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The effects of exercise intensity on the cortisol response to a subsequent acute psychosocial stressor

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ABSTRACT

Objective: The aim of this experimental study was to determine the extent to which the intensity of a single 30 min bout of exercise alters the salivary cortisol (sCort) response to a subsequently induced acute psychosocial stressor. The study further aimed to elucidate a physiological mechanism through which exercise intensity exerts stress-mitigating effects.

Methods: Eighty-three healthy men (M_{age} = 21.04 SD = 2.89) were randomly assigned to exercise on a treadmill at either 30%, 50% or 70% of their heart rate reserve (HRR) for 30 min and then underwent the Trier Social Stress Test 45 min later. sCort was measured repeatedly throughout and following the exercise bout and stressor task.

Results: ANCOVA and Multilevel Growth Curve Analysis determined that vigorous (70% HRR) exercise elicited dampened sCort responses to the stressor task, marked by lower total sCort levels, diminished sCort reactivity, and faster recovery to baseline values, as compared to less intense exercise. Moreover, exercise elicited a sCort response in proportion to the intensity at which it was performed, and this exercise-associated HPA-axis response was inversely proportional to the sCort response to the subsequent stressor task.

Conclusions: This study revealed that exercise-intensity dampens the HPA-axis stress response in a dose-dependent manner, with evidence that the cortisol released from exercising intensely suppresses the subsequent cortisol response to a psychosocial stressor.

1. Introduction

The physiological stress response helps humans adaptively mobilize energy and respond to challenging or threatening environmental, physical, and external or internal psychosocial stimuli. With prolonged, repeated, or severe exposure to psychosocial stressor stimuli, however, frequent activation of the physiological stress system can lead to clinical depression, anxiety disorders, and a host of non-communicable diseases (Miller et al., 2007). The mental and physical health burden of these forms of chronic life stress has necessitated the need for practical, stress-mitigating solutions that individuals can utilize in their everyday lives. Like social support (Hostinar and Gunnar, 2015), longterm exercise has stood out as a compelling health behavior that mitigates psychological and physiological responses to stressful events, both in the lab and everyday life (Basso and Suzuki, 2017; Puterman et al., 2017). While research has demonstrated the benefits of increased fitness and frequent aerobic exercise in terms of the physiological responses to psychosocial stressors (Puterman et al., 2012; Rimmele et al., 2009, 2007; Traustadóttir et al., 2005), in this study we explored whether the intensity of a single bout of exercise alters the physiological impact of an acute psychosocial stressor.

1.1. Normal and pathological stress responses

In response to both physical (Selye, 1998) and psychosocial stressors (Dickerson and Kemeny, 2004), the sympathomedullary pathway (SAM-p) and the hypothalamic-pituitary-adrenal axis (HPA axis) are activated. SAM-p releases catecholamines that modulate an immediate

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"fight or flight" response which optimize bodily functions for behavioral management of the stressor. The HPA axis activates a longer transient hormonal cascade that terminates with the release of glucocorticoids (cortisol in humans) from the adrenal cortex. Cortisol circulates into the peripheral blood where it mobilizes energy by releasing glucose from their storage sites. HPA axis activation improves alertness for extended durations to respond to physically or psychologically demanding events (Herman et al., 2016). Many studies have shown that salivary cortisol (sCort) serves as an effective indicator of HPA-axis activity that can be used to measure the response to psychological or physical stressors (Foley and Kirschbaum, 2010).

When a stressful event ends, inhibition of the HPA axis is paramount to prevent excessive physiological load, as excess prolonged peripheral cortisol can alter the functions and structure of many physiological systems and cause disease (McEwen, 2007). The HPA axis employs a negative-feedback circuit, whereby increased circulating glucocorticoids released from the system target receptors upstream along the HPA pathway (in the pituitary and hypothalamus) and in the cortex to shut off subsequent neuroendocrine responses. The magnitude of the negative feedback is directly proportional to the amount of cortisol released from an initial HPA-axis response (Dallman, 2010).

1.2. Physical activity and the stress response

Engagement in physical activity and exercise benefits emotional and cognitive wellbeing, and provides health benefits to the same physiological systems negatively impacted by the experience of chronic stress (Puterman et al., 2015, 2016). To characterize the effects of exercise on the physiological response to psychological stressors, researchers have theorized that repeated challenge of one type of stressor (i.e., physical exercise) can habituate *or* sensitize the physiologic stress system to heterotypic (i.e., dissimilar) stressors, such as a psychosocial stressor (Sothmann et al., 1996).

The majority of studies with humans testing this cross-stressor adaptation hypothesis with exercise and psychological stressors have been quasi-experimental, comparing sCort released in response to a psychosocial stressor task in adults pre-selected for their fitness or activity levels (Puterman et al., 2012; Rimmele et al., 2007, 2009; Traustadóttir et al., 2005; Wunsch et al., 2019). These studies assume that differences between fit and unfit adults, or active and inactive adults, can be attributed to habituated activation and recovery of neurobiological stress systems, occurring with repeated bouts of exercise, in line with the cross-stressor adaptation hypothesis. Only one study has confirmed that previously inactive participants who engage in a 12-week moderate-level aerobic exercise program show reduced overall SAM-p and HPA-axis responses to a psychosocial stressor task compared to waitlist controls (Klaperski et al., 2014).

Some researchers have studied the effects of a single bout of exercise on HPA-axis responses to a subsequently-induced psychological stressor using an experimental approach. Three studies demonstrate that various modalities of acute exercise (e.g. outdoor walking, ergometer bicycling) can reduce the HPA-axis sCort response to various psychosocial stressor tasks (Wood et al., 2018; Wunsch et al., 2019; Zschucke et al., 2015). For example, in a study by Zschucke and colleagues, overall cortisol output was reduced following a mild cognitive challenge task when participants engaged in 30 min of moderate-to-vigorous aerobic exercise (at 60–70% VO_2.peak) prior to the mental challenge compared to participants who did not engage in any exercise. (Zschucke et al., 2015). In a study by Wood et al. (2018), participants randomized to walk ~3 km outside (with 2/3 in an urban park) for approximately 30 min had reduced cortisol output to a standardized group-based speaking and math task that followed the walk, compared to those who were randomized to sit in a room for 30 min.

Since these studies primarily compared moderate-to-vigorous exercisers to a non-exercising control, a mechanism underlying the stress-mitigating effect of exercise remains elusive. Discovering the fundamental qualities of exercise (e.g. intensity, timing, modality) that best mitigate physiological responses to psychosocial stressors remains a question that, when answered, can offer insight when designing practical exercise interventions for managing psychological stressors in everyday life. Interestingly, studies have established that exercise in and of itself is a physical stressor that activates the HPA axis at intensities above 60% of VO2-peak. Above this threshold, a single bout of exercise induces a cortisol response that is linearly proportional to the intensity at which the exercise is performed (Duclos and Tabarin, 2011). We hypothesized that this cortisol response to exercise may in fact suppress the HPA axis through negative feedback and thus dampen its response to a subsequent stressor.

1.3. Current aims and investigations

In this study, healthy, young men were randomly assigned to perform 30 min of aerobic exercise on a treadmill in one of three intensity groups (light, moderate, or vigorous). All men then completed a psychosocial stressor task 45 min later. sCort was measured at several times points before, during, and after both the exercise bout and the subsequent psychosocial stressor task. We hypothesized that intensity of the bout of exercise would be inversely related to the sCort response to the psychosocial stressor across time. Secondly, we hypothesized that the cortisol response to the psychosocial stressor would be associated with the cortisol released during exercise, such that exercise-induced cortisol secretion, whose magnitude is proportional to the intensity of the exercise performed, would suppress the cortisol response to the stress task.

2. Materials and methods

The full protocols for this study was approved by the University of British Columbia's Clinical Research Ethics Board (H17–00588). A sample of 83 men was recruited for this study. Informed consent was obtained from all participants.

2.1. Inclusion and exclusion criteria

Men above 30 years old were excluded to control for the impact of age on circadian rhythmicity of plasma cortisol (Van Cauter et al., 1996). Women were excluded to control for fluctuations in sCort resulting from different phases of the menstrual cycle (Kirschbaum et al., 1999). Participants were further excluded if they answered "YES" to any section-1 or section-2 items from the Physical Activity Readiness Questionnaire for Everyone (PARQ+) (Warburton et al., 2011). A "YES" response indicated that they were either a smoker, physically disabled, had a heart condition, high blood pressure, experienced chest pains during daily activities, had a chronic medical condition and related prescriptions, or required a doctor's recommendation for supervision during exercise. Moreover, since several factors impact diurnal SAM-p and HPA-axis activity (Nater et al., 2007), participants were also excluded if they reported any psychiatric illness, substance or alcohol abuse, disorders of exogenous glucocorticoid dysregulation (e.g. Cushing's Syndrome), or use of steroid medications. Lastly, participants were required to meet a physical activity standard of moderate physical activity as assessed by the Leisure-Time Activity Categorical Item (L-CAT), (Kiernan et al., 2013) whereby participants were excluded if they responded to item-1or 2 (very low activity in daily life). Particpants were also excluded if they were physically active at high levels of 30 vigorous minutes of exercise at least 5 times per week (response of 6 on the L-CAT). All eligibility criteria were assessed either via phone interview or in-person meetings with a study-affiliated researcher.

2.2. Study protocol

All eligible participants were invited for two study visits, conducted

on separate days within 2 weeks of one another. At the beginning of each visit, participants completed the PAR-Q+ (Warburton et al., 2011) to confirm their eligibility for exercise on the appointment day. All protocols took place in quiet, comfortably lit lab rooms and suitable exercise spaces. Testing rooms were well ventilated and room temperature was kept at approximately 23ºC.

2.2.1. Study Visit 1

During the first visit to the lab, participant weight and height were recorded by a research volunteer using a *Health-O-Meter Professional* stadiometer and scale. Basic demographic information was acquired using a computer-based questionnaire. Participants were then asked to complete a modified cardiopulmonary exercise treadmill test (CPET) at maximal capacity (Kaminsky and Whaley, 1998). During this protocol, participants were incrementally ramped up to faster speeds and steeper treadmill inclines and exerted themselves until exhaustion. Work-rate (i.e., speed or incline) was incrementally increased every twenty seconds. Unlike the traditional Bruce Protocol treadmill test, in which work is increased every three minutes, participants engaged in a modified version, which required work-rate to increase incrementally every twenty seconds. The work-rates (i.e. the speed and incline) of the traditional and modified tests align at each three-minute mark. In both versions, monitoring intervals fall in accordance with the American College of Sports Medicine (ACSM) guidelines (Thompson et al., 2013). The modified test allowed for a steady, continuous increase in work-rate as opposed to a step-wise progression. Participants were monitored throughout the protocol in accordance with the ACSM guidelines. All testing took place on a COSMED T-150 treadmill, typically used for clinical and high-performance application. Omnia software suite (COSMED The Metabolic Company; Rome, Italy) was used to set stage progression. Over the course of the CPET, perceived physical exertion was assessed every 2 min using the Borg scale, ranging from 6 (very little exertion) to 20 (maximal exertion) (Borg, 1982). Chest-strapped Polar H10 Heart Rate Monitors (Polar Electro, Canada) recorded participants' heart rate (HR) continuously throughout the resting, exercise and recovery period of the test. All HR data were cleaned and processed using Kubios software (Tarvainen et al., 2014). Participant's heart rate reserve (HRR), defined as the participant's minimum HR (HR_{min}) subtracted from his maximal HR (HR_{max}), was calculated using cleaned data from the CPET protocol.

2.2.2. Randomization

Prior to their second visit, participants were randomized to one of three exercise intensity groups: (12) "Light (20–39% HRR), Moderate (40–59% HRR), and Vigorous (60–84% HRR). Participants in the light intensity group were asked to perform their treadmill exercise at 25–35% of their HRR above their HR_{min} (generally a light to moderate walk) to ensure they do not drop below or go above the HRR range for light intensity. Participants' directed range was 45–55% of their HRR in the moderate intensity group (generally a brisk walk to light jog), and 65–75% of each participant's HRR above their HR_{min} in the vigorous intensity group (generally a steady run). Randomization procedures were completed using a random-number generator in Excel. Target exercise HR ranges for each participant was calculated prior to their arrival on the second visit.

2.2.3. Study Visit 2: experimental day

The second laboratory visit was conducted to examine the main research question in this study: does the intensity of acute aerobic exercise predict the extent of HPA-axis cortisol response to a subsequent stressor? All visits were scheduled between 1:00 pm and 5:00 pm to minimize circadian variations in sCort, which are typically marked by peak rises in the morning and steady declines throughout the remainder of the day. Participants were asked if their health status had changed since their first appointment. If so, a researcher prompted them to report any physical health change or newly prescribed medications and re-administered the screening questionnaire. As requested, each participant confirmed that they had not engaged in any exercise that day, eaten any meals or drank caffeine within 2 h prior to testing, consumed any alcohol, and hydrated well prior to their appointment. Participants were fitted with the same Polar H10 HR monitor used during the first visit. HR was recorded continuously over the course of the entire protocol. Participants then quietly remained seated in a comfortable resting position for 20-minutes.

Following this baseline resting period, participants engaged in 30 min of aerobic treadmill exercise at their prescribed intensity as determined by the randomization protocol described above. Participants were not expected to maintain a perfectly stable HR but were expected to stay within the 10-percentage unit HR range defined by their respective intensity group. The researcher supervising the study ensured that participants stayed within this range by continuously monitoring participant HR and adjusting the speed of the treadmill accordingly. This exercise bout was proceeded by a 5-minute walking cool down at 2.7 km/hour. Following the cool down, participants returned to a seated resting position for 45 min. At the end of this resting phase, the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993), a well-validated psychosocial stress induction task, was administered. The task consists of a public speaking protocol and serial subtraction task in front of a panel of evaluators. Due to the social evaluative and uncontrollable nature of the task, the TSST reliably elicits a substantial HPA-axis response (Dickerson and Kemeny, 2004). Following the task, participants remained in the laboratory for an additional 70 min in order to capture cortisol reactivity and recovery post-stressor.

2.3. Measures

Salivary Cortisol: sCort was used as a biomarker indicator of the HPA-axis response to both exercise and the stressor task. Fourteen saliva samples were collected in total during the experimental day, half during the bout of exercise and half during the TSST. Specifically, seven samples were collected before, during, and after exercise ($T1_{ex}$ to $T7_{ex}$): −20 min, 0 min (start of bout), +15 min, +30 min (end of bout), +35 min (5 min post-bout), +45 min (15 min post-bout) and +60 min (30 min post-bout). Seven samples were then collected before, during, and after the TSST ($T1_{stress}$ to $T7_{stress}$): 0 min (start of TSST), +15 min (end of TSST), +25 min (10 min post-TSST), +35 min (20 min post-TSST), +45 min (30 min post-TSST), +65 min (50 min post-TSST), and +85 min (70 min post-TSST). Saliva was collected using standard commercial polypropylene, low density polyethylene swabs (Salivette®; Sarstedt, Rommelsdorf, Germany) following standard protocols and stored in −20 ºC storage freezers in the laboratory until study completion, at which point they were shipped to Dresden LabService GmbH (Germany) for biochemical analysis. At Dresden LabService GmbH, saliva samples were frozen and stored at −20 degrees C until analysis. After thawing, salivettes were centrifuged at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Salivary concentrations (nmol/L) were measured using commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). The intra and interassay coefficients for cortisol were below 5%.

2.4. Statistical analysis

An a priori power analysis, calculated using G*Power (Faul et al., 2007), was used. A MANCOVA model was used, with between- and within-group factors, to detect a medium-to-large effect, $f = 0.30$. This effect size was determined based on the calculated effect size in an exercise study examining sCort outcomes with similar experimental design (Hill et al., 2008). Power was preset at 0.8. Log transformations were applied to the sCort data to account for skewness and kurtosis. All descriptive and inferential statistics, including model diagnostic criteria, were run using SPSS Version 25. Graphs and tables were completed using R.3.4.1 and Microsoft Office, respectively. Descriptive statistics

were calculated for all measures including covariates. An alpha of.05 was set for all subsequent hypothesis testing.

First, using $T1_{stress}$ to $T7_{stress}$ log transformed cortisol data, an unconditional model (i.e. with no predictors) was constructed for sCort to determine the variance occurring within individuals across time and the variance between individuals. Given the parabolic nature of the stress response (increase in the sCort following the TSST, and then subsequent recovery), level-1 quadratic time models were constructed with time and time-squared as fixed effects and an unstructured covariate matrix was applied (Puterman et al., 2011). The general level-1 model can be written as: $Cortisol_{Stress} = \beta_o + \beta_{rise}Time + \beta_{curvature}Time^2$, where β_o is the estimated intercept, β_{rise} is the initial rate of change (i.e. reactivity), and $\beta_{curvature}$ is the curvature of the response (speed of recovery) (Singer and Willett, 2003). The estimated time for sCort was also estimated with the following equation: $Time_{peak} = -\beta_{rise}/(2 * \beta_{curvature})$.

Next, the variable of interest, exercise-intensity group, was included in a level-2 model, with each group dummy coded, and the light group serving as the referent. To compare the vigorous condition to the moderate condition, the moderate group was recoded as the referent in a separate analysis. The estimated model was as follows:

$Cortisol_{Stress} = \gamma_{00} + \gamma_{01}Moderate + \gamma_{02}Vigorous + \gamma_{10}Time + \gamma_{11}Moderate * Time + \gamma_{12}Vigorous * Time + \gamma_{20}Time^2 + \gamma_{21}Moderate * Time^2 + \gamma_{22}Vigorous * Time^2$, where γ_{00} is the intercept for the light group, γ_{01} and γ_{02} are the estimated mean differences in the intercepts of the moderate and vigorous groups compared to the light group, respectively, γ_{10} is the initial rate of change of the light group, γ_{11} and γ_{12} are the mean differences in the initial rates of change for the moderate and vigorous groups compared to the light group, respectively, γ_{20} is the curvature of the trajectory for the light group, and γ_{21} and γ_{22} are the estimated mean differences in the curvatures of the trajectory for the moderate and vigorous groups compared to the light group, respectively. Significant coefficients from the interaction model would suggest that cortisol trajectories vary as a function of each level of exercise intensity.

A subsequent set of analyses was used to address our second hypothesis: the sCort trajectory resulting from a stressor task is a function of the sCort response to a prior bout of acute aerobic exercise. sCort responses to the bout of exercise were computed as an area under the curve relative to the ground (AUC_Gs) (Pruessner et al., 2003) aggregated across the time points $T2_{ex}$ – $T7_{ex}$ (subsequently referred to as AUC_sCort_{ex}), and log-transformed. $T1_{ex}$ was excluded since this sample was collected prior to the commencement of the bout of exercise. First, ANCOVAs, with age and VO2 peaks as covariates, were used to examine whether significant mean differences in the AUC_sCort_{ex} output to aerobic exercise differed between exercise-intensity groups. Then, growth curve analyses were constructed to model the sCort response to the stress task as a function of AUC_sCort_{ex} and time. The level-1 model for this analysis was fit in the same manner as that demonstrated above; however, moderate and vigorous intensity groups were replaced with just one variable, AUC_sCort_{ex}. Simple slopes analysis was used in order to estimate the cortisol trajectories for individuals whose AUC cortisol response to exercise was at the mean, or 1 standard deviation (SD) above or below. To examine the extent to which intensity levels or the AUC_sCort_{ex} accounted for cortisol trajectories, pseudo-R^2 were calculated (Hedeker and Gibbons, 2006).

In all analyses, MIXED Syntax with restricted maximum likelihood (REML) estimates were used, as they are robust for handling skewed data (characteristic of sCort studies) and computing unbiased estimators (Raudenbush and Bryk, 2002; Singer and Willett, 2003).

3. Results

3.1. Univariate results

Mean age of the sample was 21.04 (SD = 2.89), and a majority was Caucasian (n = 40, 47.1%), with an additional 31.8% (n = 27) of Asian descent, 8.2% (n = 7) of Latin American descent,4.7 (n = 4) of Indian descent, 2.4% (n = 2) of Middle Eastern descent, 2.4% (n = 2) of African descent, and 1.2% of mixed origin. The majority of men were completing their undergraduate degrees (75%), whereas 11.8% were completing their graduate degrees, and an additional 8.2% were employed by the university.

Participants were randomized to light- (N_{light} = 28), moderate- ($N_{moderate}$ = 27), or vigorous-intensity ($N_{vigorous}$ = 28) exercise conditions. Table 1 presents mean (SDs) for age, BMI, VO2-peak, perceived stress levels, and measures taken during the second visit, including resting HR and BP, and room temperature and humidity, for participants in each group. These physiological descriptors and essential vital signs demonstrated that participants in each group represented a comparable and physiologically healthy cohort that were on average similar to healthy norms and not overly or under fit based on criteria established by the American College of Sport Medicine (Jonas and Phillips, 2009). Participants' Perceived Stress scores ($\bar{x}$ = 14.02, SD = 5.57) were normally distributed and fell in the low-to-moderate range (Cohen et al., 1983). Experimental conditions (temperature and humidity) during the second study visit were also similar between groups.

For the second visit, 91.5% of participants were able to attain their prescribed level of activity. While 8.5% (7 participants) fell outside of their set goals, none fell outside of the intensity zones outlined by the American College of Sports Medicine guidelines for exercise testing in the light (20–39%), moderate (40–59%) and vigorous intensity zone (60–84%), respectively (American College of Sports Medicine, 2018).

3.2. Unconditional means models

The interclass correlation was 0.34, indicating that 34% of the variance in sCort levels during and following the TSST was explained by differences between individuals and 66% occurred at the within person level. The design effect for the model was 3.03. Design effects above 2 are seen as requiring a multilevel structure. Level-1 and level-2 models were thus constructed.

3.3. Level-1 growth model: cortisol response as a function of time

Model fit with time and time-squared as predictors indicated that across all participants, sCort followed a curvilinear relationship (Fig. 1), suggesting successful implementation of the TSST. Time accounted for 57.4% of the unexplained variance from the null model. At time = 0,

Table 1
Means (standard deviations) for physiological and psychological descriptors by exercise intensity group.

	Total (N = 83)	Light (N = 28)	Moderate (N = 27)	Vigorous (N = 28)
Age	21.04 (2.89)	21.57 (3.11)	21.00 (2.79)	20.54 (2.77)
BMI (kg/m^2)	23.69 (2.90)	24.25 (3.25)	23.60 (2.79)	23.20 (2.62)
VO2-peak (ml/min./kg)	45.77 (6.94)	44.77 (5.85)	46.80 (8.26)	45.78 (6.65)
Perceived Stress	14.02 (5.57)	12.59 (4.45)	14.07 (6.60)	15.36 (5.29)
Heart Rate (bpm)	67.33 (9.97)	68.71 (8.54)	66.30 (9.00)	66.96 (12.15)
Systolic BP (mmHg)	115.53 (6.70)	113.91 (7.75)	116.17 (5.98)	116.68 (6.00)
Diastolic BP (mmHg)	65.20 (6.44)	65.80 (6.76)	64.19 (4.43)	65.50 (7.63)
Room Temperature (C°)	23.26 (0.80)	23.46 (0.74)	23.16 (0.94)	23.15 (0.67)
Room Humidity (%)	31.25 (7.50)	29.96 (6.61)	31.82 (9.10)	32.08 (6.59)

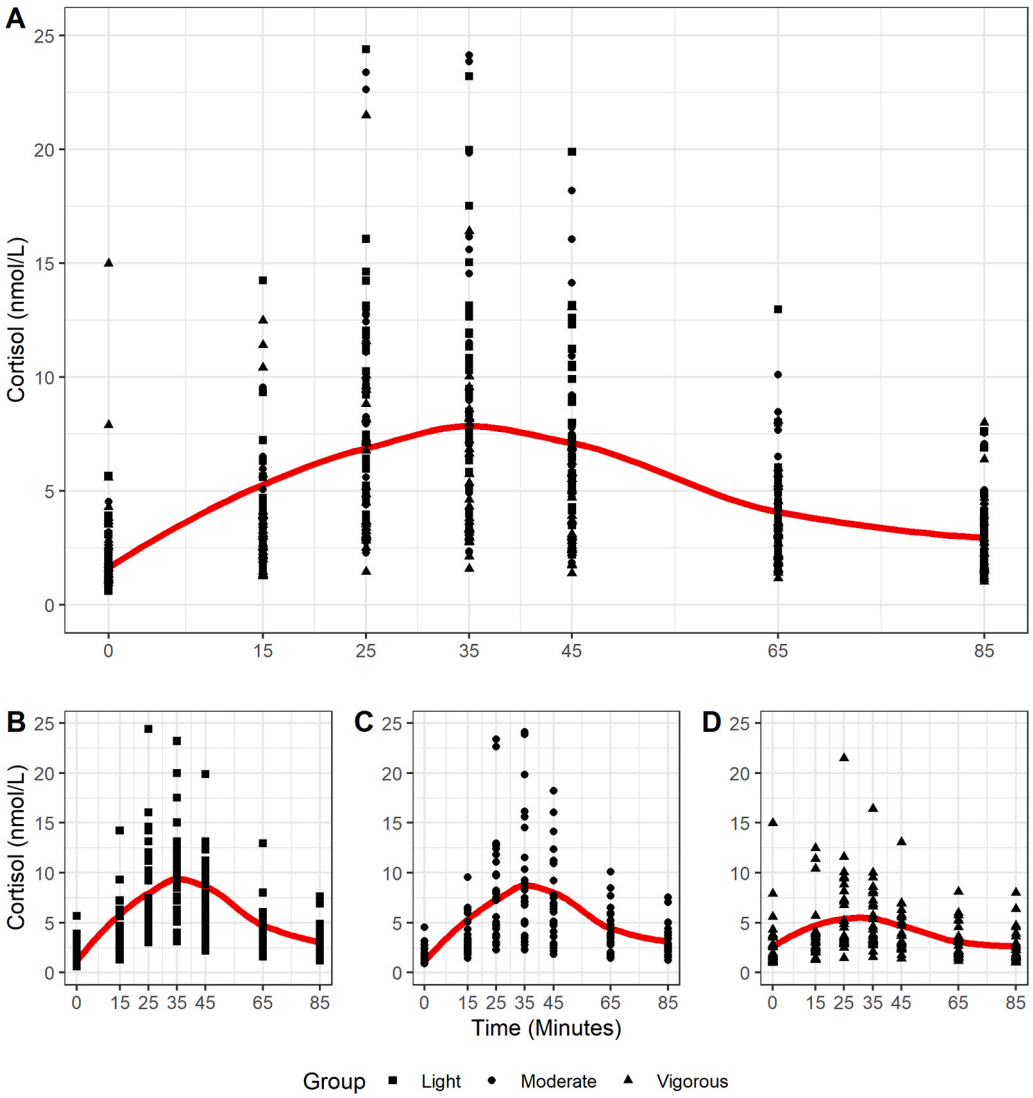

Fig. 1. Scatter plot for the cortisol stress response to the TSST as a function of time for all participants (A), and by Group (B-Light, C-Moderate, D-Vigorous). Note. Raw cortisol data presetned for all participants (Panel A), and by group (Light, B; Moderate, C; Vigorous, D) at each time point, line drawn using local polynomial regression (LOESS) fitting.

participants' estimated log-transformed cortisol was $\beta_o = 0.26$ units ($SE = 0.028$, $p < 0.001$), increased at a rate of $\beta_{rise} = 0.023$ units/minute ($SE = 9.0e\text{-}4, p < 0.001$ and showed a curvature of $\beta_{curvature} = -2.5e\text{-}4$ ($SE = 1.0e\text{-}5$, $p < 0.001$). Log-transformed cortisol peaked at an estimated 44.4 min following the onset of the stressor and reached an estimated value of 0.77 units.

3.4. Cortisol trajectories between groups

We calculated percent increases at each timepoint during the TSST compared to baseline TSST values as a function of their difference score divided by baseline TSST values. We categorized all participants whose percent increases were greater than 15.5% at times between 21 and 45 min following the onset of the psychosocial stressor as responders and all percent increases at or below that value as non-responders, as described by Miller et al. (2013). In response to the TSST, 4 of the 83 participants could be categorized as non-responders, and all 4 were within the vigorous exercise group.

To test the primary hypothesis, the level-2 moderate and vigorous intensity groups, and their interactions with time and time-squared, were included in the model (comparisons between vigorous and moderate groups were completed in a separate set of analyses, as outlined above, and all results reported here). The combined model accounted for an additional 13.7% of the variance compared to the time and time-squared only model. As depicted in Fig. 2, the estimated intercepts, initial rates of increase and curvatures for participants in all three activity groups were significant. Specifically, the vigorous group's estimated log-sCort intercepts were significantly larger than both the moderate ($\gamma_{02} = 0.147, SE = 0.066, CI = [0.017, 0.278], p = 0.027$) and light ($\gamma_{02} = 0.174, SE = 0.066, CI = [0.044, 0.303], p = 0.009$) groups, but the moderate and light groups' intercepts were not different from each other ($\gamma_{01} = -0.026$, $SE = 0.066$, $CI = [-0.105, 0.157]$, $p = 0.693$). Those who exercised at vigorous intensities had a significantly slower estimated rate of increase in log-sCort and deceleration compared to both the moderate ($\gamma_{12} = -0.013$, $SE = 0.002$, $CI = [-0.017, -0.008]$, $p < 0.001$, $\gamma_{22} = 1.2e\text{-}4$, $SE = 2.3e\text{-}5$, $CI = [7.8e\text{-}5, 1.7e\text{-}4]$, $p < 0.001$) and light ($\gamma_{12} = -0.0172$, $SE = 0.002$, $CI = [-0.021, -0.013]$, $p < 0.001$; $\gamma_{22} = 1.7e\text{-}4$, $SE = 2.3e\text{-}5$, $CI = [1.2e\text{-}4, 2.1e4]$, $p < 0.001$) groups. The moderate group also demonstrated a significantly slower estimated rate of increase ($\gamma_{11} = -0.004$, $SE = 0.002$, $CI = [-0.008, -0.0001]$, $p = 0.042$) and deceleration ($\gamma_{21} = 4.8e\text{-}5$, $SE = 2.3e\text{-}5$, $CI = [-3e\text{-}6, -9.3e\text{-}5]$, $p = 0.038$) as compared with the light group. Results were unchanged when the 4 non-responders in the vigorous group were excluded from these analyses. For

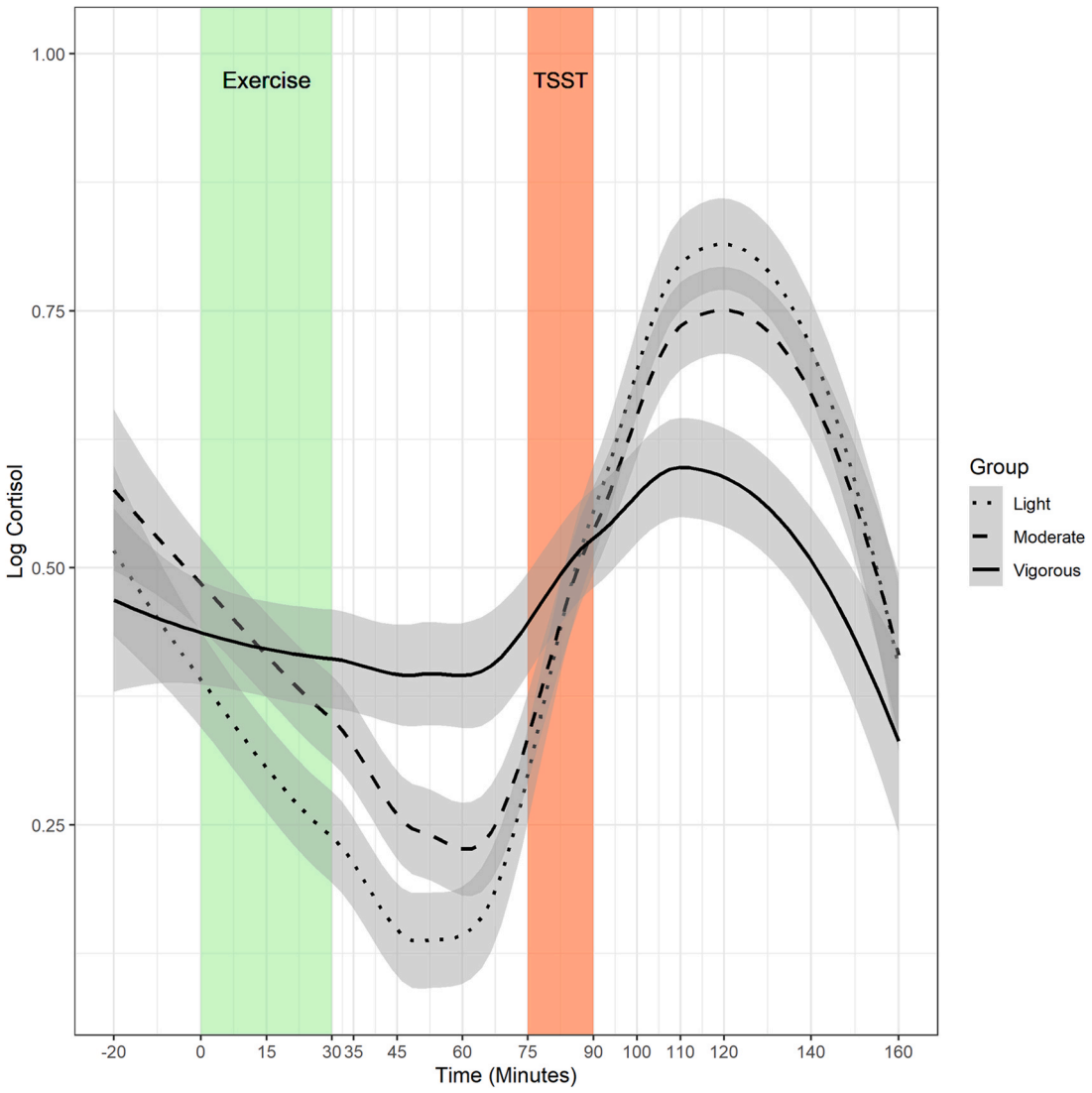

Fig. 2. Salivary cortisol response to a single bout of exercise followed by the TSST by group and time. Note: Trajectories of log-transformed cortisol during and following the single bout of exercise and the TSST for participants randomized to the light, moderate, and vigorous exercise groups. Trajectories were drawn using local polynomial regression (LOESS) fitting. Grey areas represent 95% confidence intervals.

the light, moderate, and vigorous intensity groups, the estimated sCort peak occurred at 45.5 min, 45.5 min, and 40.39 min, respectively. sCort trajectories were estimated to return to baseline for the vigorous intensity group over the course of the experiment (within 85-minutes following the TSST), with estimated returns to baseline 81 min following stressor onset. Extrapolations from the model indicated that both the light and moderate exercisers would return to their pre-stress baselines approximately 91 min following stressor onset.

3.5. *Exercise cortisol as a predictor of cortisol response to the TSST*

Trajectories of cortisol in response to the bout of exercise are demonstrated in Fig. 2. As observed, while light and moderate intensity exercise group participants decreased in their cortisol over time (as expected with midday diurnal cortisol declines), and there was an increase in salivary cortisol output in the vigorous exercise group.

An ANCOVA, with age, VO2-peak, and perceived stress as covariates, revealed that log-transformed AUC_sCort$_{ex}$ was significantly different between exercise intensity groups, $F(2, 77) = 3.94$, $\eta^2 = 0.082$, $p = 0.035$. Tukey Post-Hoc comparisons revealed that the vigorous intensity exercisers showed a significantly greater AUC_sCort$_{ex}$ response compared to light-intensity exercisers ($t(77) = -2.62$, $p = 0.028$). Vigorous intensity exercise did not significantly differ from those in the moderate group ($t(77) = -1.03$, $p = 0.561$) nor did moderate intensity exercisers differ from those in the light group ($t(77) = -1.58$, $p = 0.259$).

When level-2 growth curve models were constructed by adding AUC_sCort$_{ex}$ and its interaction with time and time-squared into the level-1 model, it accounted for an additional 3.4% of the variance relative to the level-1 model. In this model, significant effects for the intercepts ($\gamma_{01} = 0.002$, SE = 2.0e-4, $p < 0.001$), the interaction between the AUC_sCort$_{ex}$ and time (i.e. slope; $\gamma_{11} = -4.0e\text{-}5$, $SE = 7.0e\text{-}6$, $p < 0.001$), and the interaction between AUC_sCort$_{ex}$ and time-squared (i.e. quadratic; $\gamma_{21} = -3.6e\text{-}7$, $SE = 7.7e\text{-}8$, $p < 0.001$) were demonstrated to predict sCort outcomes to the stressor. Simple slopes analysis (represented in Fig. 3) revealed that estimated baseline TSST cortisol values were highest when estimated at 1 standard deviation above the mean AUC_sCort$_{ex}$ (Estimate = 0.46, SE = 0.04, 95CI = 0.39, 0.53), followed when estimated at mean AUC_sCort$_{ex}$ values (Estimate = 0.26, SE = 0.02, 95CI = 0.21, 0.31), and then followed when estimated at 1 standard deviation below the mean AUC_sCort$_{ex}$ (Estimate = 0.06, SE = 0.04, 95CI = −0.01, 0.13). Rates of increase (i.e. slope) and curvature (i.e. quadratic) were as follows: (1) when estimated at 1 standard deviation above the mean (slope Estimate = 0.018, SE = 0.001,

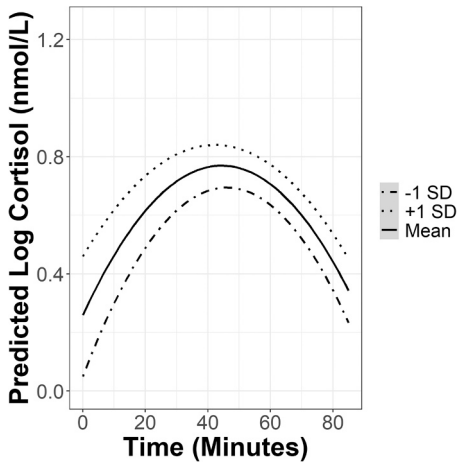

Fig. 3. Estimated salivary cortisol response to TSST across time at one standard above (+1 SD), at (Mean), and below (−1 SD) the mean of the cortisol output in response to exercise alone. Note: Trajectories of log-transformed cortisol during and following the TSST at one standard deviation below, above, and at the mean of the total outputted cortisol during and following the bout of exercise, computed as an area under the curve (AUC_sCort$_{ex}$). Trajectories were drawn using estimates for intercept, slope, and quadratic terms at each level.

95CI = 0.016, 0.020; quadratic Estimate = −2.1e-4, SE = 1.4e-5,.95CI = −2.4e-4, −1.9e-4); (2) when estimated at the mean (slope Estimate = 0.023, SE =;8.7e-4, 95CI = 0.021, 0.025; quadratic Estimate = −2.6e-4, SE = 1.0e-5, 95CI = −2.8e-4, −2.4e-4); and when estimated at 1 standard deviation below the mean (slope Estimate= 0.028, SE = 0.001,.95CI = 0.026, 0.030; quadratic Estimate = −3.0e-4, SE = 1.4e-5, 95CI = −3.3e-4, −2.8e-4). In general, rates of increase were highest at one standard deviation below the mean of AUC_sCort$_{ex}$, as was the speed of curvature at the inflection point where recovery was estimated to begin, whereas the slowest increase was at one standard deviation above the mean of AUC_sCort$_{ex}$, as was the curvature at the inflection point of recovery.

4. Discussion

4.1. Dose-dependent effect of exercise intensity

This is the first study to determine that a causal dose-response relationship exists between intensity of a bout of aerobic exercise and the HPA-axis cortisol reactivity, peak, and recovery responses to a subsequent stressor 45-minutes later. While there was a clear intensity-based dose response, it was particularly evident that vigorous exercisers experienced a slower rate of increase and lower peak, and speedier recovery back to pre-TSST levels of sCort as compared to the other groups. This dampened response is likely a result of the cortisol released in response to vigorous exercise that instigated a glucocorticoid negative feedback loop (see 4.2 for more in-depth discussion). These effects were not driven by the four vigorous intensity exercisers who displayed the lowest responses, categorized as 'non-responders' based on previous work (Miller et al., 2013). This demonstrates the particular utility of vigorous exercise in buffering the physiological response to a stressor when experienced close in time to each other.

This work addresses important limitations encountered in previous research exploring the mitigating effects of a single bout of exercise on the HPA-axis response to a stressor. Previous work primarily used quasi-experimental designs focusing on active/fit compared to inactive/unfit groups (Puterman et al., 2012; Rimmele et al., 2007, 2009; Traustadóttir et al., 2005), in which participants were asked to participate in a psychosocial stress task. When prior studies have implemented an exercise protocol prior to a stressor, they have primarily used a binary model with an exercising and non-exercising group (Wood et al., 2018; Zschucke et al., 2015). This design fails to explore the distinct features (e.g., intensity) of exercise and thus the mechanisms through which it exerts stress-mitigating effects. This work also provides the foundation for future studies that aim to explore other stress-mitigating features of acute exercise (e.g., duration, modality). Future studies may choose to employ a similar design, but manipulate the duration of exercise, the type of exercise performed, and the interval between the bout and the subsequent stressor. With respect to the latter, this study showed that acute vigorous exercise can buffer the cortisol response to a stressor up to 45 min following the bout, a time frame similar to those found in daily diary studies (Puterman et al., 2017). Future work may aim to explore the latency of this effect.

4.2. Glucocorticoid negative feedback as a mechanism of acute cross-stressor adaptation

The current study further demonstrated that exercising vigorously elicited a larger overall cortisol response to the 30-minute bout compared to the light exercisers. This finding directly mirrors previous work demonstrating that the cortisol response to exercise is proportional to the intensity at which it is performed (Duclos and Tabarin, 2011). Moreover, cortisol released during the vigorously intense bout of exercise accounted for a portion of the variance in the cortisol response to the psychosocial stressor. Participants with larger overall cortisol responses to the single bout of exercise entered the TSST with higher baseline cortisol levels but were more likely to have a dampened cortisol trajectory in response to the stressor.

Given that the cortisol response to aerobic exercise was proportional to the intensity at which the exercise was performed, and the cortisol released from the exercise bout inversely predicted the subsequent cortisol trajectory to the TSST, a mechanism of glucocorticoid negative feedback, whereby the cortisol response to the first stressor (i.e., intense exercise) dampens the response to the subsequent heterotypic stressor, is strongly implicated. While the sample size in this study was too small to assess a true mediation model, whereby cortisol released from exercise mediates the effect of exercise intensity on the cortisol response to the TSST, the study nonetheless supports a glucocorticoid negative feedback mechanism of acute cross-stressor adaption. Zschucke and colleagues (2015) were the first to posit that cross-stressor adaptation of acute exercise may occur as a result of glucocorticoid negative feedback. In their work, highly trained and sedentary men were randomized to aerobic exercise (at 60–70% VO2-peak) or placebo exercise (light stretching) prior to a mild laboratory psychosocial stressor. Men in the aerobic exercise condition showed smaller cortisol change scores from pre- to post-psychosocial stressor compared to the placebo exercise condition. Moreover, exercise-induced changes in cortisol were negatively correlated with the cortisol response to their stress task as well as fMRI activity in hippocampal regions implicated in downstream target sites of glucocorticoid negative feedback. Our findings further support the argument that glucocorticoid negative feedback might be at play here, by illustrating inverse relationships between both exercise-intensity and the cortisol response to the psychosocial stressor as well as the cortisol released from this exercise and the psychosocial stressor.

In the current study, the time frame between the exercise bout and the psychosocial stressor was limited to 45 min. Glucocorticoid negative feedback and inhibition of corticotropin releasing hormone and adrenocorticotropic hormone occurs along fast (seconds to minutes) and delayed (hours to days) timeframes, when cortisol-like steroids are administered exogenously in mouse-models in vitro. Fast-action inhibition can occur within milliseconds on neurons and within minutes at the pituitary upon steroid exposure. The magnitude of the fast inhibition reaches approximately 25% of the maximal inhibition possible and is thus very small. Fast action inhibition does not last long, allowing for responses to subsequent stressors at nearly normal levels. This fast-action negative feedback takes place at the cell membrane and is independent of genomic effects; it is not blocked by suppression of RNA or

protein synthesis (Dallman, 2010). In contrast, intermediate-action inhibition (i.e. genomic) has its onset at approximately 30-minutes to an hour after steroid administration and HPA-axis inhibition can last for hours. This intermediate-time effect weakens excitability in the HPA-axis more substantially than fast-action inhibition without fully abolishing it. It seems to rely on genomic alterations at the level of protein synthesis and epigenetic modification of intra-cellular signaling mechanisms (Gjerstad et al., 2018; Keller-Wood and Dallman, 1984). The current study included a 45 min gap between the completion of the bout of exercise and the initiation of the TSST in line with the expected genomic effects of cortisol. Future research should seek to manipulate the time between the bouts to understand how long a single bout of vigorous exercise can induce a dampened cortisol response to a psychosocial stressors.

While glucocorticoid negative feedback is a likely mechanism that accounts for our effects, other physiological and psychological processes, not measured in the current study, may have played a role as well. Brain-derived neurotrophic factor, monoaminergic neurotransmitters, and endogenous opioids are released in response to single bouts of exercise in an intensity-dependent manner and have all been implicated in the mood and stress-mitigating effects of exercise (Basso and Suzuki, 2017). Furthermore, previous research has shown that a single bout of moderate-to-vigorous exercise significantly alters cognitive and affective responses to a subsequent psychosocial stressor (Bernstein and McNally, 2017). Future work should seek to incorporate measures of affect and cognition and draw blood for measurement of circulating peripheral levels of neurotransmitters to determine the independent effects of other physiological and psychological processes that may explain the impact of a single bout of exercise on individuals' responses to a subsequent psychosocial stressor.

4.3. Exercise and chronic stress over the lifespan

The results from this study have direct implications for mitigating the cortisol response to psychological threats in a cohort of healthy, moderately fit adult males. Future work will need to explore whether the stress-mitigating features of acute vigorous exercise interact differently with the disrupted HPA-axis activity found among those particularly vulnerable to frequent stressor exposure over the lifespan. Those who are chronically stressed, like family caregivers for example, show HPA-axis diurnal rhythmicity marked by lower concentrations of morning cortisol, greater concentrations of afternoon/evening cortisol, generally flatter diurnal rhythms, and higher daily volumes over the entire day (Miller et al., 2007). Excessively elevated daily peak cortisol and slower cortisol recovery in everyday life are associated with depressive symptomatology and memory impairment, and compromised immune function and development of chronic disease (Heim and Nemeroff, 1999; McEwen, 1998). Consistent exercising at vigorous intensities (e.g. jogging, running, aerobic dance) may help to normalize daily cortisol rhythms and mitigate abnormal cortisol responses among groups particularly vulnerable to stressor exposure. To date, no study to our knowledge has explored the effects of vigorous exercise on the daily cortisol response in individuals impacted by stressors in their everyday life. The closest work, however, was a randomized control trial demonstrating that an exercise training program reduces stress reactivity to an acute stressor task among healthy individuals (Klaperski et al., 2014). Still, future work will need to assess the stress-mitigating utility of vigorous exercise among those who are chronically stressed.

4.4. Limitations

Testing a true mediation model whereby the cortisol response to exercise mediates the effect of exercise intensity on the cortisol response to a stress task was not adequately powered in the current study. Future work will need to confirm this effect to further support a glucocorticoid negative feedback mechanism of cross stressor adaptation. Moreover, this study was underpowered to explore the interaction between participants' a priori VO2-peak fitness and exercise intensity on the TSST stress response. This question would further define the populations that can benefit most from exercising vigorously to reduce stress (i.e., those who are under-fit compared to moderately or highly fit).

While operationalizing exercise intensity in terms of a percentage of a maximum value (either VO2-peak or HR) or a reserve range is common in contemporary laboratory paradigms, including this one, these measures may not correspond with the level at which true physiological changes occur. There is evidence that exercise performed at intensities above and below the ventilatory threshold, defined by respiration rate increasing to lower the partial pressure of arterial CO_2 and minute ventilation reaching a maximum level, are differentially associated with changes in sympathetic and HPA-axis activity (Schwarz and Kindermann, 1990; Urhausen et al., 1994). Future work will need to ascertain whether the ventilatory threshold is the true physiologic marker of exercise intensity that predicts changes along the HPA-axis response to a psychosocial stressor.

To identify novel physiological trends, this study prioritized internal validity at the expense of generalizability. Although future work is needed to determine the extent to which these acute effects generalize to women, prior studies that included women in their samples suggest that women who engage in moderate or high levels of physical activity in their everyday lives, compared to those low in activity, have reduced physiological (i.e. cortisol, heart rate) responses to a lab-induced psychosocial stressor (Klaperski et al., 2013) similar to previous research in men (Rimmele et al., 2009, 2007). Men and women also similarly have greater cortisol output to a single bout of exercise at high intensity as compared to lower intensity (McGuigan et al., 2004), thus it would be expected that our results would potentially extend to women as well. Future work will also need to examine the effect of exercise intensity on the stress response in normal social conditions and among diverse human groups, including older adults (who have been shown to have heightened cortisol reactivity to stress exposure (Lupien et al., 2009)), and those chronically stressed or who suffer from psychiatric disorders that are associated with little to no HPA activation to psychosocial stressors (e.g., people with posttraumatic stress disorder) (Miller et al., 2007). Future studies may attain this aim by using naturalistic studies with diverse samples to assess the effect of exercise intensity on cortisol measures taken over the course of the day.

5. Conclusion

Physical activity and exercise have been shown to boost immunity and reduce the risk of disease and mortality. This study is the first to establish that even a single bout of exercise at increased intensities has the capacity to reduce the acute HPA-axis stress response compared to milder intensity bouts, in a group of healthy young-adult men. A dose-response of exercise intensity was confirmed, whereby higher intensity exercise mitigated the cortisol reactivity and hastened the recovery to a psychosocial stressor task 45 min later. The fact that more vigorous exercise changes neuroendocrine resiliency to stress reveals a plausible pathway through which exercise preventatively contributes to bolstered health and reduce risk of chronic disease. However, whether the reductions in the cortisol response to a psychosocial stressor that have been evidenced here have future health implications is unknown, requiring exploration in future studies.

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CRediT authorship contribution statement

A. Caplin: Conceptualization, Formal analysis, Investigation, Data curation, Writing - Original draft, Project administration. **F.S. Chen:** Conceptualization, Methdology, Writing - review & editing. **M.R. Beauchamp:** Conceptualization, Writing - review & editing. **E. Puterman:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

IRB and Preregistration

The study was approved by the University of British Columbia's Clinical Ethics Research Board (H17–00588).

Conflict of Interest

Authors declare no conflict of interest.

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