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The high cortisol awakening response measured the day following high-intensity exercise

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Running title: effect of exercise intensity on CAR

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Abstract

Recent field studies have reported the effects of exercise on the cortisol awakening response (CAR); however, no study has experimentally examined the effects. The purpose of this study was to investigate the effect of exercise intensity on the CAR among 14 healthy male university students. Participants rested for 20 min (control condition) and exercised on a cycle ergometer for 20 min at 40% (low), 60% (moderate), and 80% (high) intensity of their VO\textsubscript{2}max on separate days. Saliva samples were collected 10 times as follows: 1) before and 2) immediately after the experimental session, 3) 10 min, 4) 20 min, and 5) 30 min into the recovery period, 6) the 9 PM and 7) 11 PM night after the session, 8) immediately after awakening, 9) 15 min, and 10) 30 min post-awakening the day after the session. Cortisol concentration increased after the high-intensity exercise and recovered the night after the session. The 30 min post-waking sampling point from the high-intensity exercise was higher than the control condition ($p = .039$). In addition, the higher intensity exercise condition led to the magnitude of change in CAR (CAR\textsubscript{c}) ($p = .006$) and the area of cortisol increased under the curve (AUC\textsubscript{i}) ($p = .034$), making it higher than the control condition. Furthermore, there were no significant differences in other confounders. These results suggest that the CAR showed a high value in healthy male university students the day after performing high-intensity exercise.

Keywords: physical adaptation, biomarker, endocrine, HPA axis
起床時コルチゾール反応に影響を及ぼす運動強度に関する研究

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Abstract

近年, 運動が起床時コルチゾール反応 (cortisol awakening response: CAR) に影響を及ぼすことが報告されているが, その影響を実験的に検討した研究はない。本研究の目的は, 健康な男子大学生 14 名を対象に, 強度別の運動が CAR に及ぼす影響について検討することであった。対象者は, 安静セッションと, VO₂ max の 40% (低), 60% (中), 80% (高) 強度の運動 (20 分間) を各日程で実施した。唾液サンプルは, 1) 実験前と 2) 実験直後, 3) 10 分後, 4) 20 分後, 5) 30 分後, 実験日の 6) 21 時と 7) 23 時, 実験翌日の 8) 起床直後, 9) 15 分後, 10) 30 分後 (CAR の測定) の計 10 回で採取した。実験当日の唾液中コルチゾール濃度は高強度運動条件で上昇し, 実験日の 23 時に回復した。実験翌日の起床時の測定では, 起床 30 分後の唾液中コルチゾール濃度が安静セッションと比較して高強度運動条件で有意に高かった (p = .039). 加えて, 高強度運動条件における CAR の増加量 (CAR change: CAR_c) (p = .006) と増加に関わる曲線下増加面積 (the area under the curve with respect to increase: AUC_i) (p = .034) は, 安静セッションと比較して有意に高い値を示した。運動条件以外の交絡因子にはセッション間で有意な差が認められなかったことから, 健康な男子大学生では, 高強度運動を実施した翌日に CAR が高値を示すことが示唆された。
Introduction

Physical training is essential in improving physical function and performance, whereas overtraining and lack of recovery may cause overtraining syndrome\(^1\). The treatment of overtraining syndrome takes a long time, and the only effective treatment is reduced training and complete rest\(^2\). Therefore, it is important to prevent and detect issues in the early stages. To prevent overtraining syndrome, indicators are required to determine the limits of physical adaptation to exercise and training loads. One of the indicators for monitoring physical adaptation to the exercise and training load has been psychological indicators, such as the Profile of Moods States (POMS)\(^3\). However, since the POMS only assesses mood states, objective measures are needed to monitor physical adaptation to exercise and training loads.

Cortisol, a glucocorticoid synthesized by the adrenal cortex, has a diurnal rhythm that was high in the morning and low at night\(^4\). It has been reported that there was a rapid increase in cortisol levels about 30-45 min after awakening\(^5\). This response is known as the cortisol awakening response (CAR), and has been studied as a reliable indicator that reflects hypothalamic-pituitary-adrenal axis (HPA axis) activity. CAR is regulated by understanding the demands for the following day and can be crucial in providing the necessary energy when switching from a resting to an active state\(^5,6,7\). In addition, CAR has also been investigated as an indicator reflecting chronic stress and reported to be associated with several psychological states and stressors\(^8\). Bhagwagar et al.\(^9\) and Pruessner et al.\(^10\) showed that CAR was elevated in individuals who were depressed, whereas Stetler and Miller\(^11\) reported that CAR was reduced in those individuals. In addition, CAR has been reported to be reduced in patients with chronic fatigue syndrome\(^12\) and those with post-traumatic stress disorder\(^13\). These results may reflect a
progressive process of adrenal disturbance-adaptation-maladaptation, as over or under
activity of the HPA axis may occur depending on the degree of disturbance or disease\(^8\).

Recent studies have also suggested that exercise and training load may affect CAR.
CAR was significantly increased after an intensive 7-day training period, and the greater
the CAR, the lower the rate of decline in performance among elite soccer players\(^{14}\).
Training load on the previous day was positively associated with CAR; however, it had
no measurable association with psychological indicators\(^{15}\). These results indicated that
CAR might produce over or under activity due to exercise and training load, as well as
psychological state and stressors. Therefore, CAR may be an effective indicator to
monitor physical adaptation to exercise and training loads.

Since most of previous studies related to CAR were field studies, there were no
basic findings of CAR reaction experimentally investigated. Experimental studies should
also provide fundamental findings to demonstrate the efficacy of CAR as an indicator for
monitoring exercise and training load. CAR might be affected by an intensity threshold,
since changes in CAR might have occurred with higher load interventions or in
individuals who presumably had a reduced training tolerance\(^{16}\). Therefore, it is necessary
to experimentally investigate the effects of intensity threshold on CAR in untrained
participants to characterize the responses of CAR to exercise.

With these, the purpose of this study was to experimentally evaluate the effect of
exercise intensity on CAR in healthy male university students. Anderson et al.\(^{15}\) reported
a positive correlation between training load and CAR. In addition, a VO\(_2\)max threshold
existed for the acute cortisol response to exercise and cortisol levels increased upon
exercise above the threshold\(^{17},^{18}\). In this study, we tested four experimental sessions
based on previous study\(^{17}\): (1) resting session (control condition) and three types of
exercise sessions ([2] 40%, [3] 60% and [4] 80% of their VO₂max). We hypothesized that
(1) CAR would not be changed by exercise at 40% VO₂max (that is considered below
threshold to induce CAR) and be enhanced by exercise (2) at 60% VO₂max (near
threshold), and (3) at 80% VO₂max (above threshold).

Materials and Methods

Participants. Fifteen healthy male university students (M = 21.9; SD = 1.6) volunteered
to participate in this study. Their mean height, weight, and BMI were 174.6 cm ± 4.3,
76.4 kg ± 9.6, and 25.0 ± 2.7, respectively. Their VO₂max was 39.4 ± 5.45 mL/kg/min.
Exclusion criteria included: a history of hormonal disorders, mental illness, smoking, a
diet chronically low in carbohydrates, use of anabolic steroids, or chronic nonsteroidal
anti-inflammatory drug use. Prior to this participation, all participants provided oral and
written informed consent. The study was approved by the Ethics Committee at Osaka
University of Health and Sport Sciences (approval number: 20-3). One participant was
excluded, as he mentioned taking caffeine during the experimental period. Therefore, the
data were analyzed among 14 participants.

Procedure. The experimental session, as seen in Fig. 1, lasted for five days based on
previous research (only the exercise duration was changed from 30 min to 20 min)\(^7\).
During the experiment, participants were required to refrain from exercise on the previous
day and the day of the experiment to exclude non-experimental factors. Additionally,
participants refrained from consuming caffeine and alcohol starting the day before the
experimental session until the morning of the next day. On Day 1, participants went
through screening and performing their VO₂max test. During Days 2-5, the sessions
consisted of a rest period (control condition) and the 20 min exercise bouts at 40% (low),
60% (moderate), and 80% (high) intensity of their VO2max with a cycle ergometer. All measurement was taken before (Pre) and immediately after (Post-0) the experimental session, during the recovery period from the session (every 10 min for 30 min; Post-10, Post-20, and Post-30), the night after returning home from the session (9 PM, 11 PM; recovery 1 and recovery 2), and upon waking up the day after the session. All participants completed the sessions at the same time of the day (6 PM ± 30 min) to control for biological variation. They were asked to maintain their regular diet during the experiment period and to refrain from eating at least 4 hours before the experiment and no later than 8 PM after the experiment. Each session was randomly assigned and separated by a minimum of 72h.

Instruments

**Exercise intensity during the three exercise sessions.** To evaluate the intensity of the three exercise sessions, expiratory gas, heart rate (HR) and the rating of perceived exertion (RPE) were measured during each exercise session. Respiratory gases were measured using AERO MONITOR AE300S (MINATO MEDICAL SCIENCE, Japan) and collected for 3 min periods at 7-10 min and 17-20 min. HR was monitored during the exercise (every 5 min) using a Polar HR monitor (Polar Model V800, Japan). RPE was measured using Borg’s 6-20 point rating of perceived exertion scale, which was translated into Japanese and validated19).

**Cortisol analysis.** Prior to the experiment, participants were informed of the guidelines for proper saliva collection. Saliva samples were collected using the passive drool techniques and taken as shown in Fig. 1. At saliva collection, participants collected saliva in their mouths for 2 minutes, and then poured the collected saliva into a tube by a
straw. No mouthwash was used to prevent dilution of saliva. For the late-night measurements (recovery 1 and recovery 2), participants were asked to refrain from drinking, brushing their teeth, and bathing for 1 hour prior to saliva collection. For the early morning measurement, no eating, drinking, brushing teeth, strenuous physical activity, or going back to sleep was permitted during this 30 min interval. Participants were asked to record their sleeping and awakening time, time of saliva collection, and sleeping state on a Google form. Saliva samples were initially stored by participants in a commercial refrigerator at approximately 4°C and the participants submitted their saliva sample to the laboratory within 48 hours. The received saliva samples were centrifuged at room temperature at 3000×g for 5 min to precipitate particulate matter and stored at −80°C until analysis. The saliva samples were then analysed for cortisol concentrations using an enzyme-linked immunosorbent assay (Cortisol ELISA Kit [RE52611], IBL, Germany). The magnitude of change (CAR change; CARc), relative change (CAR%), and the area under the curve according to trapezoid rule in 2 manners: relative to the ground (relative to a “zero” concentration; AUCg) and relative to the increase (relative to the first concentration; AUCi) were calculated from the samples taken upon awakening.

\[
\text{CARc} = \text{Maximum value of } [C_{15}] \text{ or } [C_{30}] - [C_0]
\]

\[
\text{CAR\%} = \frac{\text{CARi}}{[C_0]} \times 100
\]

\[
\text{AUCg} = \frac{([C_0] + [C_{15}]) \times 15}{2} + \frac{([C_{15}] + [C_{30}]) \times 15}{2}
\]

\[
\text{AUCi} = \text{AUCg} - [C_0] \times 30
\]

**Factors affecting the HPA axis.** To exclude non-experimental factors, factors that could affect CAR during awakening were measured upon awakening. The degree of
physical fatigue, muscle pain, stress, and sleep quality were measured using a visual analogue scale (VAS). Psychological condition was assessed with the total mood disturbance (TMD) score calculated from the short form of the Profile of Moods States Second Edition (POMS2-S), which was translated into Japanese and validated\(^{20}\). Participants graded 35 items on a Likert scale, from 0 (not at all) to 4 (extremely), to answer the question: “How do you feel at this moment?” The 7 dimensions assessed were: Anger-Hostility (AH), Confusion-Bewilderment (CB), Depression-Dejection (DD), Fatigue-Inertia (FI), Tension-Anxiety (TA), Vigor-Activity (VA), and Friendliness (F). The TMD score was calculated by adding 100 to the total sum of AH, CB, DD, FI, and TA minus the VA score.

**Data Analysis.** Data are expressed as means ± standard errors. Two-way repeated measures ANOVA tests were used to analyze changes in cortisol concentrations on the day of the experimental session (session [4] × sampling point [7]) and CAR on the day after the session (session [4] × sampling point [3]). Mauchly’s test was used to assess the sphericity assumption. If the assumption was violated, Greenhouse-Geisser epsilon values were used to adjust the degrees of freedom. Bonferroni corrections were used for post-hoc tests. An estimate of effect size was quantified as partial eta squared (\(\eta_{p}^{2}\)), where \(\eta_{p}^{2} = 0.01, 0.06\) and 0.14 were estimates for a small, moderate and large effect, respectively\(^{21}\). The difference between experimental sessions in CAR, CAR\%, AUC\(_{g}\), AUC\(_{i}\), VAS and the TMD score during awakening was analyzed by a one-way repeated measure ANOVA. We focused on the difference between sessions rather than sampling point, as CAR has been known as a response to elevated cortisol levels about 30-40 minutes after awakening\(^{5}\). The level of significance was set to \(\alpha \leq .05\). In addition, a more liberal \(\alpha\) value of \(\leq .10\) was used to determine marginal significance, because the cost of
making a Type II error was considered just as important as making Type I error\textsuperscript{22}). All statistical analyses were performed using SPSS version 27.0 (IBM, Japan).

**Results**

All measurements of exercise intensity during the three exercise sessions are shown in Table 1.

[Insert Table 1]

*Cortisol concentration on day of experimental session.* The changes of salivary cortisol concentration on the day of the experimental session can be seen in Fig. 2. These results showed that the main effect of session was significant ($F(3, 39) = 16.566, p = .000, \varepsilon = .653, \eta_p^2 = .560$). The main effect of sampling point was significant ($F(6, 78) = 25.375, p = .000, \varepsilon = .326, \eta_p^2 = .661$). The interaction of session and sampling point was also significant ($F(18, 234) = 12.800, p = .000, \varepsilon = .208, \eta_p^2 = .496$). Post-hoc tests of sampling point confirmed that control and moderate intensity exercise had lower levels for recovery 1 ($p = .012, p = .065$, respectively) compared to Pre, and low-intensity exercise showed significantly lower levels for recovery 1 ($p = .010$) and recovery 2 ($p = .003$) compared to Pre. In high-intensity exercise, Post-10 ($p = .001$), Post-20 ($p = .002$), and Post-30 ($p = .006$) were significantly higher, whereas recovery 2 ($p = .002$) was significantly lower compared to Pre. Post-hoc tests of session confirmed that Post-10, Post-20, and Post-30 showed higher levels for high-intensity exercise compared to the other three sessions. Recovery 1 showed a higher level for high-intensity exercise compared to the control and moderate-intensity exercises ($p < .05$, except for the moderate-intensity exercise on Post-10 [$p < .10$]), while recovery 2 showed no significant difference between sessions.

[Insert Figure 2]
Cortisol awakening response. The CAR on the day after the experimental session is shown in Fig. 3. These results showed that the main effect of session was significant \(F(3, 39) = 3.087, p = .038, \eta_p^2 = .192\). The main effect of sampling point was also significant \(F(2, 26) = 21.437, p = .000, \varepsilon = .597, \eta_p^2 = .622\). The interaction of session and sampling point was marginally significant \(F(6, 78) = 12.800, p = .091, \varepsilon = .517, \eta_p^2 = .150\). Post-hoc tests of sampling point confirmed that low-intensity exercise showed a higher \(C_{30}\) compared to \(C_0\) \((p = .027)\) and \(C_{15}\) \((p = .052)\). In moderate- and high-intensity exercise, it was significantly higher from \(C_0\) to \(C_{15}\) and \(C_{30}\) (all were \(p < .05\)). Post-hoc tests of the session confirmed that CAR was significantly higher in high-intensity exercise compared to the control at \(C_{30}\).

\[\text{CAR}_c, \text{CAR}\%, \text{AUC}_i \text{ and AUC}_g \text{ can be seen in Table 2. The main effect was significant in } \text{CAR}_c \ (F(3, 39) = 4.279, p = .001, \eta_p^2 = .248), \text{ and marginally significant in } \text{AUC}_i \ (F(3, 39) = 2.250, p = .098, \eta_p^2 = .148). \text{ Post-hoc tests of } \text{CAR}_c \text{ confirmed that it was significantly higher in high-intensity exercise compared to the control } \(p = .006\), and higher in high-intensity exercise compared to low-intensity exercise \((p = .097)\). Post-hoc tests of \(\text{AUC}_i\) confirmed that it was significantly higher in high-intensity exercise compared to the control condition \((p = .034)\). No difference was found in other factors.\]

Factors affecting CAR. Factors that affected CAR measured upon awakening are shown in Table 3 and Table 4. The main effect of physical fatigue was significant \(F(3, 39) = 4.592, p = .008, \eta_p^2 = .261\). The main effect of muscle pain was significant \(F(3, 39) = 3.325, p = .029, \eta_p^2 = .204\) at session. Post-hoc tests of physical fatigue confirmed that it was significantly higher in low- \((p = .023)\) and high- \((p = .032)\) intensity exercise.
compared to the control condition. Post-hoc tests of muscle pain confirmed that it was higher in high-intensity exercise compared to the control condition ($p = .058$). No difference was found in other factors.

**Discussion**

The purpose of this study was to investigate the effect of three different exercise intensities (40%, 60%, and 80% of VO$_{2\text{max}}$) on the CAR in healthy male university students. The results showed that CAR was higher the day after the high-intensity exercise, and there were no significant differences in other factors influencing CAR. Our findings support the hypotheses that (1) exercise at 40% VO$_{2\text{max}}$ would not change CAR and (3) exercise at 80% VO$_{2\text{max}}$ would enhance CAR. However, it did not support the part of the hypothesis: (2) exercise at 60% VO$_{2\text{max}}$ would enhance CAR. This was the first experimental study to demonstrate the effects of exercise intensity on CAR.

The three exercise sessions produced the desired physiological responses. This was clear from the VO$_2$, HR, and RPE values of the participants during the exercise sessions. The % VO$_{2\text{max}}$ of exercise session showed similar results to previous studies$^{17}$), although the actual intensity in this experiment was higher than the desired intensity.

In high-intensity exercise, salivary cortisol levels increased from Pre to Post-30 and decreased in recovery 1 and recovery 2. In contrast, salivary cortisol concentrations were not changed by low- and moderate-intensity exercise. The cortisol response has a threshold of one’s VO$_{2\text{max}}$, and exercise above the threshold increases cortisol levels$^{17}$). Most exercise physiology sources suggest that increased circulating cortisol can be
produced by exercise at or above 60% of an individual’s VO$_{2\text{max}}$. However, there other
additional studies that show that exercise at the same VO$_{2\text{max}}$’s makes no difference in
cortisol response. These differences of acute exercise can be affected by one’s fitness
level and the total duration of the workout $^{26, 27}$. The participants in this study were not
endurance-trained individuals but had a habit of physical activity about 2-3 days a week.
In addition, the total duration of the exercise was changed from 30 min to 20 min to be
considered for the exercise training status of the participants. These factors might be the
reason why exercise at 60% VO$_{2\text{max}}$ did not cause an acute cortisol response.
Additionally, the recovery of cortisol levels after exercise was related to the intensity and
duration of exercise, and that cortisol levels might or might not recover after exercise$^{28}$.
Since the increase and recovery of cortisol levels may affect by the degree of HPA
disturbances, the results suggested that the high-intensity exercise in the present study
caused acute HPA disturbances to the participants. The decrease of cortisol levels at
recovery 2 – common in all experimental sessions – may be related to diurnal rhythm.
The interpretation of recovery 1 and recovery 2 should also note the effects of diurnal
rhythm, since cortisol had characteristics of being high in the morning and low at night$^4$.

The CAR on the day after high-intensity exercise was higher than the control, at
30 min after waking, CAR$_c$ and AUC$_i$ – related to the increase of CAR – were also higher
in the high-intensity exercise than the control condition; however, those were not changed
after low- and moderate-intensity exercise. There was a positive association between
CAR$_c$ and CAR$_%$, and the training load performed the previous day$^{15}$. The results of the
present study were similar to the previous study in low- and high-intensity exercise and
supported the hypothesis. However, moderate-intensity exercise did not support the
hypothesis. Anticipated demands of the following day regulates CAR and provides the
energy need to shift from a resting to an active state\textsuperscript{5), 6), 7)}. In the present study, acute cortisol responses were observed on the day of the experiment only in high-intensity exercise and physical fatigue and muscle pain remained the day after the experiment. Delayed onset muscle soreness (DOMS), which occurs 8 to 72 hours after exercise\textsuperscript{29)}, was caused by muscular activity, especially eccentric contraction\textsuperscript{30), 31)}, but could also be present following unfamiliar exercise. Since the participants in the present study were not endurance-trained, it is likely that the unfamiliar high-intensity exercise induced physical fatigue with DOMS the following day. This suggests that the participants had not fully recovered from the physical fatigue caused by high-intensity exercise on the previous day, and that they were in a state of accumulated physical fatigue. The participants would then need to compensate for the energy for the accumulated physical fatigue in addition to the energy required under normal situations. Therefore, it appears that the energy required to shift from a resting to an active state was greater the day after high-intensity exercise, resulting an increase in CAR. Other intensity exercises did not cause acute cortisol responses and physical fatigue with DOMS. This suggests that physical fatigue did not occur or accumulate by low- or moderate-intensity exercise, which may be the reason why CAR did not change. Furthermore, there were no differences between sessions in other factors influencing CAR, which strengthened the evidence that CAR responds based on the intensity threshold of the exercise.

The limitation of the present study should be noted. First, it was not a fully-controlled experiment, as the measurements were conducted at home from the night of the experiment to the next morning. In some CAR studies, it was recommended to use accommodations to ensure accurate saliva sampling\textsuperscript{32}). On the other hand, the use of accommodations might also induce confounding factors that affect CAR, such as the level
of sleep quality and stress. Thus, there were advantages to conducting measurements at home to ensure the ecological validity of the data\textsuperscript{27}). In this study, we used measurements at the participants’ homes to reduce their stress as much as possible and control the confounding factors. However, to obtain fully-controlled experimental results, it may be necessary to use accommodations and strictly control their activity after the experiment. Second, it was difficult to secure an adequate amount of saliva samples immediately after exercise and awakening. Saliva secretion is regulated by the autonomic nervous system, and is particularly influenced by the parasympathetic nervous system. During exercise, salivary secretion decreases due to suppression of parasympathetic nerve activity\textsuperscript{33}). In addition, parasympathetic nerve activity activates during sleep, which decreases saliva secretion\textsuperscript{34}) and may also influence saliva secretion during awakening. In this study, drinking water was limited in measurements immediately after exercise and during awakening in order to prevent dilution of saliva samples. However, in order to secure sufficient saliva samples, mouthwash and drinking water may be necessary in some cases, with care taken not to affect saliva samples.

**Conclusion**

The findings of this study showed that high-intensity exercise resulted in higher CAR on the following day. This suggests that CAR may be influenced by the threshold of exercise intensity. In the future, the development of longitudinal studies and studies with athletes may also provide a more fundamental view of CAR dynamics caused by exercise, which enables us to demonstrate the effectiveness of CAR as an indicator for monitoring exercise and training load.
Acknowledgments

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References


Conflict of Interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by YO. The first draft of the manuscript was written by YO and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.
Table 1. Results of Selected Variables Measured during Each Exercise Trial (mean ± SE)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Low Intensity (40% VO\textsubscript{2max})</th>
<th>Moderate Intensity (60% VO\textsubscript{2max})</th>
<th>High Intensity (80% VO\textsubscript{2max})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workload (W)</td>
<td>80.4 ± 3.4</td>
<td>139.1 ± 4.8</td>
<td>197.7 ± 6.4</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>101.5 ± 2.9</td>
<td>128.0 ± 3.1</td>
<td>157.8 ± 2.9</td>
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<tr>
<td>VO\textsubscript{2} (mL/kg/min)</td>
<td>15.5 ± 0.7</td>
<td>24.7 ± 1.1</td>
<td>34.1 ± 1.2</td>
</tr>
<tr>
<td>% VO\textsubscript{2} max</td>
<td>39.3 ± 0.6</td>
<td>62.6 ± 1.1</td>
<td>86.8 ± 1.1</td>
</tr>
<tr>
<td>RPE</td>
<td>11.1 ± 0.5</td>
<td>13.4 ± 0.5</td>
<td>17.7 ± 0.5</td>
</tr>
</tbody>
</table>

Table 2. The Magnitude of Change, Relative Change, and the Area under the Curve of CAR (mean ± SE)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Low Intensity</th>
<th>Moderate Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR\textsubscript{c} (nmol/L)</td>
<td>7.1 ± 2.5</td>
<td>8.1 ± 2.1</td>
<td>11.9 ± 2.3</td>
<td>14.0 ± 2.1†</td>
</tr>
<tr>
<td>CAR% (%)</td>
<td>99.6 ± 28.1</td>
<td>148.1 ± 56.4</td>
<td>182.3 ± 43.7</td>
<td>153.4 ± 36.5</td>
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<tr>
<td>AUC\textsubscript{g} (nmol/L)</td>
<td>457.6 ± 73.1</td>
<td>474.3 ± 57.5</td>
<td>457.0 ± 62.8</td>
<td>557.1 ± 57.2</td>
</tr>
<tr>
<td>AUC\textsubscript{i} (nmol/L)</td>
<td>121.1 ± 35.9</td>
<td>168.8 ± 41.0</td>
<td>202.2 ± 32.5</td>
<td>186.6 ± 32.1†</td>
</tr>
</tbody>
</table>

* Significantly different (p < .05) and † marginal significance (p < .10) compared to control and low intensity, respectively

Table 3. Comparison of Factors Affecting CAR between Each Experimental Session (mean ± SE)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Low Intensity</th>
<th>Moderate Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Fatigue (mm)</td>
<td>14.0 ± 2.7</td>
<td>25.2 ± 3.8†</td>
<td>24.1 ± 5.3</td>
<td>29.9 ± 3.9*</td>
</tr>
<tr>
<td>Muscle Pain (mm)</td>
<td>3.8 ± 1.4</td>
<td>8.5 ± 2.5</td>
<td>10.4 ± 2.8</td>
<td>12.0 ± 3.2†</td>
</tr>
<tr>
<td>Stress (mm)</td>
<td>18.0 ± 3.5</td>
<td>29.0 ± 4.3</td>
<td>21.0 ± 4.9</td>
<td>28.3 ± 4.9</td>
</tr>
<tr>
<td>Sleep Quality (mm)</td>
<td>58.6 ± 7.8</td>
<td>60.2 ± 5.7</td>
<td>62.9 ± 6.9</td>
<td>61.6 ± 6.8</td>
</tr>
<tr>
<td>TMD Score</td>
<td>103.6 ± 3.8</td>
<td>104.9 ± 3.3</td>
<td>103.4 ± 3.9</td>
<td>107.1 ± 3.2</td>
</tr>
</tbody>
</table>

* Significantly different (p < .05) and † marginal significance (p < .10) compared to control

Table 4. Comparison of Awakening Time, Sampling Points and Sleeping Hours between Each Experimental Session (mean ± SE)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Low Intensity</th>
<th>Moderate Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awakening Time</td>
<td>6:44 ± 0.25</td>
<td>6:56 ± 0.15</td>
<td>7:00 ± 0.17</td>
<td>6:59 ± 0.20</td>
</tr>
<tr>
<td>First Sampling Time</td>
<td>6:46 ± 0.25</td>
<td>6:59 ± 0.15</td>
<td>7:02 ± 0.17</td>
<td>7:01 ± 0.20</td>
</tr>
<tr>
<td>Sleeping Hours (h)</td>
<td>6.2 ± 0.4</td>
<td>6.5 ± 0.3</td>
<td>6.6 ± 0.3</td>
<td>6.8 ± 0.4</td>
</tr>
</tbody>
</table>
Fig. 1 Experimental Protocol
Fig. 2 Cortisol Concentrations for Each Experimental Session. The depicted values are mean ± SE.
Fig. 3 Cortisol Awakening Response (CAR) on the Day After Each Experimental Session. The depicted values are mean ± SE