RAPID COMMUNICATION

Exercise and circulating cortisol levels: The intensity threshold effect

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ABSTRACT. This study examined the influence of exercise intensity upon the cortisol response of the hypothalamic-pituitary-adrenal (HPA) axis. Specifically, we examined exercise at intensities of 40, 60, and 80% maximal oxygen uptake (VO_{2max}) in an attempt to determine the intensity necessary to provoke an increase in circulating cortisol. Twelve active moderately trained men performed 30 min of exercise at intensities of 40, 60, and 80% of their VO_{2max}, as well as a 30-min resting-control session involving no exercise on separate days. Confounding factors such as time of day – circadian rhythms, prior diet – activity patterns, psychological stress, and levels of exercise training were controlled. Cortisol and ACTH were assessed in blood collected immediately before (pre-) and after (post-) each experimental session. Statistical analysis involved repeated measures analysis of variance and Tukey *post-hoc* testing. The percent change in cortisol from pre- to post-sampling at each session was: resting-control, 40, 60, and 80% sessions (mean±SD) =–6.6±3.5%, +5.7±11.0%, +39.9±11.8%, and +83.1±18.5%, respectively. The 60% and 80% intensity magnitude of change was significantly greater than in the other sessions, as well as from one to another. The ACTH responses mirrored those of cortisol, but only the 80% exercise provoked a significant ($p<0.05$) increase pre- to post-exercise. The calculated changes in plasma volume for the resting-control, 40%, 60%, and 80% sessions were: +2.2±3.0%, –9.9±5.0%, –15.6±3.5%, and –17.2±3.3%, respectively. Collectively, the cortisol findings support the view that moderate to high intensity exercise provokes increases in circulating cortisol levels. These increases seem due to a combination of hemoconcentration and HPA axis stimulus (ACTH). In contrast, low intensity exercise (40%) does not result in significant increases in cortisol levels, but, once corrections for plasma volume reduction occurred and circadian factors were examined, low intensity exercise actually resulted in a reduction in circulating cortisol levels.

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INTRODUCTION

Cortisol is a glucocorticoid hormone secreted by the adrenal cortex in response to physical, psychological, or physiological stressors (1, 2). One specific stressor known to drastically modify the circulating levels of cortisol in the human body is physical exercise (3, 4).

In response to exercise, the hypothalamus secretes CRH. In turn, CRH activates the anterior pituitary, stimulating the release of ACTH, and this stimulates the adrenal cortex to release cortisol (5). Once released, cortisol is taken up by a variety of tissues throughout the body, such as skeletal muscle, adipose tissue, and the liver. At these different tissues, the presence of cortisol mediates critical physiological processes which aid in exercise capacity and recovery, e.g., promoting proteins in the skeletal muscle to be broken down into amino-acids, and triglycerides in adipose tissue to be hydrolyzed into free fatty acids and glycerol (1, 4, 5). In addition, the presence of very high circulating levels of cortisol may stimulate gluconeogenesis in the liver, providing additional carbohydrate for energy production (5). The secretion of cortisol is regulated by negative feedback, in which high circulating levels signal the anterior pituitary to decrease ACTH secretion. Conversely, increased levels of ACTH and, or cortisol can signal the hypothalamus to decrease CRH secretion. This interconnected feedback loop is referred to as the hypothalamic-pituitary-adrenocortical (HPA) axis (6).

As previously noted, exercise can act as a stimulus to the HPA axis, resulting in significant increases in circulating cortisol levels. Cortisol levels increase at a rate relatively proportional to the exercise intensity, but reach a final level dependent upon the total duration (time) of an exercise session (3, 4). This acute exercise response can be influenced by exercise training status, but appears to be vastly different only when comparing sedentary individuals to highly trained individuals (1, 7).

Interestingly, there is some ambiguity in the literature regarding the minimum intensity of exercise (i.e., threshold), necessary to provoke a cortisol response from the HPA axis (1, 8). Most exercise physiology reference sources suggest that exercise at or above 60% of an individual's maximal oxygen consumption (VO_{2max}) generally produces a significant increase in circulating cortisol. However, several studies have shown exercise at or above this intensity have failed to provoke a cortisol response (9-11). It is unclear why these studies failed to see significant cortisol increases. One reason may relate to the research study design employed. Cortisol is a highly circadian hormone and in some cases researchers have not conducted an appropriate resting-control evaluation of cortisol profiles prior to exercise manipulations. Thuma et al. have stressed the need for such a resting-control evaluation to account for the circadian nature of the hormone (8). This lack of agreement between studies, where some show certain intensities of exercise increase cortisol and others do not, suggests additional research is warranted to help identifying the intensity of exercise – threshold necessary to provoke a cortisol response. Obviously, the identification of such a threshold could be a critical methodological consideration when attempting to design certain endocrine research studies.

As noted, the exercise stimulus to increase cortisol levels is due not just to the intensity but also to the duration of ex-

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ercise (or a combination of both factors interacting) (4). For this reason, it is necessary, if examining the cortisol response, to keep either the duration or intensity of exercise constant and manipulate the other in order to identify the individual effect of each contributing factor more clearly. With all of the above factors in mind, this study was designed with the intent of examining the influence of exercise intensity upon the cortisol response from the HPA axis. Specifically, we examined 30 min of exercise at intensities of 40, 60, and 80% VO_{2max} in an attempt to determine the intensity necessary to provoke an increase in circulating cortisol. Additionally, plasma volume shifts were examined as ACTH responses were in order to elucidate potential mechanistic aspects of any cortisol changes detected (i.e., hemoconcentration *vs* secretory stimuli to adrenal cortex).

MATERIALS AND METHODS

Subjects

Subjects reported to our laboratory approximately 4 h postprandial on 5 separate occasions (7). Session I included completion of an informed consent, a medical screening, skin-fold measurements for body fat estimation [skinfolds (12)] and a VO_{2max} exercise test. Approximately 1 week later, the subjects underwent experimental testing sessions consisting of 30-min cycling bouts at 40, 60, or 80% of VO_{2max} and a 30-min resting period, which served as a control session for comparing the exercise sessions. These sessions were completed in random order, occurred on different days, and were separated by at least 48 h. Subjects were instructed to maintain and control their diet (i.e., eucaloric diet with a minimum of 50% of daily calories coming from carbohydrate sources) and reduce activity level (i.e., rest) preceding the first visit and to replicate these behaviors before each ensuing experimental session visit.

Experimental procedures

Exercise duration (30 min) was fixed in order to ensure that any changes in cortisol levels were due to intensity and not to some combination of intensity and duration, and was based upon duration recommendations appearing in the literature (4). In addition, the highest intensity of exercise (80%) was selected because it was felt few people would be able to maintain a greater intensity for durations of 30 min (13).

The subjects in this study were healthy, trained males, aged 18-30 yr who were recruited from the University of North Carolina at Chapel Hill and surrounding areas. In order to be included in the study, subjects were required to pass a medical physical examination, to demonstrate normal scores on the RESTQ anxiety inventory (14), and to sign an informed consent statement prior to beginning participation in the study. During the 12 months leading up to the study, subjects trained a minimum of 3 days per week (~60 min per day), with at least 1 day including cycling exercise. Exclusion criteria included, but were not limited to, a prior history of hormonal disorders, mental illness, consistent body weight loss pattern, or a diet deficient in carbohydrate intake (assessed *via* medical history and questionnaire and nutritional survey).

Maximal oxygen consumption testing

Prior to beginning the VO_{2max} test, each subject was allowed to warm up and stretch. The warm-up was limited to 5 min of cycling at a workload below that of the first testing stage workload. Warm-up procedures were recorded and repeated for each subsequent exercise session. Following the warm-up period, the subject completed the VO_{2max} test on a Monark cycle ergometer (Monark model 818E, Sweden). The initial workload for each subject was determined based on their training history, and was increased at 3-min intervals until volitional fatigue. Heart rate (HR) was monitored each minute using a Polar HR monitor (Polar model F1, Finland) and respiratory gases were recorded every 15 sec using the TrueMax 2400 Metabolic Measurement System (Parvo Medics, UT, USA). The test was considered valid if at least two of the following criteria were met: respiratory exchange ratio >1.15, HR±10 beats of age-predicted maximum, and/or a plateau in VO_2 with an increase in workload (15).

Exercise sessions

Subjects reported to the laboratory and completed the RESTQ questionnaire. If a normal score was achieved on the RESTQ [i.e., a low level of emotional stress (14)], subjects were allowed to begin a 30-min supine rest. A 3-ml blood sample was taken at the end of the rest period (pre-sample). Subjects then completed a 5-min warm-up at approximately 10-20% of VO2max on a cycle ergometer. After the warm-up, subjects began 30-min of cycling exercise at the pre-determined workload, intensity (40, 60, or 80% VO_{2max}). Once at the prescribed intensity, respiratory measures were taken every 10 min (3 min of measurement) to ensure that the subject remained at the prescribed workload, intensity and HR was recorded at 5-min intervals. Immediately at the end of the 30-min exercise bout, a 3-ml post-exercise blood sample was taken. After blood sampling, the subject was directed to cool down by performing easy cycling for 5-10 min, and was permitted to leave the laboratory once HR dropped below 100 bpm. The time of day for all exercise testing was standardized and controlled in order to minimize circadian hormonal fluctuations. These conditions and procedures were replicated exactly for all 3 exercise experimental sessions.

Resting-control session

A randomly selected sub-sample of the subjects in this study completed a 30-min resting-control session. This session was arranged to mimic all aspects (prior diet, physical activity, time of day, *etc.*) of the experimental (exercise) sessions, except that subjects rested quietly for 30-min rather than exercising on a cycling ergometer. Blood samples were collected as noted above pre- and post- the 30-min resting-control period.

Blood procedures-analysis

Blood samples were collected *via* the venipuncture procedures using a 3-cc Vanishpoint® syringe with a 25 gauge, 1.5 cm needle (Retractable Technologies, Inc., TX, USA). All subjects were familiar with the venipuncture procedure from previous research study participation. The collected whole blood specimens were transferred into 3-ml Vacutainer® tubes treated with EDTA (Becton Dickinson Systems, NJ, USA) and placed on ice immediately.

Whole blood was used to measure hematocrit (Hct) and hemoglobin (Hb) in order to quantify any plasma volume shift using the Dill and Costill equation (16). Hct was measured in triplicate using microcapillary tubes (Fisher Scientific, PA, USA) and read using a micro-Hct reader (International

Equipment Company, MA, USA). Hb was measured in triplicate using the cyanmethemoglobin reaction procedure (17) and quantified on a Milton-Roy 1201 spectrophotometer. The remaining blood specimens were spun using a Centra 8 refrigerated centrifuge at 4 C and 3000 rpm for 10 min (International Equipment Company, MA). The separated plasma was transferred into cryo-freeze tubes and stored at –80 C in an ultra-freezer (Revco Scientific Inc., NC, USA) until all samples were ready to be analyzed. Finally, cortisol was measured in duplicate using a single-antibody, solid-phase methodology radioimmunoassay technique (DPC Inc., CA, USA). ACTH was measured in duplicate using a sandwich-style enzyme-linked immunosorbent assay technique (MD Biosciences Inc, MN, USA). All assay between and within coefficients of variation was less than 10%.

Statistical analysis

All statistical analyses were performed using SPSS [(v 15.0), LEAD Technologies, Inc., IL, USA]. Descriptive statistics (mean±SD) were computed for height, weight, age, and VO_{2max}. A repeated measures analysis of variance was used to analyze percentage of VO_2, changes in cortisol – ACTH levels and plasma volume change. A Tukey *post-hoc* test was used to determine pair wise differences (α=0.05) where appropriate.

RESULTS

Twelve male subjects participated in this study, their characteristics (X±SD) were as follows: age =26±3 yr, height =1.78±0.06 m, weight = 72.8±7.5 kg. The estimated body fat percentage and body mass index (BMI) for the subjects were 7.8±3.2% and 22.9±2.0 kg/m^2, respectively. Subjects had a mean VO_{2max} of 65.5± .1 ml/kg/min (4.80±0.63 l/min), which placed them above the 90th percentile in aerobic capacity for men between the ages of 20 and 29 yr according to the American College of Sports Medicine (15). This aerobic classification is supported by the subjects' training history, as they had been exercise training for a mean of 8.3±4.9 yr.

The actual intensities for the 3 submaximal exercise sessions the subjects completed were: 38.0±2.09%, 57.5±3.3%, and 77.4±4.0% of VO_{2max}. All exercise intensities were found to be significantly different from each other (p<0.05). These intensities were very close to the desired prescribed intensities of 40, 60, and 80% of VO_{2max}. Even though the actual exercise intensities varied slightly from the prescribed exercise intensities, the exercise trials will be referred to as 40, 60, and 80% throughout this paper. HR and rating of perceived exertion are summarized in Table 1 and further suggest that the subjects' 3 exercise bouts were of a physiological difference and caused varying amounts of stress as was desired.

Table 1 - *The heart rate (HR) and rating of perceived exertion (RPE) responses that correspond to the 3 30-min exercise sessions (mean±SD).*

	Exercise intensity					
	40%		60%		80%	
Time (min)	HR (bpm)	RPE	HR (bpm)	RPE	HR (bpm)	RPE
10	114±9	9±2	144±12	13±2	166±9	15±3
20	116±9	10±2	148±11	13±2	172±7	16±2
30	119±8	9±3	153±12	14±2	173±10	16±2

Figure 1 depicts the mean cortisol levels (±confidence interval) for the pre- and post- blood samples [exercise (no.=12) and resting-control (no.=6) sessions]. The pre- cortisol levels were not found to be significantly different from one another for each of the 4 experimental sessions (p>0.05). The post-sample cortisol level in the 30-min resting-control session was significantly reduced (p<0.05) from its respective pre-sample. In the 40% exercise session there was no change in the post-sample cortisol compared to the pre-sample. However, exercise caused a significant increase (p<0.01) in cortisol levels (post-) compared to pre-exercise samples in both the 60 and 80% sessions. Furthermore, the post-sample cortisol levels in the 60 and 80% sessions were significantly different from one another as well as being greater than both the 40% and resting-control sessions post-samples. Finally, the latter 2 post-samples (40% and resting-control) did not differ from one another. The percent change in cortisol from pre- to post-sample at the resting-control, 40, 60, and 80% sessions was –6.6±3.5%, +5.7±11.0%, +39.9±11.8%, and +83.1±18.5%, respectively.

The ACTH hormonal responses are depicted in Table 2. These responses mirrored closely those of cortisol. In the resting-control session ACTH decreased (non-significantly, p>0.05), was unchanged pre- to post-exercise in the 40% session (p>0.05), and was significant or approaching significant changes in the 60% (p<0.08) and 80% sessions (p<0.05). There was, however, a greater degree of variability in these data [most likely due to the small sample size (no.=4)] which mostly likely compounded the chance to find further statistical significance. Regrettably, methodological problems prevented further blood specimen analysis for this hormone.

The calculated changes in plasma volume for the resting-control, 40, 60, and 80% sessions were: +2.2±3.0%, –9.9±5.0%, –15.6±3.5%, and –17.2±3.3%, respectively. The plasma volume changes in response to all exercise sessions were significantly greater than those observed during the resting-control session. Also, the 60 and 80% reductions were significantly greater than in the 40% exercise session (p<0.05), but did not significantly differ from one another.

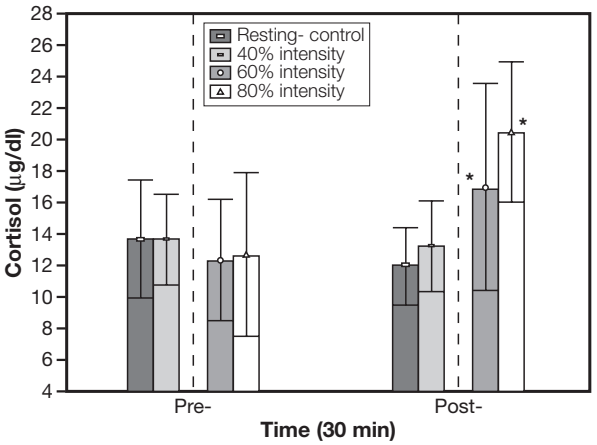

Fig. 1 - *The mean cortisol (±confidence intervals) response for each of the experimental sessions used in this research study. The increases at post-exercise sampling at 60% and 80% intensity are significantly greater than at pre-exercise sampling. The * denotes statistical significance (p<0.05) in respective pre- to post- comparisons.*

Table 2 - *The ACTH responses at the control-resting and the three 30-min exercise sessions (mean±SD). The * denotes statistical significance (p<0.05) and the γ denotes approaching significance (p<0.08) in respective pre- to post- comparisons.*

Hormone	Experimental sessions							
	Resting-control		40% intensity		60% intensity		80% intensity	
	Time							
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
ACTH (pg/ml)	13.5±4.4	9.1±4.9	12.2±4.3	10.8±5.4	12.3±4.1	20.1±6.0γ	12.9±6.3	43.2±11.3*

DISCUSSION

The intent of this study was to examine the influence of exercise intensity on the cortisol response of the HPA axis to 30 min of exercise at intensities of 40, 60, and 80% of VO_{2max} in order to determine which intensity elicited an increase in circulating hormonal levels. The findings support the concept that moderate to high intensity (60%, 80%) exercise will provoke significant increases in circulating cortisol. These increases seem to be brought about by a combination of hemoconcentration and well as enhanced secretion stimuli (i.e., ACTH) within the HPA axis. In contrast, low intensity (40%) exercise does not result in significant increases in circulating cortisol levels. In fact, if one considers the resting-control cortisol response, and the plasma volume reduction within this exercise session, then the 40% intensity actually results in a reduction in circulating hormonal levels.

The current findings are in agreement with several similarly designed studies. Specifically, those conducted by Davies and Few (3), Bloom (18), Luger et al. (19), and Rudolph and McAuley (20). Many aspects of the present study were constructed to model after the classic work of Davies and Few just mentioned. For this reason, we feel that the conclusions reached by those investigators are appropriate here too. Specifically that: (a) a "threshold intensity" of ~60% or greater VO_{2max} will elicit elevations in circulating cortisol; (b) the elevations observed most likely represent increases in the rate of glandular secretion and are not due to decreases in the metabolic clearance rate (MCR); and (c) the lack of elevation at the low intensity exercise may reflect increased MCR, in particular target tissue uptake of the hormone (3).

Divergent findings from the present study, however, have been reported by some investigators. Jacks et al. (10) found 60 min of exercise at 45, 60, and 75% of VO_{2max} resulted in significant cortisol increases at only the highest exercise intensity. Duclos et al. (9) also found no change in cortisol levels with 20 min of exercise at either 50 or 80% of VO_{2max}. Interestingly, in this same study, when the 50% intensity exercise was extended to 120 min there was still no significant increase in cortisol levels. The lack of agreement between the present study and those seeing either no increase with exercise, or an increase at only very high exercise intensities could be due to several factors. One such factor is exercise training background (2, 18, 21). More highly trained individuals typically have a higher intensity threshold necessary to provoke an increase in cortisol (5, 21). The level of training in the subjects, as assessed by VO_{2max} levels reported, seems to differ broadly across these various studies just mentioned. Another critical factor affecting hormonal outcomes is the timing used in the blood sampling protocol. Specific to cortisol, Daly et al. (22) reported that the time-point at which blood samples are collected with respect to exercise (immediately at the end, 10 min, 20 min into recovery, *etc.*) can greatly affect the interpretation of data outcomes due to the hormonal half-life. The collection time of blood samples was also vastly different across the present and the comparative studies noted above. In a similar fashion, the highly circadian aspects of cortisol make it necessary to conduct an appropriate resting-control evaluation of cortisol profiles prior to exercise manipulations (8), which not all of these studies with divergent results incorporated into their research designs.

As mentioned earlier, some researchers question the validity of certain studies concerning the influence of various exercise intensities upon circulating cortisol (1, 8). Some of this criticism is based upon the lack of control of confounding factors which interact with exercise to affect cortisol responses. The present study attempted to negate these confounding factors; that is, we controlled for time of day – circadian rhythms, prior diet – activity patterns, levels of exercise training, psychological stress, and utilized resting-control session to determine "normal" cortisol responses when exercise is not conducted. Because of the care we took in conducting our research, we strongly feel that our data are highly valid.

In conclusion, the present work supports the view that moderate to high intensity exercise will provoke increases in circulating cortisol levels. In contrast, low intensity exercise does not result in increases in cortisol levels. Once corrections for plasma volume reduction are conducted and circadian factors examined, low intensity exercise of 40% VO_{2max} results in a reduction in circulating cortisol levels.

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