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A single bout of hard RPE-based cycling exercise increases salivary alpha-amylase

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ABSTRACT

Exercise exerts beneficial effects on cognition, in part by stimulating an arousal response that includes the release of catecholamines. Sympathetic nervous system arousal and activation of the noradrenergic system in particular may enhance cognitive performance. Measurement of salivary alpha-amylase, a non-invasive biomarker of central noradrenergic activity, is a promising avenue for characterizing the arousal-mediated effects of exercise on cognition. However, the effectiveness of high-intensity acute exercise, and the time course of sAA concentrations following exercise, has not been clearly described. The purpose of this study was to determine the effects of 20 min of perceived exertion-based high-intensity cycling exercise on salivary alpha-amylase levels in healthy young adults. We utilized a repeated-measures design to examine the sAA response to cycling exercise, rest, and an emotional picture viewing task. Thirty-two participants between the ages of 18–30 viewed pleasant, neutral, and unpleasant pictures from the International Affective Picture System. Before and after the task, participants completed either 20 min of seated rest or cycling exercise at an intensity corresponding to 15 ("hard") on Borg's Ratings of Perceived Exertion scale. Salivary alpha-amylase was assessed at time points immediately before and after rest, exercise, and the picture viewing task. Exercise elicited a robust increase in salivary alpha-amylase approximately six times higher than that induced by emotional picture viewing. Importantly, the observed exercise-induced increase in salivary alpha-amylase returned to a level comparable to baseline after ten minutes. These findings have meaningful implications for future work characterizing the relationship between exercise and arousal-mediated effects on cognitive performance.

1. Introduction

In response to physical and psychological stressors of sufficient intensity, the sympathetic nervous system initiates a fast adaptive response, commonly referred to as the "fight or flight response." A hallmark of this autonomic response is an elevation in circulating catecholamines, primarily epinephrine, and central release of norepinephrine (NE) from neurons of the locus coeruleus. The locus coeruleus, located in the pons of the brainstem, sends noradrenergic projections to several regions of the cortex, limbic system, and other brainstem nuclei [1]. Through these diverse projections, the noradrenergic system influences behavior and benefits several domains of cognition, including prefrontal cortex-dependent executive functions [2,3], and hippocampal-dependent learning and memory [for review, see [4]]. Studies in humans have demonstrated that laboratory-based stressors [5] and peripheral epinephrine infusions [6], which presumably increase central NE release, improve memory. In animals, infusing NE into the hippocampus or peripherally injecting epinephrine has a powerful influence on neural plasticity, enhancing memory performance and reducing the threshold for long-term potentiation of synapses [7,8].

Interventions in humans that use reliable methods to stimulate noradrenergic activity may be useful to maintain or improve cognitive health. Exercise is a potential non-invasive, low-cost approach to modulate the noradrenergic system and therefore beneficially influence cognitive function [9]. Acute physical exercise has well-documented benefits on several domains of cognition [10–12], yet the effects of acute exercise on cognitive performance may be moderated by parameters of the exercise stimulus (e.g., duration, mode, intensity), the experimental paradigm (e.g., timing of cognitive task relative to exercise), and the fitness of the participants. Exercise-induced noradrenergic arousal may be an important factor to consider in moderating

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the relationship between acute exercise and cognition [13]. While exercise of sufficient intensity and duration has been associated with an increase in circulating catecholamines and central noradrenergic activity [14], very few investigations in humans have quantified and demonstrated the time-course of central noradrenergic activity in response to moderate-to-high intensity acute exercise.

One challenge of investigating the relationship between exercise-induced arousal and cognitive faculties in humans is the inability to directly measure concentrations of stress hormones and noradrenergic signaling in the brain. Circulating epinephrine cannot pass the blood-brain barrier, but stimulates central noradrenergic activity in the locus coeruleus via its action on β-adrenoceptors of the vagus nerve [15,16]. While the central release of NE cannot be directly measured in human studies, salivary alpha-amylase (sAA) is a useful non-invasive biomarker for sympathetic nervous system activation. Secretion of sAA, an enzyme primarily involved in the digestion of starch and carbohydrates, is stimulated by sympathetic nerves innervating the salivary glands [17,18]. Studies of sAA in humans have shown a detectable elevation of the enzyme in response to various psychological [19,20], pharmacological [21,22], and physical stressors including exercise [23,24]. This elevation is associated with parallel increases in plasma epinephrine and NE [23,25].

While the exact autonomic mechanisms orchestrating sAA release are not clearly elucidated, there is substantial evidence that sAA is reflective of sympathetic nervous system activity [see [26] for review]. It has been demonstrated in rats that sAA secretion is mediated by stimulation of vagal β-adrenoceptors [27,28]. Work in humans has demonstrated increased sAA in response to direct NE infusion, which mimics the NE response to stress [29]. Complementary pharmacological studies have observed sAA increases after administration of yohimbine, an α-2-adrenergic receptor antagonist that stimulates NE release in the central and peripheral nervous system [21,22,30]. Ehlert and colleagues [21] reported an increase in sAA after yohimbine administration, but did not find a correlation between sAA and peripheral norepinephrine measured in blood. The lack of correlation between sAA and peripheral measures suggests that sAA reflects central rather than peripheral increases in NE; however, there are equivocal findings in this regard [e.g. [25]]. Importantly, pharmacological blockade with β-adrenoceptor antagonists (i.e. phentolamine, propranolol, or atenolol) prevents the increase in sAA observed after infusion with NE in humans [31,32]. These studies provide strong evidence for the reliability and validity of sAA as a central noradrenergic activation-sensitive biomarker.

Exercise is a non-invasive, low-cost, and controllable approach to elevate stress hormones and potentially influence behavior. The purpose of this study was to determine the effects of 20 min of high-intensity cycling exercise, subjectively perceived as" hard" using Borg's 6–20 Rating of Perceived Exertion Scale [33], on central noradrenergic activity indexed by sAA in healthy young adults.

2. Methods

2.1. Participants

Forty young adults ages 18–30 were recruited through undergraduate courses and advertisements at the University of Maryland, College Park. Exclusion criteria included: 1) self-report of a past or current diagnosis of anxiety, depression, or mood disorder; 2) contraindications to moderate-to-high intensity exercise; 3) sedentary status, defined as self-reported participation in moderate-to-vigorous physical activity < 2 days/week; 4) indication of suicidal ideation (score of ≥ 1 on Item 9) on the Beck Depression Inventory II [34]; or 5) participation in a previous study that utilized a similar set of stimuli. Participant eligibility was determined using responses to a 15-item pre-screening questionnaire administered prior to participation using Qualtrics software (Qualtrics, Provo, UT, USA) and confirmed with a health history and demographics questionnaire administered during the first study session. The Institutional Review Board approved the study procedures, and all participants provided written informed consent.

2.2. Study design

This study utilized a mixed two-way repeated measures design to determine the effects of acute RPE-based high-intensity cycling exercise on sAA. These data were collected within a study designed to investigate the time-dependent effects of acute exercise on long-term emotional memory; therefore, participants completed an emotional picture viewing task during these procedures. Unfortunately, a design flaw in the memory task prevented meaningful interpretation of the results. The current report is focused on the sAA response to RPE-based exercise compared to rest. Emotional picture viewing has been utilized as one means of inducing psychological arousal in the lab [e.g., [20,35]]; therefore, this design also provides the opportunity to compare the effects of exercise against viewing emotionally provocative picture stimuli. Group was treated as a between-subjects factor with three levels; participants were randomly assigned to one of two exercise groups (pre-task, post-task), or a resting control group. Random assignment was applied separately for male and female participants to ensure equal distribution between groups. Time was the within-subjects factor with four repeated measurements (see Fig. 1).

2.3. Procedures

Participants were instructed to arrive prepared to exercise, and were asked to abstain from caffeine, nicotine, and exercise for two hours before the scheduled testing visit. After providing informed consent, participants completed a health history and demographics questionnaire, State-Trait Anxiety Inventory Forms Y1 and Y2 [STAI-Y1, STAI-Y2; [36]], Beck Depression Inventory II [BDI; [34]], Godin Leisure

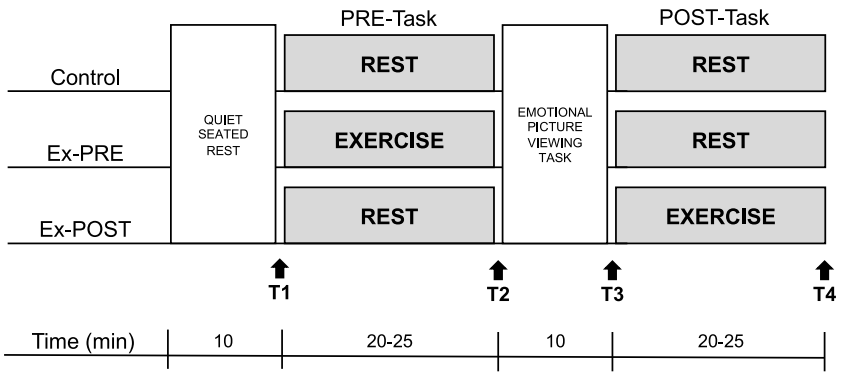

Fig. 1. Study design. Arrows indicate timing of saliva sample collection.

Time Exercise Questionnaire [LTEQ; [37]], and 7-Day Physical Activity Recall Interview Questionnaire [7-Day PAR; [38]]. Participants were fitted with a heart rate monitor (Polar® RS800CX, Polar Electro, Kempele, Finland) for the remainder of the session. The technology used in Polar® monitors to detect and wirelessly transmit heart rate information demonstrates excellent validity with electrocardiogram, the gold standard of heart rate measurement, during rest and exercise [39]. The procedures for all participants included quiet seated rest, a pre-task interval, emotional picture viewing task, and post-task interval (Fig. 1). Saliva samples were collected at baseline (T1) and after the pre-task interval (T2), picture viewing task (T3), and post-task interval (T4). Pre- and post-task conditions were completed in accordance with group assignment: the pre-task exercise group performed the acute exercise session between T1-T2 while the post-task exercise group exercised between T3-T4. Participants in these groups rested during the alternate interval, while those in the control group rested during both intervals. Participants in all groups completed ten minutes of quiet seated rest before providing the baseline saliva sample (T1). Water was provided ad libitum, except during the ten minutes prior to each saliva collection to avoid sample dilution.

After completing the appropriate pre-task condition and providing the second saliva sample (T2), each participant viewed 90 images from the International Affective Picture System [IAPS; 40]. A total of 180 images were selected to create two picture sets, each comprising 30 neutral and 60 emotionally arousing (30 pleasant, 30 unpleasant) pictures (see Supplementary Material). These stimuli were not necessarily selected to strategically provoke a catecholamine response; however, their inclusion offered an opportunity to examine the exercise-induced response measured with sAA relative to an emotional picture viewing task, which has been reported in previous work [19,35,20]. Presentation of each picture set was counterbalanced across participants. Picture sets were matched on normative valence and arousal ratings and on semantic content. During the picture viewing task, participants were seated at a desk with a button response box positioned comfortably for use with the right hand. Stimuli and task prompts were presented electronically on a 15″ monitor using *E*-Prime 2.0 Software (Psychology Software Tools, Pittsburgh, PA). Individual images were presented in a pseudorandomly determined order for a duration of 3000 ms. A fixation cross was displayed during the interstimulus interval for a randomly selected duration of 3000, 3500, or 4000 ms. During presentation, participants rated each picture as *arousing*, *somewhat arousing*, or *not arousing* using the button response box. The picture viewing task lasted approximately 10 min. Immediately after completing the task, participants provided the third saliva sample (T3). After completing the post-task exercise or rest condition, participants provided the fourth saliva sample (T4).

2.3.1. Acute exercise condition

The acute exercise condition consisted of high-intensity cycling exercise on a mechanically braked cycle ergometer (Ergomedic 828 E, Monark Exercise AB, Vansbro, Sweden). A 2-min warm-up and cool-down were completed at a participant-selected work rate. During the 20-min high-intensity exercise interval, participants cycled at a perceived exertion of 15 (“Hard”) on the Borg Rating of Perceived Exertion (RPE) scale. Pedaling cadence was maintained between 60 and 70 rpm, while participants controlled the resistance on the cycle ergometer throughout the exercise session to maintain the perception of working “Hard.” Heart rate (HR) and RPE were recorded at baseline, at the end of the warm-up (i.e. the 0-min time point), at 5, 10, 15, and 20-min time points during the high-intensity interval, and after the cool-down. At the same time points, participants rated their affective experience on the Self-Assessment Manikin Valence (SAM-V) and Arousal (SAM-A) dimension scales [40]. Post-exercise saliva samples were collected as promptly as possible after the 20-min time point during the cool-down. Immediately after exercise cessation, participants were escorted to a neighboring laboratory, where blood lactate concentration was measured with a finger prick (Lactate Plus Lactate Analyzer, Nova Biomedical, Waltham, MA). During the rest condition(s), participants were seated quietly in a chair for 20 min with no access to technology or reading materials while HR was recorded at 0, 5, 10, 15, and 20-min time points.

2.3.2. Saliva collection and analysis

Saliva samples were obtained using the passive drool method. The passive drool method requires participants to allow saliva to pool in the floor of the mouth and gently direct saliva into a tube through a saliva collection aid (Salimetrics, State College, PA). This method is recommended over the alternative oral swab method as a means of collecting unstimulated whole saliva from adults in a supervised laboratory setting [33]. After collection, samples were immediately stored on ice and subsequently frozen at −40 °C. Alpha-amylase activity was quantified using an enzymatic assay (Salivary Alpha-Amylase Kinetic Enzyme Assay Kit, Salimetrics, State College, PA). On the day of the assay, samples were thawed and diluted with the provided alpha-amylase diluent (1:200) and combined with the provided alpha-amylase substrate (37 °C) immediately before absorbance measurement. Absorbance at 1- and 3-min time points was measured with a 405 nm filter on a programmable microplate reader (ELx808, BioTek, Winooski, VT) set to incubate at 37 °C. Each saliva sample was analyzed in duplicate across the pair of strips during the same absorbance reading cycle. For quality control purposes, high and low concentration controls provided by the manufacturer were included on each strip. Sample duplicates with a coefficient of variation (CV) $< 15\%$ were averaged together for each measurement. Duplicates with a CV $\geq 15\%$ were excluded. The detection limit was equivalent to a change in absorbance < 0.01. The linearity limit at the given dilution was 400 U/mL. Samples that fell outside this range were excluded from statistical analysis. Only participants with intact data points for all four sAA measurements were included in statistical analyses. Data from 8 participants were excluded due to: failure to provide a saliva sample of adequate volume ($n = 4$), sAA values exceeding the upper linearity limit ($n = 2$), or duplicate sAA samples with CV $\geq 15\%$ (n = 2). There were no samples that fell below the detection limit.

2.4. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 24. An alpha value of 0.05 was set as the threshold for significance. All variables were examined for normality prior to conducting statistical tests. Demographic variables were compared between groups using one-way analysis of variance (ANOVA) and Fisher's exact tests. HR_{avg}, RPE_{avg}, $SAM\text{-}V_{avg}$, and $SAM\text{-}A_{avg}$ were calculated from the average of 5-, 10-, 15-, and 20-min time points during exercise to capture the high-intensity interval. HR_{avg} values for the rest condition were calculated from the same time points. To confirm the equivalence of the exercise condition between exercise groups, HR_{avg}, RPE_{avg}, $SAM\text{-}V_{avg}$, $SAM\text{-}A_{avg}$, and blood lactate concentration were compared using independent samples *t*-tests. As a manipulation check, HR_{avg} was compared between groups within the pre- and post-task intervals using one-way analysis of variance (ANOVA). Within-group comparisons of HR_{avg} during exercise and rest conditions were conducted using paired *t*-tests.

Review of the Shapiro-Wilk statistics and Q-Q plots suggested that normality could not be reasonably assumed for sAA measurements within groups; therefore, all sAA data were square root transformed. Transformed (√sAA) values were utilized in the remainder of statistical analyses, unless otherwise noted. Repeated measures ANOVAs were conducted to examine the effect of time on √sAA within each group.

One-way ANOVAs were conducted to compare √sAA at T1 and T3 between groups, as these measurements correspond to time points prior to pre- and post-task intervals. Change in sAA across pre-task ($\Delta\sqrt{sAA}_{PRE\text{-}TASK} = \sqrt{sAA}_{T2} - \sqrt{sAA}_{T1}$), emotional picture viewing ($\Delta\sqrt{sAA}_{TASK} = \sqrt{sAA}_{T3} - \sqrt{sAA}_{T2}$), and post-task ($\Delta\sqrt{sAA}_{POST\text{-}TASK} =$

$\sqrt{sAA_{T4}} - \sqrt{sAA_{T3}}$) intervals was calculated for each group. We then compared $\Delta\sqrt{sAA_{PRE\text{-}TASK}}$ and $\Delta\sqrt{sAA_{POST\text{-}TASK}}$ between groups using one-way ANOVAs. Independent samples *t*-tests were conducted to confirm the equivalence of $\Delta\sqrt{sAA}$ associated with exercise ($\Delta\sqrt{sAA_{EX}}$) and rest ($\Delta\sqrt{sAA_{REST}}$) conditions between the exercise groups. In order to examine the potential role of sex in the $\Delta\sqrt{sAA}$ response, these data were submitted to a two-way ANOVA with condition as the within-subjects factor and sex as the between-subjects factor. Subsequently, $\Delta\sqrt{sAA_{EX}}$ and $\Delta\sqrt{sAA_{REST}}$ were submitted to a paired samples *t*-test. Pearson's correlation coefficients were calculated to assess the relation of ΔsAA_{EX} with HR_{avg}, RPE_{avg}, $SAM\text{-}V_{avg}$, and $SAM\text{-}A_{avg}$, and blood lactate concentration. To ease interpretation, ΔsAA_{EX} was calculated from non-transformed sAA values and examined for normality prior to conducting correlations.

Significant omnibus tests were further deconstructed through post-hoc *t*-tests. We performed Bonferroni correction to reduce the chance of type I errors in the case of multiple comparisons (critical $\alpha = \alpha_c = 0.05/c$, where c = number of comparisons). We chose to use Bonferroni control of family-wise error rate as a conservative method of minimizing false positives due to our limited sample size. In instances where Levene's test indicated unequal variances between samples, the results of Welch's *t*-tests are reported. For ANOVA procedures with more than two repeated measures, Huynh-Feldt adjustment was used when Mauchly's test of sphericity met significance.

3. Results

3.1. Participants

Sample characteristics for participants included in analyses (N = 32, 21F/11M) are shown in Table 1. One-way ANOVAs revealed no between-group differences in age (M = 21.9, SD = 3.1), self-reported anxiety or depression symptoms, or self-reported physical activity level. Fisher's exact-tests revealed no association between group assignment and sex (p = .580), current over-the-counter or prescription medication status (p = .899), or oral contraceptive use in female participants (p = .649).

3.2. Physiological and psychological acute exercise measures

Paired *t*-tests detected no significant differences between the exercise groups on HR_{avg}, RPE_{avg}, $SAM\text{-}V_{avg}$, $SAM\text{-}A_{avg}$, or blood lactate concentration (Table 1). One way ANOVAs revealed significant differences in HR_{avg} during pre-task (F(2, 31) = 202.676, p < .001) and post-task (Welch's F(2,17.614) = 100.359, p < .001) intervals. Post hoc comparisons (α_c = 0.0167) indicate that HR_{avg} during the pre-task interval was higher in the pre-task exercise group (M = 151.3, SD = 17.2) than in the control (M = 68.4, SD = 6.5, p < .001) and post-task exercise (M = 61.4, SD = 7.9, p < .001) groups. HR_{avg} during the post-task interval was higher in the post-task exercise group (M = 153.1, SD = 18.6) than in the control (M = 66.8, SD = 7.4, p < .001) and pre-task exercise (M = 85.6, SD = 9.8, p < .001) groups. HR_{avg} was also higher in the pre-task exercise group than in the control group during the post-task interval (p = .007). Within-group comparisons of HR_{avg} during exercise compared to rest revealed significant differences in the pre-task (t(9) = 15.329, p < .001) and post-task (t(10) = 19.000, p < .001) exercise groups. Within-group comparison of HR_{avg} during pre- and post-task intervals revealed no difference between intervals in the control group (t(10) = 1.185, p = .264). The average HR during high-intensity exercise was 152 ± 17 beats per minute, corresponding to 79.3% of this sample's age-predicted maximum HR estimated using the Tanaka equation. This relative exercise intensity falls within the range expected of vigorous-intensity exercise according to the American College of Sports Medicine's guidelines [41].

3.3. Salivary alpha-amylase response

Square root-transformed and non-transformed sAA data and calculations are available in Supplementary Tables 1 and 2. Repeated measures ANOVAs revealed a significant effect of time on √sAA in the control (F(3, 30) = 4.570, p = .009, η^2 = 0.314), pre-task exercise (F(3, 27) = 12.755, p < .001, η^2 = 0.586), and post-task exercise (F(2.054, 20.54) = 25.337, p < .001, η^2 = 0.717) groups (Fig. 2). Post hoc paired *t*-tests were conducted to compare √sAA at baseline (T1) with T2, T3, and T4 measurements within each group (α_c = 0.0167). In the

Table 1
Demographic and physiological variables by group.

Variable	Group			p value[a]
	Control	Pre-Task Exercise	Post-Task Exercise	
n	11	10	11	
Sex	8F, 3 M	5F, 5 M	8F, 3 M	0.455[b]
Age (y)	20.3 ± 1.7	22.9 ± 3.4	22.6 ± 3.5	0.102
Trait anxiety (STAI-Y2)	36.6 ± 10.9	34.7 ± 7.6	34.6 ± 6.3	0.833
State anxiety (STAI-Y1), Day 1	33.3 ± 10.5	32.7 ± 10.2	26.9 ± 5.0	0.201
State anxiety (STAI-Y1), Day 2	27.9 ± 7.4	31.8 ± 8.1	26.1 ± 6.1	0.200
Depression symptoms (BDI-II)	5.1 ± 6.1	5.6 ± 4.4	4.8 ± 4.8	0.941
Leisure time physical activity (LTEQ, hrs/wk)	77.6 ± 30.9	53.9 ± 18.6	56.2 ± 31.0	0.108
Leisure and occupational physical activity (7-Day PAR, kJ/kg/day)	134.9 ± 12.7	142.2 ± 57.9	118.3 ± 35.4	0.365
Physiological and psychological variables				
HR_{avg} (bpm)	67.6 ± 6.6[c]	151.3 ± 17.2	153.1 ± 18.6	0.821[d]
RPE_{avg}	–	14.3 ± 0.6	14.5 ± 0.6	0.252
$SAM\text{-}V_{avg}$	–	6.2 ± 1.4	6.5 ± 1.4	0.606
$SAM\text{-}A_{avg}$	–	6.0 ± 1.2	5.0 ± 2.2	0.223
Blood lactate concentration (mmol/L)[e]	–	6.29 ± 2.45	5.65 ± 3.20	0.637

Outcomes reported as Mean ± SD. Exercise variables represent average of 5, 10, 15 and 20-min measurements. STAI, State-Trait Anxiety Inventory; BDI-II, Beck Depression Inventory II; LTEQ, Godin Leisure Time Exercise Questionnaire; 7-Day PAR, 7-Day Physical Activity Recall Interview Questionnaire; HR, heart rate; RPE, Borg's Ratings of Perceived Exertion, 6 to 20 scale; SAM-V and SAM-A, Self-Assessment Manikin Valence and Arousal dimensions, 1 to 9 scale.

[a] p values correspond to F or t statistic representing between-group mean difference.

[b] p value corresponds to χ^2 statistic.

[c] HR_{avg} combined across pre- and post-task intervals.

[d] p value corresponds to t statistic between exercise groups.

[e] Blood lactate concentration measured 3.2 ± 1.7 min after exercise cessation.

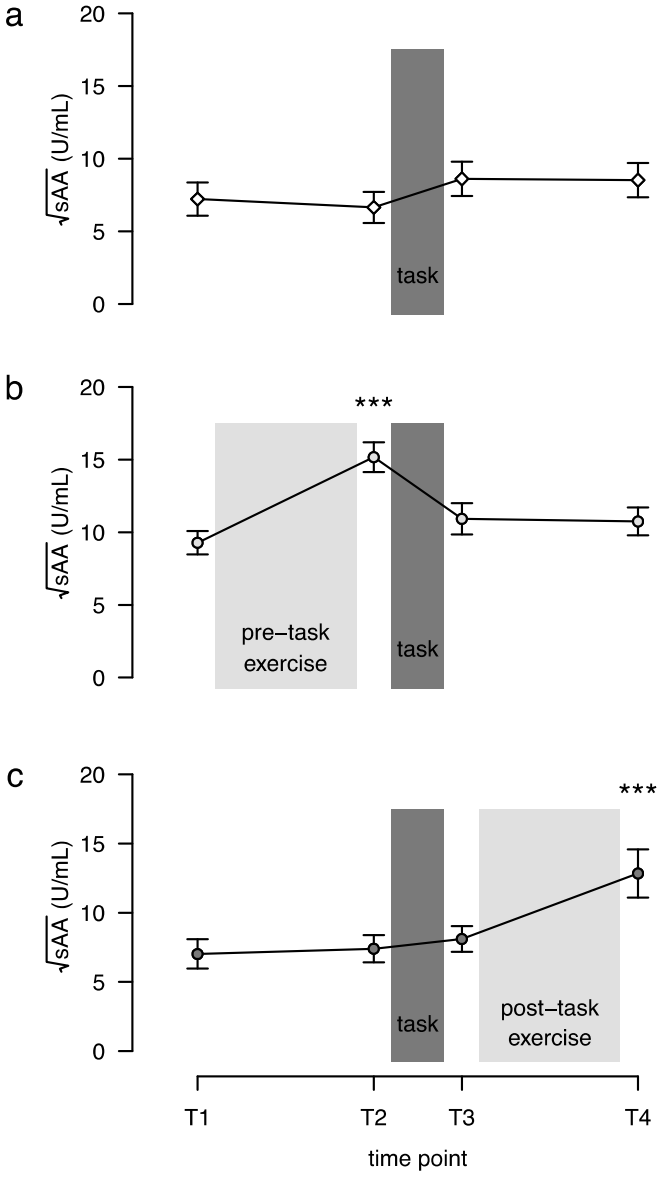

Fig. 2. Change in square root-transformed sAA (Δ√sAA) over time in a) control group, b) pre-task exercise group, and c) post-task exercise group. Bars represent 95% confidence interval. *** indicates $p < .001$.

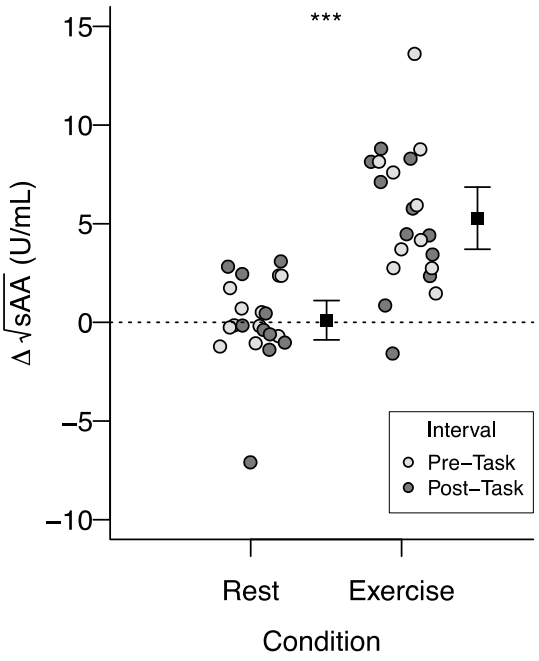

Fig. 3. Change in square root-transformed sAA (Δ√sAA) during rest and exercise conditions collapsed across pre- and post-task intervals. Bars represent 95% confidence interval. *** indicates $p < .001$.

control group, there was no significant difference between √sAA at baseline and T2 ($t(10) = -1.407$, $p = .190$) or T4 ($t(10) = 1.991$, $p = .074$). The difference between √sAA at baseline and T3 was significant at the $\alpha = 0.05$ level but did not meet the Bonferroni-corrected threshold for significance ($t(10) = 2.546$, $p = .029$). In the pre-task exercise group, √sAA was significantly increased at the post-exercise time point (T2) relative to baseline ($t(9) = 5.042$, $p = .001$). Significant differences in √sAA were not detected between baseline and T3 ($t(9) = 1.865$, $p = .095$) or between baseline and T4 ($t(9) = 0.180$, $p = .180$). The post-task exercise group exhibited a complementary pattern of change over time, such that √sAA was significantly increased at the post-exercise time point (T4) relative to baseline (t(10) = 6.306, $p < .001$), while no significant changes in √sAA were detected at T2 ($t(10) = 0.971$, $p = .355$) or T3 ($t(10) = 1.858$, $p = .093$).

No significant between-group differences in √sAA at T1 ($F(2, 31) = 1.425$, $p = .257$) or T3 ($F(2, 31) = 1.921$, $p = .165$) were detected. One-way ANOVAs revealed a significant effect of group on $\Delta\sqrt{sAA}_{PRE\text{-}TASK}$ ($F(2, 31) = 23.107$, $p < .001$) and $\Delta\sqrt{sAA}_{POST\text{-}TASK}$ ($F(2, 31) = 10.166$, $p < .001$). As expected, post hoc comparisons ($\alpha_c = 0.0167$) revealed that the pre-task exercise group had higher $\Delta\sqrt{sAA}_{PRE\text{-}TASK}$ (M = 5.89, SD = 3.69) than the control (M = −0.57, SD = 1.35, $p < .001$) and post-task exercise (M = 0.38, SD = 1.29, $p < .001$) groups. Similarly, the post-task exercise group had higher $\Delta\sqrt{sAA}_{POST\text{-}TASK}$ (M = 4.73, SD = 2.51) than the control (M = −0.09, SD = 2.39, $p = .002$) and pre-task exercise (M = −0.18, SD = 2.93, $p = .002$) groups. There was no significant difference in $\Delta\sqrt{sAA}_{EX}$ ($t(19) = 0.756$, $p = .459$) or $\Delta\sqrt{sAA}_{REST}$ ($t(19) = -0.575$, $p = .572$) between the pre-task and post-task exercise groups. Collapsing across exercise groups, a two-way ANOVA revealed a main effect of condition ($F(1, 19) = 30.762$, $p < .001$, $\eta^2 = 0.618$) on Δ√sAA. There was no main effect of sex or interaction of sex and condition on Δ√sAA measures. Paired *t*-tests revealed a significant difference between $\Delta\sqrt{sAA}_{EX}$ and $\Delta\sqrt{sAA}_{REST}$ ($t(20) = 5.828$, $p < .001$; Fig. 3). Non-transformed values of ΔsAA_{EX} revealed a mean exercise-induced increase in sAA of 239.57 U/mL (SD = 95.17) and 195.31 U/mL (SD = 147.49) in the pre- and post-task exercise groups, respectively. Pearson's correlations revealed a moderate positive correlation between non-transformed ΔsAA_{EX} and blood lactate concentration measured 3.2 ± 1.7 min after exercise cessation ($r(18) = 0.502$, $p = .034$; Supplementary Fig. 1). HR_{avg}, SAM-V_{avg}, and SAM-A_{avg} were not significantly correlated with ΔsAA_{EX}.

Change in sAA associated with the emotional picture viewing task ($\Delta\sqrt{sAA}_{TASK}$) was examined only in the control and post-task exercise groups to avoid the confound of exercise during the pre-task interval. An independent samples *t*-test revealed no between-group difference in $\Delta\sqrt{sAA}_{TASK}$ ($t(20) = 1.506$, $p = .148$). A one-sample *t*-test revealed a significant non-zero increase in $\sqrt{sAA}_{TASK}$ (M = 1.34, SD = 2.02) when collapsed across control and post-task exercise groups ($t(21) = 3.110$, $p = .005$). Evaluation of non-transformed ΔsAA_{TASK} and ΔsAA_{EX} data demonstrated that the average increase in sAA associated with exercise (M = 133.67, SD = 100.78) was approximately six-fold higher than the sAA increase associated with the emotional picture viewing task (M = 21.27, SD = 36.32).

4. Discussion

The purpose of this study was to determine the effect of subjectively regulated "hard" cycling exercise on noradrenergic activation, indexed using a non-invasive salivary biomarker (sAA). Here, we demonstrate that a 20-min bout of RPE-based "hard" cycling exercise is sufficient to increase sAA in a sample of healthy young men and women. The increase in sAA induced by RPE-based acute exercise was approximately six-fold higher than the increase induced by an emotional picture viewing task, a frequently encountered stimulus in studies of noradrenergic arousal. Examination of the time course revealed a return of the exercise-induced sAA to a baseline-comparable level after approximately ten minutes. This finding has important implications for studies examining the effect of acute exercise on cognitive tasks, particularly those affected by arousal-mediated processes.

Our results complement those of previous studies, which have reported observable increases in sAA as a result of moderate-to-high intensity aerobic [23,25,42,43] and resistance [44] exercise. This study demonstrates that a robust increase in sAA is elicited by subjectively regulated (i.e. RPE-based) high-intensity cycling, but that this increase is short-lived. To our knowledge, few if any examinations have opted to measure sAA in response to exercise prescribed using subjective ratings of perceived exertion. RPE has been validated as a practical and affordable means of monitoring and prescribing exercise intensity across modalities [45], and may be a preferable means of prescribing exercise to promote adherence [46]. In this study, HR during exercise increased to a rate expected of high-intensity exercise, and we observed an association between exercise-induced sAA and post-exercise blood lactate concentration. These findings support the validity of RPE as a means of prescribing high-intensity exercise and extend on previous work to suggest that Borg's RPE of 15 ("hard") is a sufficient exercise prescription to increase sAA in healthy young adults.

The exercise-induced increase in sAA observed in this study was notably higher than a recent study that measured sAA before and after ten minutes of treadmill walking exercise [47], and of those that have measured the sAA response to emotional picture viewing [19,20,35]. While the sAA response to exercise was robust, it was short-lived; examination of the pre-task exercise group suggests that sAA levels returned to baseline within ten minutes of exercise cessation. Interestingly, Segal and colleagues [19] demonstrated a return of sAA to baseline that occurred between 8 and 18 min after an emotional picture viewing task, corroborating our finding of a short-lived increase in sAA that reflects the fast response and recovery of the sympathetic arousal response [17]. It should be noted, however, that the time course for detection of sAA may not necessarily represent the time course of availability of circulating NE and epinephrine in blood, or noradrenergic metabolism and signaling in the brain. Future research directions should include exploration of the determinants of the magnitude and duration of the sAA response.

There are several limitations of this study. While it is widely accepted that exercise influences several domains of cognition, the data presented here preclude our ability to draw conclusions about the impact of exercise-induced arousal on cognition or memory performance. Future studies should aim to systematically explore the effects of varying levels of sAA-indexed arousal on different domains of cognition. We also acknowledge that the true relation between sAA and levels of central NE in specific brain tissues is yet to be clearly elucidated. While there is strong evidence that sAA does reflect central noradrenergic activity, the fact remains that sAA is an indirect biomarker which may also be influenced by parasympathetic effects [48]. The choice of cycling exercise in this study was based on meta-analytic findings suggesting larger effect sizes of cycling exercise on cognition relative to running [10]. Additional work is necessary to characterize the dependence of sympathetic arousal on exercise mode, intensity and duration. Further, it has been demonstrated in animal models [49] and in human studies [see 14 for review] that exercise training and cardiorespiratory fitness are associated with increased noradrenergic responsiveness to exercise. We did not conduct exercise testing on the participants in this study, therefore we are unable to draw conclusions about the sAA response in relation to cardiorespiratory fitness or relative exercise intensity based on direct measures of cardiorespiratory fitness. The relative intensity limitation is mitigated by our use of RPE-based exercise. However, these individuals self-reported participation in least two sessions of moderate-to-vigorous physical activity in a typical week, and there was no difference in habitual physical activity level assessed on the LTEQ [37] and 7-Day PAR [38] between groups. While the aim of this study was not to examine the relation between fitness and the sAA response, future work should examine this question in the context of RPE-based exercise. Similarly, previous work has demonstrated equivocal findings regarding sex differences in acute sAA responsiveness [18,19]. Due to the already limited sample size and relatively low enrollment of male participants in this study, we were unable to examine sex-related differences and did not control for sex in these analyses. Replication of these findings in a larger sample will be necessary to determine the moderating role of sex on exercise-induced sAA response.

Appropriately prescribed exercise modulates activity of the noradrenergic system and may therefore exert beneficial effects on cognition. In examining these effects, it is important to consider features such as the duration, mode, and intensity of the exercise. Further, the timing of an exercise session relative to performance of a cognitive task is a critical factor influencing the direction and strength of cognitive effects. Exercise-induced effects that are contingent on sympathetic arousal, specifically noradrenergic activation, may therefore depend on the magnitude and duration of increased noradrenergic activity. Our results suggest perceptually hard exercise reliably increases salivary alpha-amylase, more so than viewing emotional pictures, and furthermore, that this increase reliably returns to baseline after about 10 min. These findings have meaningful implications for future work characterizing the relationship between exercise and arousal-mediated effects on cognitive performance.

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Appendix A. Supplementary data

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