The effect of acute exercise on the cortisol awakening response

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Abstract:

The effects of acute exercise on the cortisol awakening response (CAR), characterized by the rapid increase in cortisol concentrations within the 30–45 min following sleep offset has yet to be fully elucidated. Thus, our study investigated the effects of late-evening acute exercise on the CAR the following morning. We hypothesized that exercise would have a significant effect on the CAR the following morning. Twelve participants (mean (SD): age = 23 (4) years; mass = 76.8 (8.7) kg; height = 175.6 (5.0) cm; $\dot{V}O2max$ = 48.9 (7.5) ml.kg−1.min−1) reported to the laboratory in the evening (1800 h) on two occasions and were randomly assigned to either exercise for one hour (70–75% of maximal power output) or rest condition. Blood and saliva samples were assayed for cortisol. Mixed-effects models determined the effect of exercise on the cortisol response post-waking in both blood and saliva. Participants demonstrated an average exercise-induced increase in circulating cortisol of 477.3%, with actual mean (SD) heart rate relative to maximum of 87.04% (6.14%). Model results demonstrated a negative effect for exercise condition when modeling the serum and salivary cortisol responses to awakening via a quadratic growth model (serum, βCondition = − 42.26 [95% CI − 64.52 to − 20.01], $p < 0.001$; saliva, βCondition = − 11.55 [95% CI − 15.52 to − 7.57], $p < 0.001$). These results suggest that cortisol concentrations in saliva and blood are significantly lower the morning following a prior evening exercise session. Therefore, the CAR may serve as a useful biomarker to monitor responses to exercise training, although the underlying mechanism for these decreases in the CAR should be investigated further.

Keywords: circadian | cycling | hormonal profile | sleep

Article:

Introduction

Physiological and psychological inputs to the hypothalamus can stimulate the hypothalamic–pituitary–adrenal axis (HPA-axis) neuroendocrine cascade, ultimately increasing cortisol concentrations. Exercise is considered a physiological stressor, as it induces many perturbations to

biological homeostasis, including alterations in metabolic requirements, thermoregulation, and cardiovascular adjustments. An increase in total cortisol concentration in response to acute exercise is well documented (Lutoslawska et al. 1991; Fahrner and Hackney 1998; Inder et al. 1998; Gozansky et al. 2005; Starkie et al. 2005; Hill et al. 2008) and tends to be proportional to the volume of exercise completed, requiring both a minimum exercise intensity (Jacks et al. 2002; Hill et al. 2008) and duration (Viru et al. 1992; Duclos et al. 1997; Tremblay et al. 2005).

These acute exercise responses occur against a background of the robust circadian rhythm of cortisol. In addition to the diurnal profile, cortisol also tends to present a marked increase within the hour after waking from nocturnal sleep. This increase in cortisol concentration, termed the cortisol awakening response (CAR), has been implicated in a range of psychopathological conditions, including depression (Adam et al. 2010; van Santen et al. 2011; Vrshek-Schallhorn et al. 2013; Dedovic and Ngiam 2015), chronic fatigue syndrome (Roberts et al. 2004; Nater et al. 2008; Heim et al. 2009; Hall et al. 2014) and burnout (Pruessner et al. 1999; Grossi et al. 2005; Oosterholt et al. 2015), as well as exercise-related conditions such as the overtraining syndrome (Anderson et al. 2021).

However, very few investigations (Anderson et al. 2018; Juliff et al. 2018; Ucar et al. 2018) have assessed the effect of acute exercise on the CAR. Of these studies, no study has (a) had a laboratory-controlled exercise stimulus, (b) had a supervised CAR sample collection, or (c) assessed both salivary (i.e., free cortisol) and blood (i.e., total cortisol) biomarkers. The elucidation of these points would inform the use of the CAR as a biomarker to monitor acute and chronic exercise responses, as well as improve general guidance for CAR assessment in other domains. Therefore, to address the current gap in the literature, the purpose of this study was to examine the effects of a late-evening lab-controlled acute exercise bout on the CAR, measured via supervised collection in both blood and saliva. We hypothesized that late-evening exercise would have a significant effect on the CAR, but due to the paucity of research on this question, made no hypothesis regarding the direction of the effect.

Materials and methods

Study design

The present manuscript and reported results are a small subsection of data collected as part of a more extensive study. The larger study had three specific aims, each to be addressed separately. First, the study aimed to conduct a laboratory-controlled exercise trial and assess the effect on the CAR; this specific aim is addressed in the current manuscript and thus includes analysis of the participant demographics, exercise trials, and cortisol awakening responses from serum and saliva. The second and third specific aims address the use of alternative salivary biomarkers (manuscript currently in review), and non-invasive correlates of the endocrine responses to exercise and awakening, respectively. The larger study therefore included the measurement and assessment of additional blood, saliva, and autonomic nervous system non-invasive biomarkers, to be analyzed and reported separately. The present study specific aim was investigated using a randomized, cross-over design experiment in which participants completed both an exercise condition and control condition. The study protocol required participants to report to the exercise physiology laboratory at the University of North Carolina at Greensboro for two sets of visits, with two visits per condition for a total of 4 separate visits. For each condition, participants completed a Screening visit and an Experimental visit, separated by approximately 7–10 days. Experimental conditions

were separated by at least eight weeks. The Institutional Review Board approved this study at the University of North Carolina at Greensboro (#20-0244). All participants signed written informed consent before participating.

Participants

Participants (Table 1) were delimited to males between 18 and 30 years of age who were not taking any medications that would disrupt any aspect of the endocrine or autonomic nervous systems, including but not limited to non-steroidal anti-inflammatory pharmaceuticals and centrally acting autonomic nervous system agents, such as methylphenidate. In addition, participants were required to self-report a regular sleep cycle and not engage in any shift work that requires either a delay in the onset of nocturnal sleep or a shortening of sleep duration. Because some evidence exists for the role of obesity on the CAR (see Rodriguez et al. 2015 for review) participants were also restricted to < 25% body fat, as measured by air displacement plethysmography. To complete the exercise intervention, participants were also delimited to having a $\dot{V}O2max > 35$ ml.kg−1.min−1, and to avoid the role of depressive symptomology, participants were excluded if Beck Depression Inventory score of was > 20. Lastly, participants were required to restrict participation in moderate-vigorous exercise and have typical (7–9 h) sleep for 24 h before each experimental visit. Efforts were made to schedule sessions to assist in these behavioral requirements, but visits were rescheduled if the experimental screening questionnaire suggested this requirement was not met. There was an average of 10.3 days (7.2) and 7.4 days (2.0) between screening and experimental visits for the Control and Exercise conditions, respectively. Seven participants were randomly assigned to the Control condition in the first set, whereas 5 participants were assigned to the Exercise condition. Subsequently, in the second set, 5 participants were assigned to the Control condition, and 7 participants were assigned to the Exercise condition, although only 6 Exercise visits were completed.

Procedures

Screening visit

Participants arrived at the laboratory between 0700 h and 1000 h. Participants first completed a series of questionnaires (Medical History, Beck Depression Inventory II, Munich Chronotype Questionnaire, International Physical Activity Questionnaire, Pittsburg Sleep Quality Index, and Hollingshead Index; data not reported here) and had their body composition assessed by a 7-site skinfold assessment (Jackson and Pollock 1978) and air displacement plethysmography (BodPod; COSMED USA, Concord, CA). Participants donned a heart rate monitor (Polar H10 Heart Rate Sensor; Polar Electro Inc., Bethpage, NY) and were fitted to the cycle ergometer (Velotron RacerMate; Quarq Technology, Spearfish, SD) to complete a $\dot{V}O2max$ test to calculate the desired power range for use during the experimental trial and to account for any changes in aerobic capacity between experimental trials. Following a 5-min warmup at 75 W at a self-selected cadence, participants began the test starting at 50 W with wattage increasing by 1 W every 2 s. Participants were asked to maintain their cadence as close as possible to 90 rpm with the test continuing until the subject could no longer maintain a cadence > 50 rpm. Expired gas data was collected and analyzed via indirect calorimetry (TrueOne 2400 Metabolic Measurement System; ParvoMedics, Murray, UT) and included $\dot{V}O2$, $\dot{V}CO2$, and respiratory exchange ratio. The purpose

of this test was to account for any potential differences in cardiorespiratory fitness capacity between the two sets of visits, and to prescribe the exercise intensity for the exercise intervention in the Experimental Visit.

Table 1 Comparison of demographic data, body composition, and maximal exercise test results between screening visits

	Control	Exercise	Overall
Age (years)	24 (5)	24 (4)	24 (4)
Height (cm)	174.1 (6.07)	175.28 (4.97)	174.67 (5.48)
Mass (kg)	74.16 (10.69)	75.53 (8.98)	74.81 (9.71)
Resting heart rate (bpm)	65 (7)	67 (7)	66 (7)
Systolic blood pressure (mmHg)	111 (6)	115 (12)	113 (10)
Diastolic blood pressure (mmHg)	76 (6)	76 (8)	76 (7)
BodPod fat mass (kg)	9.72 (2.84)	10.29 (3.59)	9.99 (3.16)
BodPod fat-free mass (kg)	64.44 (9.57)	65.23 (9.35)	64.82 (9.25)
BodPod fat (%)	13.08 (3.37)	13.76 (5.2)	13.41 (4.25)
BodPod fat-free mass (%)	86.92 (3.37)	86.24 (5.2)	86.59 (4.25)
Skinfolds fat (%)	12.48 (3.98)	12.87 (5.18)	12.67 (4.49)
Urine specific gravity (A.U.)	1.010 (0.007)	1.009 (0.006)	1.010 (0.006)
Urine color (A.U.)	3.00 (1.04)	3.27 (1.35)	3.13 (1.18)
Urine osmolality	410 (277)	398 (259)	404 (263)
V̇VO2max (ml.kg−1.min−1)	50.21 (7.83)	49.64 (7.5)	49.94 (7.5)
Maximal heart rate (bpm)	187.42 (7.61)	187.18 (8.92)	187.3 (8.07)
Respiratory exchange ratio	1.18 (0.07)	1.2 (0.06)	1.19 (0.07)
Peak power (Watts)	304.08 (39.26)	310.73 (42.72)	307.26 (40.14)

Experimental visit

Participants reported to the exercise physiology laboratory by 1800 h for each experimental visit. Participants first answered a series of questions to ensure compliance with the research protocol. Failure to adhere to the exercise restriction guidelines (e.g., not refraining from moderate to vigorous exercise within 24 h of the visit) or atypical sleep prior to the experimental visit resulted in the visit being rescheduled.

Participants provided a spot urine sample to assess hydration status via urine specific gravity (TS Meter-D refractometer, Reichert Technologies, Depew, NY, USA) and urine osmolality (Model 3320, Advanced Instruments, Norwood, MA, USA), followed by assessment of their nude body mass (WB-800S plus, Tanita Corporation, Tokyo, Japan). An indwelling intravenous (I.V.) catheter was placed in the forearm to permit for blood sampling (5 mL) every 15 min (Q15). A keep-vein-open (KVO) protocol administered normal saline (0.9% NaCl) at a drip rate of 20–30 ml.h−1 to maintain line patency without heparin. Blood collection began at 1845 h and continued for the remainder of the visit. All blood samples were permitted to clot at room temperature and then centrifuged at 4 °C at 3000 rpm for 15 min. Serum was pipetted into cryovials in 0.5 ml aliquots. In addition, beginning at 1900 h, subjects produced a saliva sample via passive drool (Gallagher et al. 2006) every waking hour. Serum and saliva samples were stored at − 80 °C until analysis.

Participants were randomized to either the exercise or control condition in their first visit and completed the alternate condition at the second visit. If assigned to the exercise condition, following the catheter placement, participants entered the environmental chamber (Cantrol Environmental Systems, Markham, ON, Canada) with conditions set at 30 °C and 50% relative humidity and were seated on the cycle ergometer by 1855 h. Participants began the exercise intervention at 1900 h. The exercise protocol required participants to complete a warmup of cycling for 5 min, starting at 50 W and increasing intensity every 30 s to a power output corresponding to 70–75% of the maximal power obtained in the V̇O2max assessment from the screening session (V̇O2max mean (SD): 49.94 (7.5) ml.kg−1.min−1). Participants then maintained this power output range for 55 min (1 h of total exercise duration). Cadence was modified (increased or decreased) in 5 W increments as necessary to maintain cadence within the range of 60–90 rpm to maximize the total workload across the exercise bout. After completing the exercise protocol and the 2000 h saliva sample, participants rested in a seated position for another 30 min within the environmental chamber. In the control condition, participants entered the environmental chamber by 1855 h and remained seated in the environmental chamber until 2030 h. Heart rate and RPE were recorded every 5 min. No fluid intake was permitted while in the environmental chamber in either the exercise or the control condition. Immediately after exiting the chamber (2030 h), participants provided a second nude body mass measure, after which participants were permitted to drink water ad lib.

Biochemistry

As part of a more extensive data collection sequence, Q15 blood samples were collected throughout the visit, but given the focus on CAR in the current manuscript, only samples collected at and after waking were considered in the analysis. Thus, selected blood and saliva samples were assayed for cortisol using a commercial competitive enzyme-linked immunoassay (IBL-America, Minneapolis, MN). Awakening saliva samples were first thawed and centrifuged at 14,000 rpm for 15 min before assay. All samples were assayed in duplicate and were reanalyzed if any individual intra-replicate coefficient of variation was > 20%. All participant samples from a single overnight visit were analyzed on the same day.

The intraassay coefficient of variation (CV) for the serum cortisol samples was 5.28%, and the inter-assay CV was 6.24%. Because assay kits were from two kit lots, all samples from a single person were assayed within a single kit lot to ensure quality control. The inter-lot CV was determined by assaying the same controls across lots and was calculated as 7.78%. The intraassay and inter-assay CV for salivary cortisol were 5.25% and 13.11%, respectively.

Data reduction

For awakening response blood (b) and saliva (s) samples, the area under the curve with respect to the ground (AUCG) and with respect to the increase (AUCI) was calculated via the trapezoidal method as described by Pruessner et al. (2003). In addition, the Δ score was also calculated for blood and saliva samples as the difference between the first awakening sample (+ 0 min) and the greatest concentration during the awakening period (13% of serum CAR peaked at + 0 min, 43.5% at 15 min, 26.1% at 30 min, 8.7% at 45 min, and 8.7% at 60 min). For observations wherein the peak occurred at + 0 min, the Δ score was set equal to zero. Lastly, plasma volume shifts from pre to post-exercise (and corresponding time points in the control condition) were calculated according

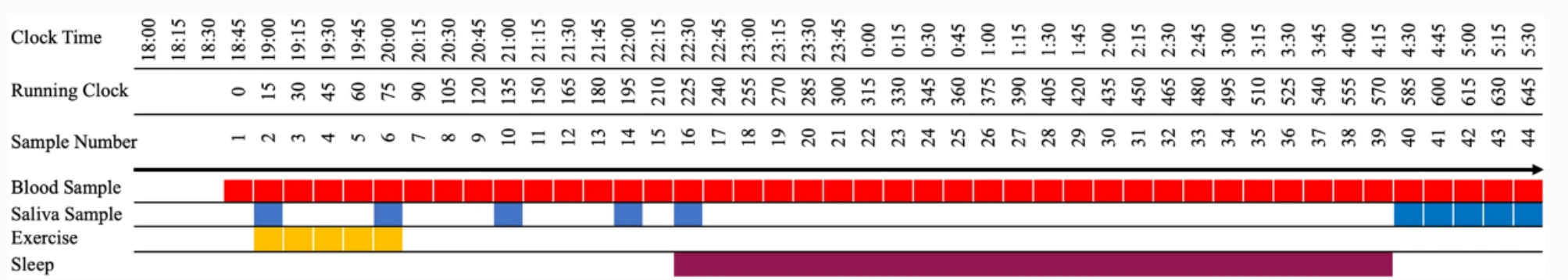

Fig. 1 An example of the Experimental Visit timeline for a theoretical participant who wakes at 04:30 h. Only samples 40–44 (collected between 04:30 h and 05:30 h) are reported and analyzed in the present manuscript.

to the method of Dill and Costill (1974). Hematocrit was determined immediately after blood collection by centrifuging (HemataSTAT-II, EKF-diagnostic, Barleben, Germany) microhematocrit capillary tubes and CritoSeal (VWR International, West Chester, PA, USA) and calculated as the proportion red blood cells relative to whole blood. Hemoglobin was determined using HemoCue microcuvettes via the HemoCue 201 + Analyzer (HemoCue, Ängelholm, Sweden).

Statistical approach

Separate mixed-effects conditional growth models were used to test the effect of condition (fixed effect) on serum and salivary cortisol as a function of and interaction with time (fixed effect). Here, time included both a linear and quadratic effects (orthogonal coefficients) to capture the expected increase and decrease in cortisol concentration. In addition, separate mixed-effects models were used to test the effect of condition on $CARb_{\Delta}$, $CARb_{AUCG}$ and $CARb_{AUCI}$, $CARs_{\Delta}$, $CARs_{AUCG}$, and $CARs_{AUCI}$.

All data analysis was completed via the statistical software R (R Core Team 2020). Mixed-effect models were fit using the lmer function in the lme4 package (Bates et al. 2015, p. 4). For all mixed-effects models, varying random-effects structures were tested, and the best-fitting model, as assessed by Akaike Information Criterion (AIC), −2log-likelihood (−2LL), and $\chi 2$ was selected for further analysis and interpretation of parameter estimates. All inferential statistical analyses were set at $\alpha = 0.05$.

Result

Eleven participants completed all components of this study. One participant completed only the Control condition, and thus there are 23 hormonal profiles across the two conditions (n = 12 control profiles, n = 11 exercise profiles). The participant missing the Exercise condition is thus excluded from analyses contrasting the two conditions but included in analyses assessing relations between variables for which this subject contributes observations from the single condition. Participants did not differ (as assessed via paired-samples t test) by age, height, mass, resting heart rate, or resting systolic or diastolic blood pressure between the two conditions. The average time between screening visits was 11.7 weeks. Further, there were no significant differences between screening visits for any body composition assessment or between any variable assessed in the maximal exercise test on the cycle ergometer (Table 1).

Of the 839 serum samples scheduled to be collected and analyzed, four samples were missed from a single subject in the Control condition (+ 15, + 30, + 45, and + 60 min post waking) due to issues with the I.V. catheter. However, the salivary samples during this period were collected, and so the missing serum samples were imputed from the saliva samples via multiple imputations using chained equations (Azur et al. 2011). In addition, one saliva sample was excluded for having a concentration > 5.4 standard deviations above the mean, possibly indicating blood contamination. Following deletion, this saliva value was then imputed using the same methods described above. Following this imputation, serum data from all 12 participants were used in analyses.

There were no significant differences between conditions for urine biomarkers of hydration, indicating similar hydration levels across experimental conditions (Table 1). The one-hour exercise session resulted in increases in objective internal (heart rate) and relative (RPE) load

metrics. Despite the target of 70–75% of peak power for the one-hour duration, the exercise session resulted in an average relative power output of 51.1% of peak power. This was due to the power being reduced during the exercise session when participant cadence dropped below 60 rpm. HR was on average 87.04% for the 1-h exercise session compared to maximal HR from their maximal exercise test.

Results from the exercise intervention in comparison to the control condition can be seen in Table 2. Of note, average HR, relative HR, peak RPE, and average RPE were all significantly elevated in the exercise condition relative to the control condition (all $p < 0.001$). The exercise condition also resulted in a significantly greater change in nude body mass ($t(10) = 4.46$, $p = 0.001$). Plasma volume change was significantly greater ($t(9) = 4.25$, $p = 0.002$) in the exercise condition (− 9.07% [4.22]) compared to the control condition (− 0.88% [4.87]).

Table 2 Comparison of internal and external load between control and exercise conditions

	Control	**Exercise**
Peak power (Watts)	0.00 (0.00)	225.62 (26.78)†
Average power (Watts)	0.00 (0.00)	155.07 (31.62)†
Average relative power (%)	0.00 (0.00)	51.1 (3.7)†
Exercise energy expenditure (kcals)	–	658.93 (126.44)
Average relative VO2 (% of V˙V˙O2max)	–	55.52 (8.05)
Peak heart rate (bpm)	108.14 (13.54)	183.12 (7.47)†
Average heart rate (bpm)	73.60 (8.12)	161.48 (10.04)†
Average relative heart rate (%)	39.08 (4.23)	87.04 (6.14)†
Peak RPE (AU.)	6.25 (0.46)	19.12 (0.99)†
Average RPE (AU.)	6.20 (0.39)	16.95 (0.98)†
NBM change (%)	0.11 (0.30)	− 0.82 (0.79)†

†Significantly different from Control condition. RPE = Rating of Perceived Exertion Borg 6–20 scale. NBM = nude body mass. Relative Power and Relative Heart Rate are calculated as the percentage of the average heart rate relative to the maximal value achieved during the maximal exercise test from the screening visit specific to that set of visits. Exercise energy expenditure is calculated from Velotron power profiles, assuming a cycling efficiency of 20%. Average Relative VO2 is calculated by corresponding the average power output to power during the VO2max assessment

The purpose of the exercise session was to stimulate the HPA-axis and maximize the cortisol response to exercise. Cortisol concentrations increased, on average, by 477.33% during the exercise condition. Cortisol concentrations during the 1-h exercise and 30 min recovery period were on average 367 nmol.l−1 greater compared to the equivalent time points during the control condition. Pearson product moment correlation revealed a significant association between salivary and blood cortisol concentrations ($r = 0.77$, $p < 0.001$).

Mixed-effects growth models were first used to model the change in serum cortisol across the 1-h waking period and test for main and interaction effects with the exercise condition. Unconditional models demonstrated an intraclass correlation (ICC) of 65%, justifying the use of random-effects models. Random intercept only, and random intercept and slope random-effects structures were compared. Including the random effect of time (i.e., slope), did not improve model fit (random intercept only: AIC = 1324.9, −2LL = − 657.45; random intercept and slope: AIC = 1327.5, −2LL = − 656.72; $\chi 2(2) = 0.483$). Therefore, models with random intercepts only were retained and examined further. Models including only the effect of time demonstrated a

significant quadratic effect of time (βLinear = 234.89, [95% CI 119.28 to 350.50], $p < 0.001$; βQuadratic = − 245.42, [95% CI − 356.28 to − 134.56], $p < 0.001$, Conditional $R2 = 0.705$, Marginal $R2 = 0.050$). There was no significant main effect of exercise condition, when the main effect was also included as an interaction effect with the linear and quadratic effects of time, necessary to gauge its impact on the rise and fall of cortisol, (βCondition = − 43.99 [95% CI − 90.56 to 2.57], $p = 0.064$, Conditional $R2 = 0.744$, Marginal $R2 = 0.080$). Further, condition did not interact with linear or quadratic time. When included as a main effect only, a significant negative main effect of exercise condition was observed (Fig. 2; βCondition = − 42.26 [95% CI − 64.52 to − 20.01], $p < 0.001$, Conditional $R2 = 0.747$, Marginal $R2 = 0.080$).

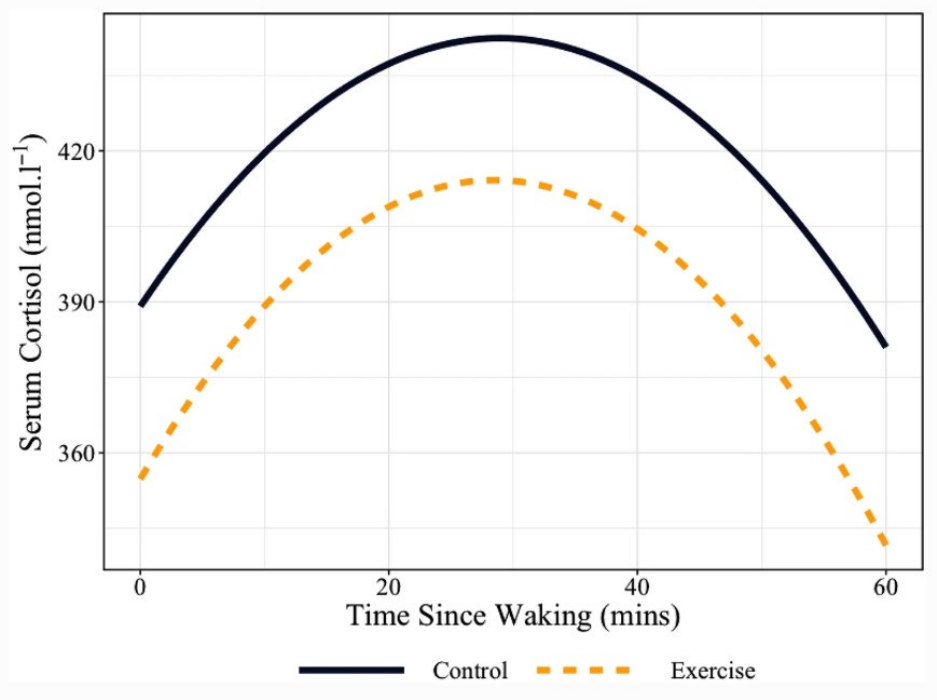

Fig. 2 The effect of exercise on the serum cortisol awakening response

In comparing CARb indices between conditions, random slope and intercept models failed to converge and therefore mixed-effects models with random intercepts only are reported here. Models demonstrated that there was no significant effect of exercise condition on CARbAUCG (βCondition = − 153.32 [95% CI − 349.97 to 43.32], $p = 0.126$, Conditional $R2 = 0.687$, Marginal $R2 = 0.034$), CARbAUCI (βCondition = 21.56 [95% CI − 158.37 to 200.90], $p < 0.566$, Conditional $R2 = 0.174$, Marginal $R2 = 0.002$), or CARbΔ (βCondition = 10.38 [95% CI − 42.86 to 63.62], $p = 0.702$, Conditional $R2 = 0.086$, Marginal $R2 = 0.006$; Fig. 3).

Mixed-effects growth models were used to model the change in salivary cortisol across the 1-h waking period and test for main and interaction effects with exercise condition. Unconditional models demonstrated an ICC of 48%. Preliminary analyses indicated that random slopes did not improve fit, and thus, random intercept models were used. Models with random intercepts only were retained and examined further. Models including only the effect of time demonstrated a significant quadratic effect of time (βLinear = 52.25, [95% CI 29.93 to 74.56], $p < 0.001$; βQuadratic = − 42.04, [95% CI − 63.44 to − 20.65], $p < 0.001$, Conditional $R2 = 0.576$, Marginal $R2 = 0.092$). When included as a main effect only, a significant negative main effect of exercise

condition was observed (βCondition = − 11.55 [95% CI − 15.52 to − 7.57], p < 0.001, Conditional R2 = 0.695, Marginal R2 = 0.178; Fig. 4).

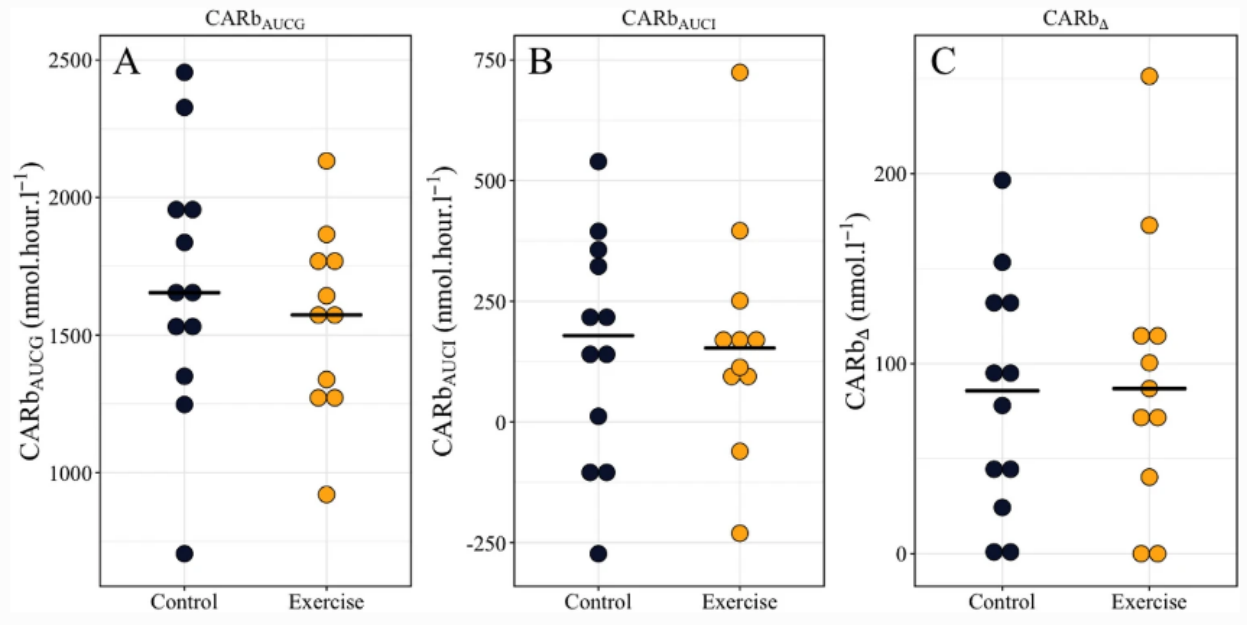

Fig. 3 Comparison of CARb$_{AUCG}$ (A), CARb$_{AUCI}$ (B), and CARb$_{\Delta}$ (C) between conditions

When included as an interaction effect with the linear and quadratic effects of time, there was no longer a significant main effect of exercise condition observed (βCondition = − 5.05 [95% CI − 13.23 to 3.14], p = 0.227, Conditional R2 = 0.701, Marginal R2 = 0.186), or significant interactions with linear or quadratic time.

In comparing CARs indices between conditions, only mixed-effects models with random intercepts only converged and thus are reported here. An analysis of the effect of condition on salivary indices of the CAR demonstrated that there was a significant negative effect of exercise condition on CARsAUCG (βCondition = − 45.20 [95% CI − 82.44 to − 7.96], p = 0.017, Conditional R2 = 0.590, Marginal R2 = 0.108), CARsAUCI (βCondition = − 27.78 [95% CI − 47.42 to − 8.13], p = 0.006, Conditional R2 = 0.629, Marginal R2 = 0.133), and CARsΔ (βCondition = − 8.79 [95% CI − 16.67 to − 0.92], p = 0.029, Conditional R2 = 0.533, Marginal R2 = 0.104; Fig. 5).

Discussion

The primary purpose of this study was to assess the effect of late-evening exercise on the cortisol awakening response on the following morning in a laboratory-controlled environment. We hypothesized that the late-evening exercise would have a significant effect on the CAR, assessed in both serum and saliva. It was demonstrated that prior evening exercise resulted in a lower cortisol concentration across the awakening period, although this was more evident in the salivary CAR metrics than the corresponding serum indices.

Little is known regarding the impact of exercise on the CAR. In one of the preeminent investigations of intensive exercise and the CAR, fifteen elite soccer players underwent seven days of intensive training (a total of ~ 21 h), as would be typical of a preseason camp environment, and

provided awakening saliva samples for two days before and after this training period (Minetto et al. 2008). While the two days of sampling showed high stability, the post-training CAR was significantly greater compared to before training (AUC; 12.4 + 2.4 vs. 16.43.3 nmol.l−1), as was

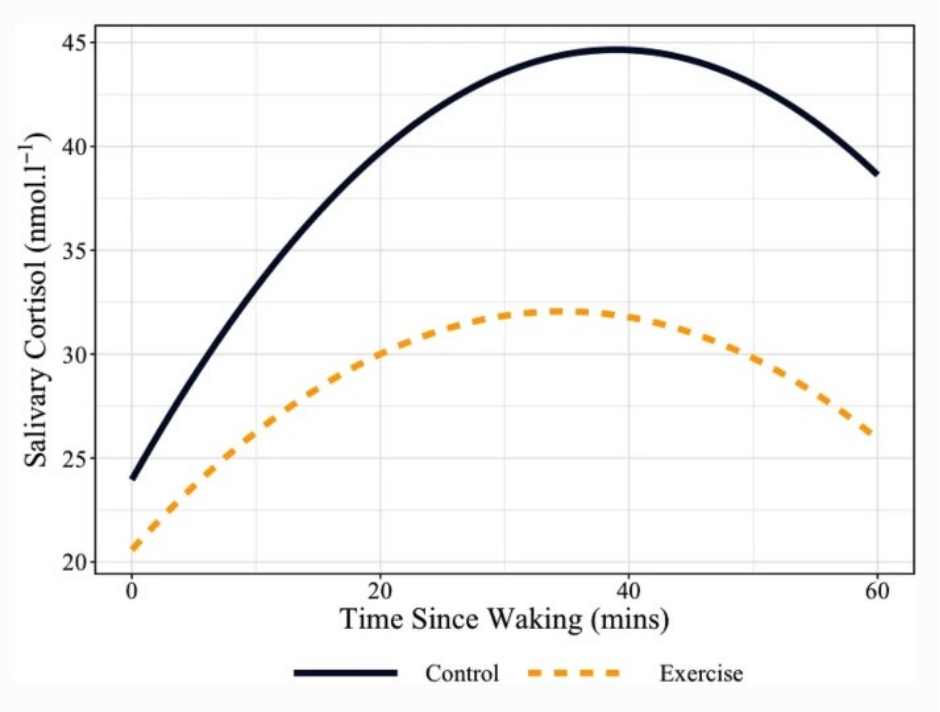

Fig. 4 The effect of exercise on the salivary cortisol awakening response

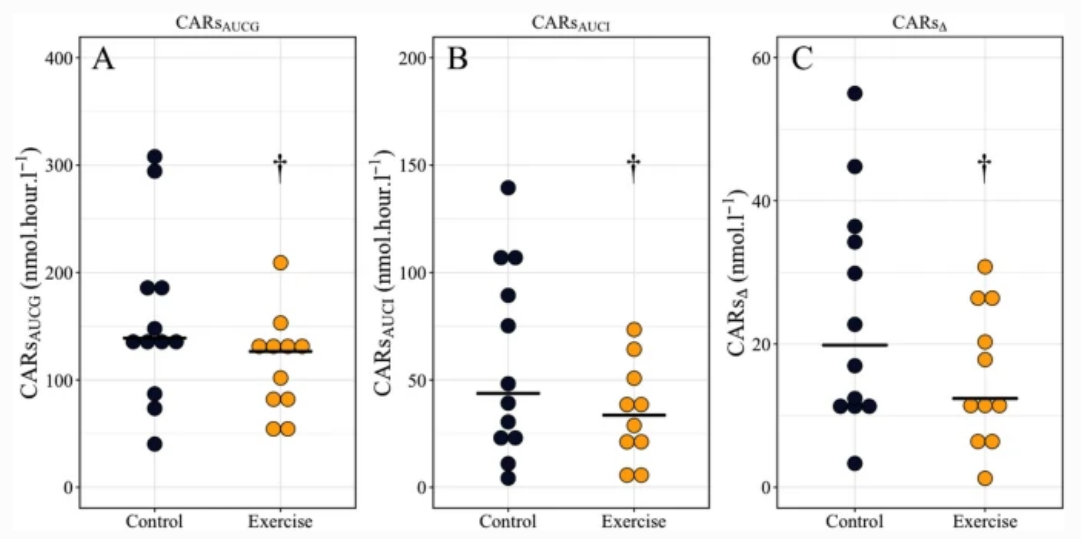

Fig. 5 Comparison of CARsAUCG (A), CARsAUCI (B), and CARsΔ (C) between conditions. † = significant difference from Control condition

the first sample after waking (Minetto et al. 2008). These researchers also demonstrated relations between the change in CAR and change in performance variables such as 20 m speed and counter-

movement jump (Minetto et al. 2008). Prior to this, Gouarné et al. (2005) presented data suggesting that triathlon athletes (n = 2) diagnosed with the overtraining syndrome had a substantially lowered CAR (36.4% and 0% increase 30 min post-waking) at the end of the season, compared to 151% and 64% at the start of the triathlon season. Comparatively, the non-overtrained athletes had a progressively increasing CAR across the season. Further, a recent study in professional Rugby players completing a preseason intensive training camp demonstrated a blunted salivary cortisol response to awakening on the 4th and final day of the training camp (Serpell et al. 2019), although common CAR metrics were not calculated or assessed.

Yet, despite these chronic exercise training studies, few studies have investigated whether acute exercise would affect the CAR. Researchers have previously shown that there was no change in the CAR the morning following a late-night soccer match (Ucar et al. 2018) or netball competition (Juliff et al. 2018). However, it must be noted that (a) these interventions were real-world activities and thus the work completed for each subject cannot be known nor controlled for; and (b) the saliva samples were collected in the individual's homes, introducing possible sources of sampling error. In the present study, we have now demonstrated for the first time that acute, laboratory-based, moderate to high-intensity and moderate duration exercise in the late evening can significantly decrease the cortisol output during the first hour after waking.

The present results contrast with the reported relationship between the previous day's training load and the CAR, which demonstrated a positive association between Banister's training impulse score and CARΔ (Anderson et al. 2018). However, in the current study, a laboratory-controlled exercise bolus was applied to the participants, designed to elicit a robust cortisol response compared to a control condition using a within-subjects design. In contrast, Anderson et al. (2018) assessed free-living exercise participation, wherein participants self-selected the exercise intensity and duration, and analyses were conducted at the between participant level. Thus, it is conceivable that participants who regularly participate in higher-intensity or longer-duration exercise have an increased CAR, while an acute exercise bout that elicits a robust cortisol response causes an acute reduction in the cortisol output the following morning.

The results of this study do, however, agree with recent observations of the modification in the cortisol dynamics after waking in overtrained athletes compared to healthy athlete control participants (Anderson et al. 2021). Overtrained athletes displayed a significantly blunted cortisol output, similar to the effect observed in the current study, confirming early observations of morning cortisol output in overtrained athletes (Gouarné et al. 2005) and more recent work observing the effect of progressively intensified swimming training on urinary cortisol in elite athletes (Izov et al. 2020). These results also agree with the findings of Serpell et al. who reported a progressive blunting of the CAR during a 4-day preseason rugby training camp (Serpell et al. 2019). Taken together, it appears that the CAR may be positively associated with exercise loads typical for a specific individual but can be significantly reduced given large acute exercise loads or sustained exercise loads above which the individual can reasonably recover (e.g., intense acute exercise or the process of overreaching).

The reduction in cortisol concentrations following large increases in cortisol induced by acute exercise is not a novel finding. Resting cortisol concentrations have been previously reported to be lower than baseline concentrations 24-h following a laboratory-based exhaustive exercise session (Anderson et al. 2016). This pattern (i.e., initial increases in cortisol concentrations in response to the exercise followed by decreases in resting cortisol concentrations), has also been observed in real-world exercise investigations with decreases in cortisol concentrations below baseline observed in the days following competition for collegiate American football (Kraemer et

al. 2009), rugby (Elloumi et al. 2003; Cunniffe et al. 2010), marathon (Bae et al. 2019) and Ironman races (Neubauer et al. 2008; Hooper et al. 2019), and a functional fitness competition (Tibana et al. 2019). An analysis of this body of work suggests multiple plausible explanations for the observation of lower cortisol following late-evening exercise, but center on two processes: (1) cortisol may be removed from circulation through cellular uptake or metabolism at a higher rate following exercise or (2) cortisol synthesis and secretion may be reduced after significant elevations due to negative feedback loops operating at multiple levels of the HPA-axis.

This negative feedback and suppression of the HPA-axis has also been proposed by Cunniffe et al. (2010) as an explanation for the reduced basal cortisol concentrations in rugby athletes 38 h following a competition. Teleologically, the reduction in cortisol output was speculated to permit a more anabolic endocrine milieu due to the ability of cortisol to inhibit testosterone production by influencing both the hypothalamic-pituitary–gonadal axis and steroidogenesis at the testicular Leydig cells (Daly et al. 2005; Hackney et al. 2017).

The reduction in cortisol concentration across the awakening period may also be a result of alterations to sleep architecture. The typical circadian decreases of cortisol observed during the early sleep period occur concomitantly with slow-wave sleep (Buckley and Schatzberg 2005). A recent meta-analysis has suggested that evening exercise results in a relative reduction in stage 1 sleep and a relative increase in slow-wave sleep (Stutz et al. 2019), consistent with previous reviews that reported increases in slow-wave sleep following acute exercise (Youngstedt et al. 1997). Despite a statistical difference, however, the clinical relevance of these effects has nonetheless been questioned (Stutz et al. 2019), and more recent well-controlled studies suggest that 30 min of moderate-intensity cycling 90 min before sleep has no effect on slow-wave sleep (Miller et al. 2020). If the present exercise intervention did result in participants spending more time in slow-wave sleep, this would potentially be inhibitory on the HPA-axis and may serve to at least partially explain the decreased cortisol concentrations observed after the early sleep period. Unfortunately, we did not also assess sleep architecture in the current study, and thus, this remains an area for future investigation.

The absence of any significant differences in any serum CAR indices between conditions is a curious finding, given the effect of exercise observed in the growth model. A potential explanation for this finding is the mathematical derivation of these indices. The mixed-effects growth model fits a quadratic model across time for the observed cortisol concentration, with a separate intercept estimated for each individual, and then assesses average differences in cortisol concentrations after accounting for this quadratic change across time. In comparison, the CAR indices first summarize the dynamics of the waking hormonal profile and, in the case of AUCG and AUCI, incorporate the effect of time into the metric. While the growth model summarizes the change across time via a continuous quadratic function, the calculation of the AUCG and AUCI indices uses linear associations between each time point to approximate the integral. The differences of these summarized metrics between conditions are then tested, allowing the baseline (i.e., the value of the index in the control condition) to vary between individuals. These fundamental differences in analysis technique may contribute to the discrepancy in the results, particularly for the CARbAUCG metric—which, based on the main effect of condition in the growth model, would be expected to be lower following exercise. However, differences were observed between conditions for salivary CAR. Therefore, a more plausible explanation is that while exercise appears to affect total cortisol concentrations (as measured in serum), it differentially impacts the free portion of the hormone that is subsequentially quantified in saliva.

The results suggested that salivary cortisol dynamics, as opposed to only average concentration over the post-awakening period, are impacted by acute exercise. To explore why salivary cortisol may respond differently than serum cortisol, it is imperative to understand the role of binding proteins in the blood. Most cortisol is bound to carrier proteins (corticosteroid binding globulin [CBG] and albumin), with only a small portion of the hormone unbound and free and able to diffuse into the saliva. Increasing total cortisol concentrations (especially beyond the binding capacities for these carrier proteins), changing the concentration of CBG, and affecting the affinity of CBG for cortisol, will all substantially affect the free portion of the hormone.

The CBG ~ cortisol affinity has been demonstrated to be significantly impacted by temperature, whereby temperature increases will reduce the affinity between the hormone and binding protein, consequently increasing the availability of the free portion of the hormone (Cameron et al. 2010). Although not measured in this current study, participants likely experienced a significant increase in core body temperature, indicated by the larger decreases in nude body mass following the exercise trial. In addition, some evidence suggests that late-night exercise can increase core body temperature throughout the nocturnal period (O'Connor et al. 1998), which may subsequently increase the free portion of the hormone. However, not all studies have demonstrated this alteration in nighttime core temperature (Miller et al. 2020), so this effect of altered core body temperature on free cortisol should be investigated further.

The affinity between CBG and cortisol is also acutely affected by inflammation. Neutrophil elastase, an enzyme secreted from stimulated neutrophils, can cleave CBG and decrease the affinity for cortisol (Perogamvros et al. 2012; Bikle 2021). With prolonged exercise, circulating neutrophil counts and plasma elastase levels increase immediately post-exercise, despite that the lipopolysaccharide-stimulated neutrophil elastase release is reduced on a per cell basis (Bishop et al. 2002; Cunniffe et al. 2010). However, data from Cunniffe et al. (2010) sheds light on the prolonged effects on elastase during recovery from exercise. In this study, a 14 h post-exercise assessment of neutrophil counts showed near total recovery of counts to pre-exercise levels and a rebound decrease in plasma elastase concentration well below the pre-exercise levels (Cunniffe et al. 2010), suggesting lower levels of free cortisol compared to non-exercise conditions and perhaps providing a potential explanation for decreased CAR the morning after vigorous exercise. Both variables had recovered completely by 38 h post-exercise. We did not assess any of these variables in the current study, but given that Hammond et al. (1990), proposed that local elastase actions could provide a path for targeting cortisol delivery to specific sites of inflammation, the effects of exercise and recovery on these markers should be investigated further. Due to the role CBG may have in further explaining the discrepancies between salivary and blood CAR indices, it is recommended that future studies collecting both blood and saliva samples during the peri- and post-awakening period quantify circulating CBG concentrations for the calculation of the free cortisol index (Coolens et al. 1987). Although resources are limited, it may also be prudent to quantify circulating free and bound cortisol using techniques such as ultrafiltration or equilibrium dialysis (Levine et al. 2007).

Limitations

Despite the numerous strengths of this study, including the exact quantification of the exercise load, the randomized cross-over design where each participant serves as their own control, and the exact timing of awakening and the collection of waking samples, several limitations should be acknowledged. First, although there are substantial benefits to a laboratory-controlled

environment, the sleep experienced by participants is not necessarily comparable to the sleep duration or sleep quality one may expect if they were to sleep in their usual sleep environment. The extensive nature of the current protocol did not permit additional sleep trials to allow habituation to the laboratory sleep environment. In addition, even if the sleep environment per se was not disruptive, participants did sleep with the intravenous line in the forearm, some of whom may have had difficulty sleeping due to the worry or concern of the catheter itself or participants' preferred sleeping positions becoming disrupted during the overnight blood draws.

Additionally, all biochemical assessments used in the present study were completed using immunoassays. Recommendations and guidelines in recent years have encouraged the use of liquid chromatography-tandem mass spectroscopy (LC–MS/MS) to quantify steroids (Monaghan et al. 2014; Casals and Hanzu 2020; Haq et al. 2020). The use of LC–MS/MS avoids common issues with immunoassays, such as cross-reactivity with other steroids. The salivary cortisol ELISAs used in the present study report relatively low cross-reactivity for cortisone (0.8%), and the immunoassay used to quantify salivary cortisone has a reported cortisol cross-reactivity of < 0.1%. However, the serum cortisol assay used reports a serum cortisone cross-reactivity of 78%, potentially affecting the strength of the association between serum cortisol and salivary cortisone. However, cortisol concentrations are approximately 4.3-fold greater in serum than cortisone (Debono et al. 2016), and so it is unclear the extent to which this would affect the present analyses. Regardless, future investigations should replicate and contrast the present findings with those obtained using LC–MS/MS. Finally, although adequately powered to detect the main effects of exercise, the present study may have limited statistical power to observe more complex interaction effects, particularly in the serum quadratic models.

Conclusions and future directions

In this study, we demonstrated that cortisol concentrations are significantly reduced during the awakening period following evening exercise and that the free portion of circulating cortisol may be differentially affected by exercise as indicated by effects on the CAR measured in saliva. The results of this study have the potential to significantly improve our understanding of the CAR and its utility in monitoring the physiological strain of exercise. Furthermore, since we demonstrated a similar and perhaps more informative effect in saliva than serum, this further supports the notion that non-invasive biomarkers can be utilized in this capacity. To further understand the ability of the CAR to reflect acute exercise responses, it is recommended that future studies investigate the effects of alternative exercise modalities and exercise occurring earlier in the day, and explore the impact of varying environmental condition exposures, with and without exercise, on the following day CAR. Furthermore, given the limitations of immunoassays, future studies replicating these findings should consider LC/MS, test for CBG and other circulating binding globulins, and utilize larger and more diverse sample sizes, including replication in females. The addition of sleep architecture measures may further elucidate mechanisms or pathways through which exercise is impacting the CAR.

Abbreviations

−2LL: −2 Log-likelihood

95% CI: 95% Confidence interval

AIC: Akaike information criterion

AUCG: Area under the curve with respect to the ground

AUCI: Area under the curve with respect to the increase

CAR: Cortisol awakening response

CARb: Cortisol awakening response in blood

CARs: Cortisol awakening response in saliva

CBG: Corticosteroid binding globulin

CV: Coefficient of variation

HPA-axis: Hypothalamic–pituitary–adrenal axis

HR: Heart rate

LC–MS/MS: Liquid chromatography-tandem mass spectroscopy

RPE: Rating of perceived exertion

VO2max: Maximum volume of oxygen uptake

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