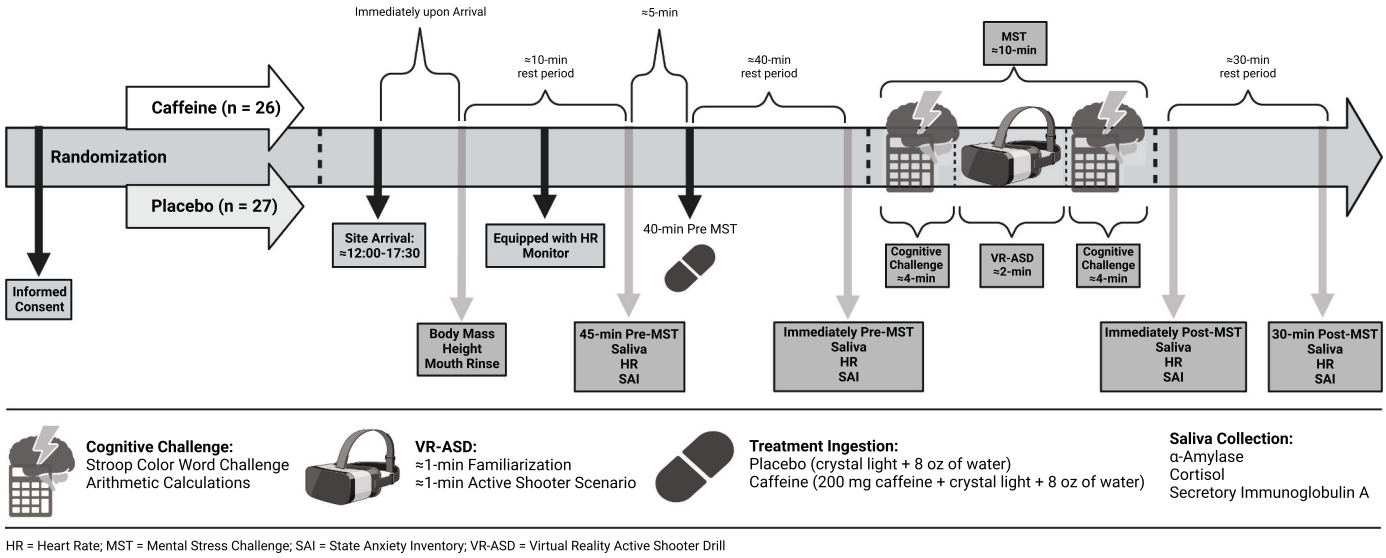


Fig. 1. Overview of experimental procedures.

as a reliable and valid scale to assess subjective stress (Marteau and Bekker, 1992; Tluczek et al., 2009).

2.5. Supplement ingestion

Caffeine supplement capsules containing 200 mg caffeine powder (Hard Eight Nutrition, LLC, dba, Bulk Supplements, Henderson, NV) were used for the present study. The capsules were broken into halves and the caffeine powder inside was mixed with 8 oz of water and 5/8 tsp of calorie free lemonade flavored crystal light (Chicago, IL). For the placebo treatment, ½ tsp of the same flavor of crystal light was mixed with 8 oz of water. The recipes for the treatments were pilot tested in advance to ensure flavor matching prior to implementation. The subjects were required to ingest within a two-minute period.

2.6. Saliva sample collection and analysis

Saliva samples were collected via a passive drool collection method (Salimetrics, PA, USA) at the following timepoints: 1) 45-minutes pre, 2) immediately pre, 3) immediately post, and 4) 30-minutes post mental challenge. These timepoints were selected based on previously published work (McAllister et al., 2022). The subjects were instructed to tilt their head forward while allowing saliva to "pool" in the mouth, and then, passively drool into the mouthpiece adaptor attached to the collection tube. Subjects were asked to provide ~1.0 mL of saliva per each timepoint. The saliva samples were immediately stored at −80 °C until subsequent analysis.

The saliva samples were thawed and centrifuged at 1500 x *g* for 15-minutes at 4 °C, and then subsequently analyzed in duplicate for sAA activity as well as concentrations of sAA, SIgA, and sCORT using commercially available kits (Salimetrics, PA, USA). Saliva samples were shipped overnight, on dry ice to a commercial laboratory for analysis for SIgA and sAA (Salimetrics, PA, USA). Samples were shipped overnight back to the primary research laboratory for analysis of sCORT. Saliva was analyzed in house for sCORT concentrations. Absorbance was determined via a colorimetric plate reader (BioTek, Winooski, VT, USA). An automated washer was used for assays that required washing (BioTek, Winooski, VT, USA). The intra-assay and inter-assay % coefficient of variation for these assays was between 4.7% and 8.7%.

2.7. Statistical analysis

All statistical data procedures conducted using SAS v 9.4 (Cary, NC, USA). A 2 × 4 (treatment x timepoint) factorial repeated measures ANOVA (with repeated measures on timepoint) was used to compare the changes across time for the caffeine and placebo treatments. In the instance of a significant main effect or interaction ($p < 0.05$), Fisher's Least Significant Difference test was conducted to compare means. Effect size was calculated and reported as partial eta squared (ηp^2). In terms of interpretation, it should be noted: $\eta p^2 = 0.01$ indicates small effect; $\eta p^2 = 0.06$ indicates medium effect; $\eta p^2 = 0.14$ indicates large effect. Since previous work has shown that biological sex can impact stress related biomarkers including salivary stress markers (McAllister and Martaindale, 2021), a secondary exploratory analysis was also conducted to compare male/female responses over time and between treatments via 3 way (sex *x* treatment *x* time) factorial ANOVA. A power analysis from our previous work (McAllister et al., 2022) indicated that a sample size of 27 was sufficient to achieve a statistical power of 0.88 with an alpha level at 0.05.

3. Results

A total of 53 subjects (n = 53; male = 24; female = 29; height: 170 ± 9.6 cm; weight: 164.6 ± 31.9; age = 21 ± 3 yrs. Fig. 2) completed the experimental testing. The subjects self-reported activity levels (exercise at least three times per week = 46; No = 7), if they used any nicotine products (No = 46; Yes = 7), and if they consumed caffeine (coffee, pre-workout, soda) on a regular basis (Yes = 39; No = 14). Subject descriptive characteristics are as follows: caffeine treatment: height = 167.7 ± 9.2 cm; mass = 72.0 ± 12.7 kg; BMI = 25.7 ± 4.8 kg/m^2; placebo treatment: height = 172.0 cm; mass = 77.1 ± 15.8 kg; BMI = 25.9 ± 4.6 kg/m^2. All data are shown as mean ± standard deviation.

3.1. Saliva data

A significant treatment *x* time interaction was noted for mean sAA concentrations ($F = 2.96$, $p = 0.03$, $\eta_p^2 = 0.05$). Post hoc analysis indicated a significant increase in sAA concentrations from immediately pre to immediately post MST in the caffeine treatment ($p < 0.001$) as well as the placebo treatment ($p = 0.02$). There was a significant decrease in sAA activity from immediately post to 30 min post MST in the caffeine treatment ($p < 0.001$). The decrease in sAA activity from immediately post to 30 min post MST in the placebo treatment approached significance ($p = 0.05$). Moreover, sAA concentrations were significantly higher immediately post MST in the caffeine treatment compared to placebo ($p < 0.001$). Mean sAA concentrations are shown in Fig. 3.

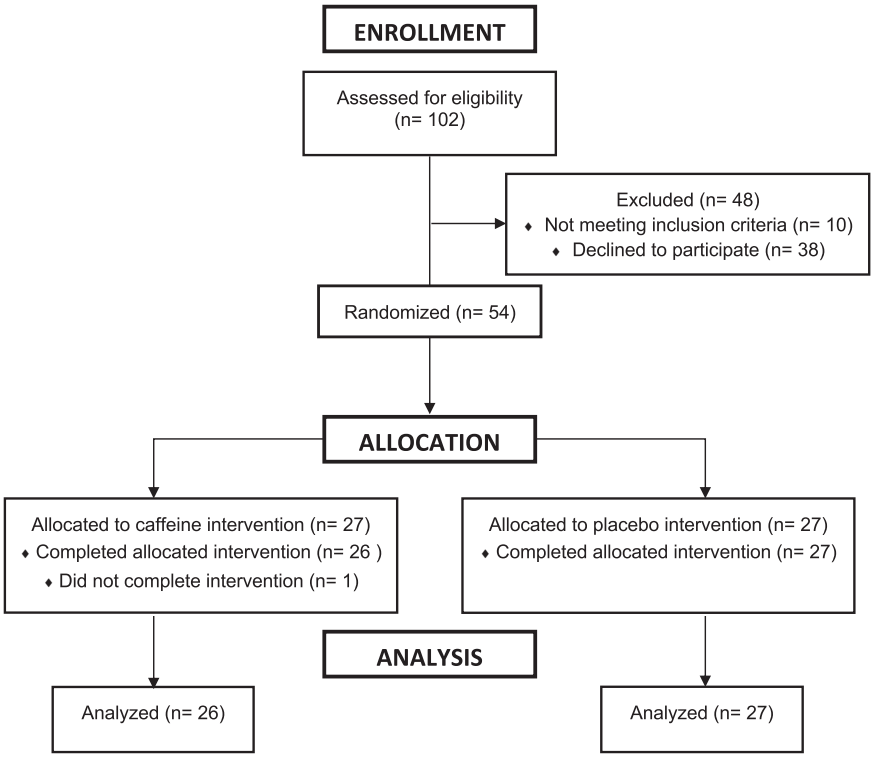


Fig. 2. Study design and consort diagram.

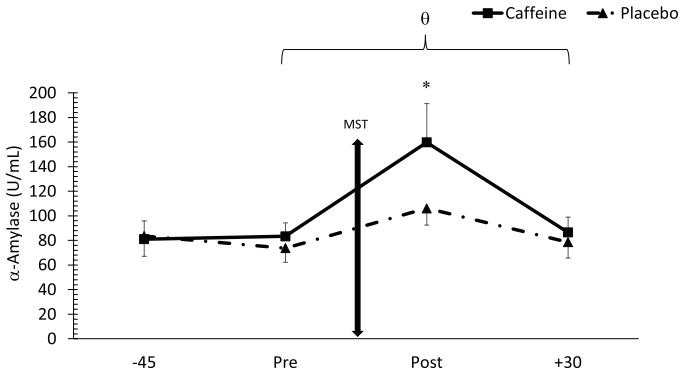


Fig. 3. Mean salivary α-amylase (sAA) concentrations across time and between treatments. MST = stress task. θ indicates a significant treatment *x* time interaction ($p < 0.05$). *Indicates significantly greater sAA concentrations immediately post MST compared to the caffeine treatment. Both groups experienced a significant ($p < 0.05$) increase in sAA activity from immediately pre to immediately post MST. − 45 = 45 min pre-MST, pre = immediately pre-MST, post = immediately post MST, + 30 = 30 min post MST.

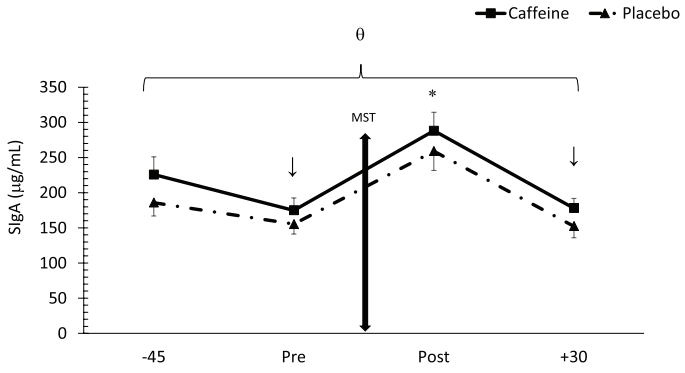


Fig. 4. Mean SIgA concentrations across time and between treatments. MST = mental stress task. θ indicates a significant main effect for treatment group ($p < 0.05$) with higher SIgA concentrations in the caffeine group. ↓ indicates a significant decrease in SIgA concentrations compared to the previous timepoint ($p < 0.05$). *Indicates a significant increase from immediately pre to immediately post MST. −45 = 45 min pre-MST, pre = immediately pre-MST, post = immediately post MST, + 30 = 30 min post MST.

Regarding mean SIgA concentrations, there was no significant treatment *x* time interaction ($p = 0.78$). However, there was a significant main effect for time ($F = 32.5, p < 0.001, \eta_p^2 = 0.41$) and treatment ($F = 8.66$, $p = 0.003$, $\eta_p^2 = 0.05$). Post hoc analysis demonstrated a significant decrease in SIgA concentrations from 40 min pre to immediately pre-MST ($p = 0.001$), followed by a significant increase in SIgA from immediately pre to immediately post MST ($p < 0.0001$). A significant decrease in SIgA concentrations was noted from immediately post MST to 30 min post MST ($p < 0.0001$). Finally, the subjects in the caffeine treatment demonstrated significantly higher SIgA concentrations overall compared to the placebo treatment ($p = 0.003$). Mean SIgA concentrations are shown in Fig. 4.

With respect to sCORT concentrations, there was no significant treatment *x* time interaction ($p = 0.25$). However, a main effect for both treatment ($F = 51.1, p < 0.001$) and time ($F = 2.7, p = 0.04$) was found. Post hoc demonstrated significantly higher sCORT concentrations in the caffeine treatment compared to placebo treatment ($p < 0.001$). In addition, there was a significant decrease in sCORT from immediately post MST to 30 min post MST ($p = 0.005$). Results from the time effect post hoc analysis from the time effect also demonstrated an 18% increase in sCORT concentrations from 45 min pre-MST to immediately post MST (0.142–0.167 µg/dL, respectively); however, this change only approached significance ($p = 0.07$). Mean sCORT concentrations for caffeine and placebo treatments are shown in Fig. 5.

3.2. Heart Rate

No treatment *x* time interaction was noted for mean HR ($p > 0.05$).

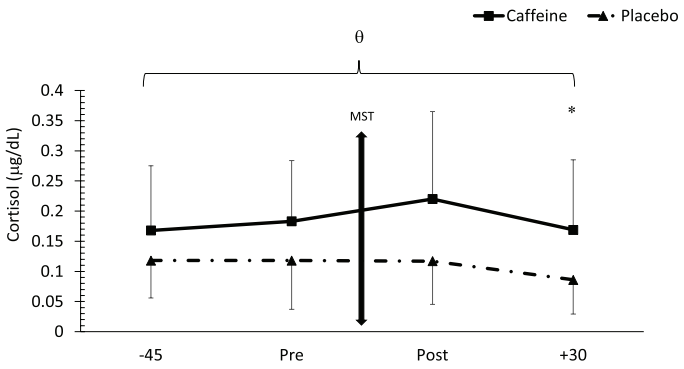
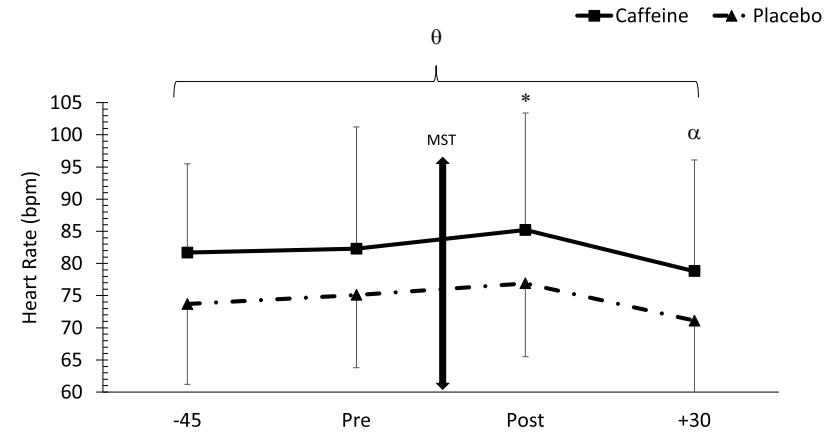


Fig. 5. Mean cortisol (sCORT) concentrations across time and between treatments. MST = mental stress task. θ indicates a significant main effect for treatment group ($p < 0.05$) with higher sCORT concentrations in the caffeine treatment compared to placebo. *Indicates a significant decrease from immediately post to 30 min post MST. − 45 = 45 min pre-MST, pre = immediately pre-MST, post = immediately post MST, + 30 = 30 min post MST.

However, there was a significant main effect for treatment (F = 45.51, $p < 0.0001$, $\eta_p^2 = 0.23$) and time (F = 5.58, $p = 0.001$, $\eta_p^2 = 0.09$). Post hoc analysis demonstrated significantly higher HR immediately post MST compared to 40 min pre-MST ($p = 0.03$) and 30 min post MST ($p < 0.0001$). Mean HR was higher in the caffeine treatment compared to the placebo treatment ($p < 0.001$). Mean HR data are shown in Fig. 6a.

3.3. State anxiety inventory

In terms of subjective anxiety, no treatment x time interaction was found ($p > 0.05$). However, there was a significant main effect for treatment (F = 28.93, $p < 0.0001$, $\eta_p^2 = 0.16$), and time (F = 13.81, $p < 0.0001$, $\eta_p^2 = 0.22$). Post hoc analysis revealed significantly higher SAI immediately post MST compared to all other timepoints ($p < 0.0001$). In addition, significantly higher SAI was noted overall among the subjects in the caffeine treatment compared to placebo ($p < 0.0001$). Mean SAI data are shown in Fig. 6b.



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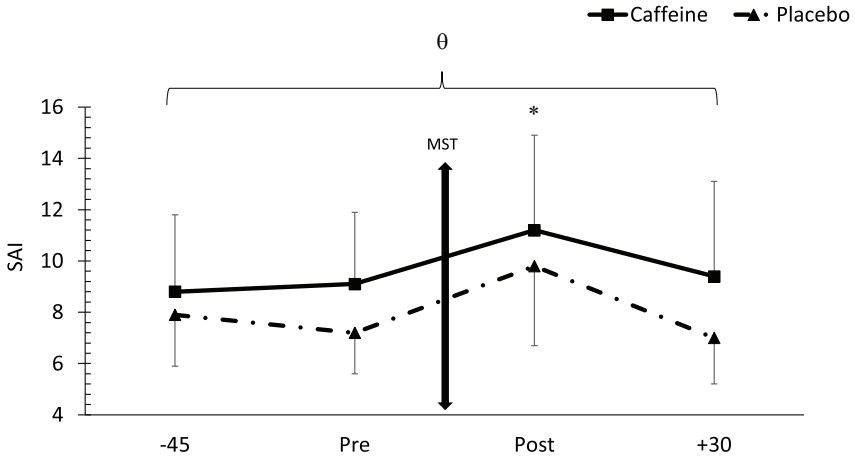

B.

Fig. 6. a. Mean heart rate responses across time and between treatments. MST = mental stress task. θ = significantly higher ($p < 0.05$) heart rate in the caffeine treatment group compared to placebo group. *Indicates a significant increase ($p < 0.05$) from 40 min pre to immediately post MST in both groups. α indicates a significant decrease from immediately post MST to 30 min post MST. − 45 = 45 min pre-MST, pre = immediately pre-MST, post = immediately post MST, + 30 = 30 min post MST. Fig. 6**b**. Mean state anxiety inventory (SAI) values across time and between treatments. MST = mental stress task. θ = significantly higher ($p < 0.05$) SAI in the caffeine treatment group compared to placebo group. *Indicates significantly higher SAI values immediately post MST compared to all other timepoints ($p < 0.05$). − 45 = 45 min pre-MST, pre = immediately pre-MST, post = immediately post MST, + 30 = 30 min post MST.

3.4. Male/female comparison

There was no significant treatment *x* time *x* sex, treatment *x* sex, or time *x* sex interaction for HR, SAI, SIgA, or sAA concentrations ($p > 0.05$). However, the treatment *x* sex interaction for sAA approached significance ($p = 0.06$). There was a main effect for sex regarding HR ($F = 58.6$, $p < 0.0001$) with significantly higher HR overall in women compared to men (81bpm vs. 71bpm, $p < 0.0001$). In addition, a significant treatment *x* sex interaction was found for sCORT ($F = 13.21, p < 0.001$). Post hoc analysis revealed no difference between men and women with respect to sCORT concentrations in the caffeine treatment. However, in the placebo treatment, women demonstrated significantly higher sCORT concentrations compared to men (0.15 vs. 0.08 μg/dL, respectively; $p < 0.001$). In addition, men in the caffeine condition, demonstrated significantly higher sCORT concentrations compared to men in the placebo treatment (0.19 vs. 0.08 μg/dL, respectively; $p < 0.001$). However, it should be acknowledged that the sample size for these subgroups (male/female within each experimental treatment) varied significantly and thus, the present analysis was completed as an exploratory measure. Note, the caffeine treatment included 8 men and 18 women; the placebo treatment involved 16 men and 11 women.

3.5. Mental challenge data

No treatment *x* time interaction, or main effect for treatment, or time was noted for mental arithmetic: number of answers incorrect, missed responses, or response time ($p > 0.05$). With respect to Stroop responses, no treatment *x* time interaction, or main effect for time was noted for number of incorrect and missed responses. The main effect for time for Stoop response time approached significance ($p = 0.05$). There was a significant main effect for treatment for incorrect responses ($F = 5.32$, $p = 0.02$ $\eta_p^2 = 0.09$). Post hoc analysis demonstrated significantly higher number of incorrect responses in the placebo treatment. However, it should be noted that one outlier was detected (> 3 SD from mean) for each of the following: mental arithmetic incorrect, mental arithmetic response time and Stroop number of incorrect responses. Therefore, ANOVAs were conducted again after removing this outlier and results demonstrated no significant interaction, treatment, or time effect for any variable ($p > 0.05$). However, the treatment *x* time interaction for mental arithmetic incorrect responses approached significance ($p = 0.07$). Mean incorrect responses pre to post MST was 2.9–2.7 in the caffeine group, compared to 2.6–3.5 in the placebo group.

4. Discussion

The main findings of this study suggest that: 1) the MST resulted in significant elevations in markers of physiological stress including: sAA, SIgA, HR, and SAI, and 2) caffeine consumption results in significantly higher sAA concentrations immediately post MST (indicated by a significant treatment *x* time interaction). Significant treatment effects were also found, which included: higher SIgA and sCORT concentrations, higher HR, SAI, and higher mental performance (quantified by number of incorrect responses during the Stroop test) in the caffeine treatment compared to placebo. Thus, based on these data, it can be concluded that caffeine consumption results in significantly greater sAA concentrations post MST. While higher SIgA, sCORT, HR, and SAI were noted in the caffeine treatment, this should not be solely attributed to caffeine ingestion since there was no significant treatment *x* time interaction found for these variables. Finally, the impact on mental performance was not significantly impacted as there was no significant treatment *x* time interaction for mental performance data.

Caffeine consumption has been shown to increase sympathetic nervous system activity resulting in elevated concentrations of blood catecholamines, cortisol, and blood pressure (Klein et al., 2014). Past work from Klein et al. (2010) demonstrated that caffeine consumption significantly increases sAA activity in response to a mental challenge in a dose responsive manner (i.e., 0 mg, 200 mg, 400 mg). These findings differ from a follow up study reported by Klein et al. (2014), which reported no significant treatment *x* time interaction when habitual caffeine consumers were provided the same doses of caffeine and exposed to a non-stressful task. The present findings are in agreement with those from Klein et al. (2010), which involved a 20-minute mental arithmetic challenge as a mental stressor. It should be acknowledged that the present study incorporated MST that was substantially shorter in duration than the ~20 min mental arithmetic task previously used (Klein et al., 2010) our MST was ~10 min duration. Based on these findings, it appears as though the magnitude of sAA response from caffeine ingestion depends on the presence or absence of a mental stressor.

sAA has been studied as a marker to respond to acute stress and acts as a non-invasive and valid indicator of sympathetic nervous system activity (Suska et al., 2012). Fasting sAA concentrations have been shown to be positively correlated with adverse markers of cardiometabolic health including elevated fasting and postprandial glucose concentrations, markers of insulin resistance, and blood pressure (Ikeda et al., 2021). While such markers of cardiometabolic health were not included in the present study, it should be noted that acute/transient exposure to stressors that can facilitate increases in sAA, HR, and blood pressure, which are not necessarily contributing factors to metabolic syndrome and cardiovascular disease. However, regular and chronic exposure to such stressors may increase risk for developing cardiometabolic disease (Huang et al., 2013). Thus, future studies should examine long term impact of caffeine consumption on markers of stress and cardiometabolic health among individuals working in high stress tactical occupations. However, the source of the caffeine should be considered as coffee for example has been well documented to demonstrate anti-oxidative and anti-inflammatory properties that may attenuate risk for chronic disease including cardiovascular disease, metabolic disease and cancer (Azam et al., 2003; Yashin et al., 2013).

The findings from the present study demonstrate that the MST caused significant increases in SIgA, HR, and SAI, which were not impacted by caffeine ingestion (no significant treatment *x* time interactions were noted). Regarding SIgA, significantly higher concentrations were found in the caffeine group compared to placebo; however, considering the lack of significant interaction, and the timing of the measurements (taken both pre and post caffeine ingestion) this finding should not be attributed to caffeine ingestion. Previous work has shown that caffeine ingestion prior to 90 min of endurance exercise causes significant elevations in plasma epinephrine and SIgA at a dose of 6 mg/kg body mass (Bishop et al., 2006). However, comparatively, this is a larger dose than what was used in the present study as the average body mass for our subjects was 74.8 ± 14 kg; thus, this would have equated to an average of ~460 mg of caffeine ingestion, while we only administered 200 mg. A follow up study done by Dulson et al. (2019) reported that caffeine ingestion of 4, 6, and 8 mg/kg of body mass results in significant elevations in sAA compared to 0 and 2 mg/kg doses. However, SIgA concentrations were unchanged by various doses of caffeine intake prior to submaximal exercise (Dulson et al., 2019). Based on the conflicting results, future studies should investigate the impact of various doses of caffeine ingestion on sAA and SIgA concentrations in response to mental stress.

With respect to sCORT, a main effect for time effect was found, which revealed a significant decrease in sCORT from immediately post to 30-minute post MST, and an 18% (but non-significant, $p = 0.07$) increase in sCORT from 45 min pre to immediately post MST. These findings suggest the possibility that the subjects anticipated stress exposure from the MST, which explained the lack of significant change from 45 min pre to post MST. Moreover, while the male/female comparison between treatments across time was conducted as an exploratory measure, these findings should also be considered especially since women were found to have significantly higher overall sCORT concentrations in the placebo

treatment compared to men. Previous work has demonstrated that caffeine ingestion can elevate cortisol concentrations concurrent with increases in epinephrine (Mustafa and Lovallo, 2004) while the change in sCORT concentrations can be impacted by factors including habitual caffeine use (Lovallo et al., 2005). Further, men tend to demonstrate a higher cortisol response to acute stress compared to women (Reschke--Hernández et al., 2017), but caffeine ingestion enhances cortisol response to stress in both men and women (Lovallo et al., 2006). Regardless, since men and women have been shown to differ significantly with respect to the cortisol response to acute stress (Reschke--Hernández et al., 2017), future studies should further examine the impact of caffeine ingestion in men and women separately when exposed to a MST involving a VR-ASD.

The present study did have some limitations that need to be mentioned. First, sAA and sCORT concentrations can be impacted by various factors such as age, sex, menstrual cycle, and time of day due to diurnal changes (Suska et al., 2012). In attempt to minimize this effect, the age range of the subjects in the present study were relatively uniform (18—40 yrs.), and the time of day of testing was standardized and occurred in the early afternoon (between 12:00 and 17:30). It should be noted that the variation in time of day can still impact these biomarkers. In addition, the present study utilized a between-subjects design, which was chosen in attempt to minimize any potential inoculation/adaptation effect from multiple bouts of exposure to the MST. Follow up studies should determine whether exposure to this form of mental stress results in an adaptative effect that may impact stress marker responses from repeated exposure. In addition, those that may seek to incorporate a crossover design should aim to maintain consistent testing times in attempt to minimize the impact of diurnal changes in sAA and sCORT concentrations. In addition, a secondary aim of the present study was to analyze the cognitive challenge data (mental arithmetic and Stroop number of incorrect and response time). However, the cognitive challenge that was presently used incorporated a four-minute session (2 min of mental arithmetic and 2 min of a rapid Stroop challenge) has not been validated in terms of reliability and validity and thus, caution is warranted when interpreting these results.

5. Conclusions

The findings from the present study suggest that the ~10-minute MST resulted in significant increases in several markers of biological and subjective stress including sAA, SIgA, HR, and SAI and caffeine ingestion at 200 mg prior to the MST resulted in significantly greater sAA concentrations post MST. While mental performance was not significantly impacted by the VR-ASD (i.e., no change in mental performance from pre to post VR-ASD), or by caffeine ingestion, the treatment x time interaction did approach significance. Therefore, future studies should investigate the effects of various doses in relation to a similar MST protocol than what was presently used.

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CRediT authorship contribution statement

All authors contributed to conceptualization of study design, data analysis, interpretation, and initial and final manuscript write up.

Declaration of Competing Interest

None.

Data Availability

Data will be made available upon reasonable request.

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