IMMUNOENDOCRINE RESPONSES TO A SINGLE AND REPEATED PHYSICAL STRESS

Arsalan Damirchi¹, Hamid Arazi¹, and Parvin Babaei²

¹Dept of Physical Education, University of Guilan
²Dept of Physiology, Cellular and Molecular Research Center, Guilan University of Medical Sciences.

Corresponding author:
Parvin Babaei, PhD
Postal address: Iran, Rasht, Department of Physiology, Faculty of Medicine, Guilan. University of Medical Sciences
Tel: 09113313747
Email: p_babaei@yahoo.com

Submitted for publication: May 2008
Accepted for publication: July 2009

ABSTRACT
DAMIRCHI, A.; ARAZI, H.; BABAEI, P. Immunoendocrine responses to a single and repeated physical stress Brazilian Journal of Biomotricity, v. 3, n. 3, p. 253-260, 2009. Background: The purpose of this study was to investigate the effect of single and repeated physical exercise on serum C-Reactive Protein, IL6 and Cortisol. Methods: Eight physical education students (mean age 21, s= 1.51 yrs, mean weight 74.25, s= 8.85 kg, mean height 178.2, s= 5.57 cm) volunteered to participate in the present study. In a periodized design, during 21 days subjects completed two trials and one control condition. Trial one, performing concurrent endurance (cycling for 45 min with 75 percent of maximal heart rate and resistance (6 exercise in 3 sets and 8 repetition with 80% IRM for 45 min) exercises in the afternoon, and trial two performing above exercises in the morning and afternoon. Blood Samples were taken before, immediately and 3 hours after exercises, while the subjects were overnight fast before first blood sampling (at least 12 h). Related variables of acute phase response (C-Reactive Protein, Interleukin 6 and Cortisol) were measured. Results: According to obtained data there were no significant changes in C-Reactive Protein, Interleukin 6 and cortisol after single and repeated bouts of selected exercises (P>0.05). Also, there were no significant differences in mentioned variables between single and repeated bouts in a day (P>0.05). Conclusion: An acute phase response does not seem to occur as a consequence of training protocols of this study.

Key Words: Acute phase response, acute stress, exercise, Immune system

INTRODUCTION
Two bouts of exercise in one day is a normal training procedure for elite athletes in many fields of sport. A few studies have documented immuno -endocrine responses, specially the acute phase response after a single trial exercise (BRENNER et al., 1998; PEDERSEN & HOFFMAN–GOETZ, 2000; MACDONALD et al., 2003) and also limited data are available on repeated training (MARLIS et al., 1991; KACIUBA-USCILKO et al., 1992; KANALEY et al., 1997; KELLER et al., 2001; MCKUNE et al., 2006). The acute phase response is a common reaction to a range of threats to homoeostasis including...
bacterial infection, surgery, inflammatory diseases (KUSHNER & RZWNICKI, 1994), and prolonged exercise (KUSHNER, 1982). One of the most important acute phase proteins, is C-reactive protein (CRP) which has a role in induction of anti-inflammatory and suppression of pro-inflammatory cytokines in immune cells (PUE et al., 1996). Cortisol is a glucocorticoid which releases after stressful stimuli and exhibits anti-inflammatory effects. It has been known that Cortisol is involved in the redistribution of immune cells during exercise (CUPPS & FAUCI, 1982) and attenuate the initial response of immune system to inflammation (SHEPHARD et al., 1991). Another protein which is postulated to be important in acute phase response is interleukin-6 (IL-6). This cytokine is secreted by various cells (KELLER et al., 2001) and it is involved in regulation of energy metabolism, and also in evoking inflammatory responses (SUZUKI et al., 2000).

Susceptibility to respiratory tract infections after periods of heavy training is one of the main problems in sport medicine, and has been reported by previous studies (NIEMAN et al., 1990; NIEMAN & PEDERSEN, 1999). Based on temporary immunosuppressive changes that have been observed a few hours after prolonged heavy exertion (GLEESON et al., 1995; MACKINNON, 1997), the theory of “open window” has been proposed. This theory suggests that repeated bouts of exercise with incomplete immunological recovery might leave an athlete with increased risk of infections (NIEMAN et al., 1990; NIEMAN & PEDERSEN, 1999). To our knowledge a training protocol with two bouts of endurance and resistance concurrent exercises lasting more than 1 h, has not been studied. Therefore the aim of this study was to determine the presence or absence of the acute phase response measuring CRP, IL6 and Cortisol, after single and repeated physical exercises.

**METHODS**

- **Participants**

Eight male physical education students took part in the study. They were not specifically endurance or resistance trained but actively involved in various sports such as volleyball, handball and football on average 6-8 hours weekly. Before the start of the study, participants provided written informed consent. They were asked not to perform any strenuous exercise or consume medication 2 days prior to each trial. The protocol was approved by the Ethics Committee of Guilan University.

- **Preliminary Measurements**

Maximal oxygen uptake was estimated by means of a continuous incremental exercise test on a cycle ergometer (Monark 874E, Monark Exercise AB, Sweden) to volitional exhaustion. Participants began cycling at 70 Watt with increments of 35 Watt every 3 min. The cadence remained at 70 revolutions per minute (rpm) and heart rate was monitored using radiotelemetry. During the 3rd min of each work rate increment, expired gas was collected in Douglas bags. An oxygen/carbon dioxide analyzer (Servomax 1400B, Crowborough, UK) was used along with a dry gas meter (Harvard Apparatus, Edenbridge, UK) for determination of VO\textsubscript{2} and VCO\textsubscript{2}. From the VO\textsubscript{2} work rate relationship, the work rate equivalent to 60% VO\textsubscript{2max} was interpolated.

At the beginning of the study muscular strength was measured. Strength was assessed by one repetition maximum (1-RM) for bench press, leg press, leg extension, leg curl, lat pull-down, biceps curl. Following warm-up, for six resistance exercises testing, participants selected a weight with which they felt they could complete three repetitions. At this weight, they only performed one repetition. Participants then selected a weight they felt would be their 1-RM and attempted one repetition with this weight. Following successful attempts,
weight was increased by 2-5 kg for subsequent 1-RM attempts. The 1-RM was usually reached in less than 6 sets, including the warm-up set. Between each attempt three min of rest was allowed. Two assistants changed the weight on the bar between attempts.

- Experimental Procedures

The participants completed two experimental trials and one control condition in a counterbalanced order, each separated by 7 days. For trial one or one bout exercise, they reported to the laboratory at 13:30 after fasting from 22:30 the previous day to 10:30 A.M. Then performed 90 min cycling at 60% VO\(_{2\text{max}}\) followed by 90 min free-weight resistance exercise: bench press, leg press, leg extension, leg curl, lat pull-down, biceps curl consisted of three sets of 8 repetitions (80% 1-RM), with two min rest between sets and three min rest between exercises starting at 14:00. In trial two or two bouts exercise, subjects reported to the laboratory at 08:30 after an overnight fast, then performed two bouts of exercise (EX1 started at 09:00 and EX2 started at 14:00). During control condition or resting period participants were allowed to rest. All participants were asked to empty their bladder before measurement of body mass, and performed cycling at 70 rpm on the same ergometer used to determine VO\(_{2\text{max}}\). Heart rate was recorded continuously during exercise by radiotelemetry. During resting period, blood samples were taken at 09:00, 10:30, 14:00, 15:30 and 18:30. They were given a simple standardized breakfast (300 kcal) at 10:30 A.M and lunch (2000 kcal) at 12:00 P.M. also, water ingestion was allowed ad libitum during the trials. The laboratory temperature and relative humidity were 21.4 ± 0.4\(^\circ\)C and 64 ± 3%, respectively.

- Blood Collection and Analysis

Venous blood samples were taken from an antecubital vein by venepuncture, and were collected into 3 Vacationer’s tubes (Becton Dickinson, Oxford, UK). Plasma volume changes were calculated according to Dill & Costill (1974).

Whole blood was spun at 1500 g for 10 min in a refrigerated centrifuge at 4\(^{\circ}\)C. The obtained serum was immediately stored at –80\(^{\circ}\)C prior to analysis.

Cortisol (IT kit and gama counter LKB, Finland) was determined using radio immuno assay method (RIA) and IL –6 (DRG kit GmbH instrument, Germany) and CRP (DBC, SLT-Spectria Instrument, Austria) were determined using enzyme-linked immunosorbant assay (ELISA) methods. The intra-assay coefficient of variation was 3.5%, 8.3%, 4.7%, and inter-assay coefficient of variation was 5.1%, 7.6%, and 6.4% for cortisol, IL-6, and CRP respectively.

- Statistical Analysis

All results are presented as mean values and standard errors of the mean (exception to participant’s characteristics). Before statistical analysis data were checked for normality, homogeneity of variance, and sphericity using Kolmogorov-Smirnov test (P>0.05), and, where appropriate, the Huynh-Feldt method was applied for adjustment of degrees of freedom for the \(F\)-tests. Data were analyzed using 1-factor repeated measure ANOVA test. Statistical significance was accepted at \(P\leq0.05\).

RESULTS

- Participant’s characteristics: Participants had a mean age of 21, \(s=\ 1/51\) yrs, a mean height of 1.78, \(s=\ 5.57\) cm, a mean weight of 74.25, \(s=\ 8.85\) kg, a mean body fat content of 12.31%, \(s=\ 3.65\), and a mean peak aerobic power of 46.21, \(s=\ 6.77\) ml.kg\(^{-1}\).min\(^{-1}\).

- Plasma volume changes: There was no significant decrease in plasma volume \([F_{1, 7} =

\begin{align*}
...\
\]
3.6, \( P=0.055 \) in trial two (mean 53.17\%, \( s_x=0.31 \)) Compared with trial one (mean 52.38\%, \( s_x=0.63 \)) and control (mean 53.78\%, \( s_x=0.54 \)) at 15:30 P.M. Furthermore, no significant differences between control (mean 53.64\%, \( s_x=0.57 \)), trial one (mean 54.07\%, \( s_x=0.78 \)) and trial two (mean 54.49\%, \( s_x=0.74 \)) was observed for plasma volume at 18:30 P.M \([F_{1,7} = 1.15, P=0.34] \).

- C-reactive protein response: There was slight, but insignificant increase in CRP concentration between trial two (mean 101.12, \( s_x=16.32 \) ng.mI\(^{-1}\)) \([F_{1,7}= 4.02, p=0.052] \) and trial one (mean 90.22, \( s_x=18.80 \) ng.mI\(^{-1}\)), at 15:30 P.M. compared with control (mean 67.28, \( s_x=10.2 \) ng.mI\(^{-1}\)). No significant differences was observed in plasma concentrations of CRP \([F_{1,7} = 1.91, P=0.20, \text{Figure 1A}] \) between control (mean 73.37, \( s_x=14.90 \) ng.mI\(^{-1}\)), trial one (mean 89.03, \( s_x=15.81 \) ng.mI\(^{-1}\)), and trial two (mean 85.50, \( s_x=14.60 \) ng.mI\(^{-1}\)) at 18:30 P.M.

- Interleukin-6 response: As figure 1B shows there was no significant change in IL-6 in trial two (mean 3.67, \( s_x=0.47 \) pg.mI\(^{-1}\)) compared with trial one (mean 3.99, \( s_x=0.41 \) pg.mI\(^{-1}\)) and control (mean 3.50, \( s_x=0.59 \) pg.mI\(^{-1}\)) at 15:30 P.M \([F_{1,7} = 0.50, P=0.52] \). Furthermore, the results of IL-6 concentration in trial two (mean 4.70, \( s_x=0.51 \) pg.mI\(^{-1}\)) compared with trial one (mean 3.60, \( s_x=0.41 \) pg.mI\(^{-1}\)) and control (mean 4.70, \( s_x=0.51 \) pg.mI\(^{-1}\)) at 18:30 P.M \([F_{1,7} = 1.57, P=0.25] \) were not significant.

- Cortisol response: As figure 1C shows there is no significant differences in Cortisol level \([F_{1,7} = 2.98, P=0.08] \) between trial one (mean 391.87, \( s_x=27.93 \) nmol.l\(^{-1}\)), trial two (mean 277.37, \( s_x=49.39 \) nmol.l\(^{-1}\)) and control (mean 388.12, \( s_x=43.15 \)) at 15:30 P.M. Almost, the similar insignificant results was observed in trial two \([F_{1,7}=1.60, P=0.24] \) (mean 381, \( s_x=38.50 \) nmol.l\(^{-1}\)) compared with trial one (mean 475, \( s_x=50.43 \) nmol.l\(^{-1}\)) and control conditions (364.25, \( s_x=56 \) nmol.l\(^{-1}\)) at 18:30 P.M.

Figure 1 - Changes in serum concentrations of CRP (A), IL-6 (B) and Cortisol (C) among control, immediately and 3 h after exercises (Trial one and two) conditions. Values are means ± standard error of the mean (n=8); (p≤0.05).
DISCUSSION

The main purpose of this study was to test the hypothesis that a second bout of endurance exercise would evoke the acute phase response compared with one bout of similar exercise. The findings do not support the mentioned hypothesis. This is suggested by a slight and insignificant increase in serum concentration of CRP, IL6 and Cortisol after performing second bout of endurance and resistance concurrent exercise.

The finding of Tomaszewski et al. (1998) showing slight increase in CRP level just after an ultra marathon is consistent with our findings, whereas Okita and his colleagues (2004) found that exercise training combined with weight reduction, significantly decreased CRP levels. According to Albert et al (2004) prolonged aerobic physical activity may lower CRP level by favorable effects on lipid profile in blood, body mass index, insulin metabolism, and blood pressure.

Differences between effects of training on serum CRP level in studies may be due to the involvement of different mechanisms in the regulation of acute phase responses in particular conditions (GLEESON et al., 1995; KELLER et al., 2001; OKITA et al., 2004). In our study the basal level of C-reactive protein was within the normal range and increased slightly just after the cessation of exercise. The serum IL-6 concentration was not elevated significantly after exercise in any of trials. These results are inconsistent to Pedersen et al. (2001) study. Their experiment was carried out on prolonged strenuous exercise and showed a marked elevation in the plasma IL-6 level. Recent studies have demonstrated that IL-6 is produced in contracting skeletal muscle, released into the circulation (STEENSBERG et al., 2000) and acts as a hormone to promote hepatic glucose production and also stimulate lipolysis in adipose tissue during exercise (PEDERSEN et al., 2001). The other source for producing interleukin-6 is adipose tissue (FRIED et al., 1998), and it probably does not play a major role in immunological and inflammatory responses, but more likely is involved in the regulation of energy utilization and functions as an anti-obesity cytokine (AHIMA & FLIER, 1998; PEDERSEN et al., 2003). The degree of increase in serum IL-6 heavily depends on the protocol of physical exercise. After marathon races, serum level of IL-6 increases more than 100 times (SUZUKI et al., 2000), while, in the present study it only increased very slightly. It has been known that various protocols of physical exercise induce different types of energy metabolism, so metabolic demand is very important in determining IL-6 response to physical exercise (PEDERSEN et al., 2001; MACDONALD et al., 2003). There is more controversy about the importance of exercise – induced IL-6. Some data suggest that IL-6 is an anti-inflammatory cytokine and causes inhibitory effects on low-grade inflammation, and also speculated to exert metabolic control by increasing energy supply during exercise (PEDERSEN et al., 2001) and facilitating glycogen phosphorylase and lipase (KELLER et al., 2001; MOHAMED-ALI et al., 2001, LINGSO et al., 2002).

Although there was no direct evidence that activity used in trial two of our study, caused glycogen depletion, it can be assumed that the subjects’ glycogen stores would have not been depleted at the end of trial.

In this study there were no significant differences in the serum concentrations of cortisol between trial one and two. This finding is consistent with Brenner et al. (1998) and Marliss et al. studies (1991). They used two bouts of moderate exercise with interval rest of 45 min, and found no differences in the cortisol responses between the first and second bout of exercise.

In our study, participants had two meals with 2300 kcal during the 3.5 hours of rest between the two bouts exercise in trial two, which is sufficient for complete restore of
muscle glycogen before initial of the training (VOLESTAK et al., 1989; DHABHAR et al., 1994). It seems participants were not relying on hepatic glycogen break-down toward the end of the second exercise in trial two compared with trial one (WINDER & GALBO, 1986).

It has been known that cortisol is involved in the redistribution of immune cells during exercise (CUPPS & FAUCI, 1982). Inhibitory effects of cortisol on migration of neutrophils out of the circulatory compartment (BRENNER et al., 1997) could consequently reduce the initial response of immune system to inflammation, and trauma (SHEPHARD et al., 1991). Our observation of a non pronounced neuroendocrine response elicited by the second bout of exercise compared with the first bout might relates to the lack of decreased muscle glycogen levels, and reciprocal immuno-neuroendocrine regulation. Based on insignificant changes in immunoendocrine response to a second bout of exercise after 3.5 h of recovery between the two bouts we can conclude: 1) two bouts exercise performed in the morning and afternoon does not induces acute phase response, 2) a 3.5 hours rest is sufficient for recovery of immune system after 90-min endurance resistance concurrent exercises. Further research is warranted to study the other immunoendocrine factors related to this kind of exercise. The results have important clinical implications, for athletes who commonly perform training sessions used in this experiment.

REFERENCES


SHEPHARD, R. J.; VERDE, S. T.; THOMAS, S. G.; SHEK, P. Physical activity and the


AUTHORS BIOGRAPHY

**Name:** Parvin Babaei  
**Employment:** Guilan University of Medical Sciences  
**Degree:** Ph.D  
**Research interests:** exercise immunology, molecular biology, obesity  
**E-mail:** p_babaei@yahoo.com

**Name:** Arsalan Damirchi  
**Employment:** Guilan University  
**Degree:** Ph.D  
**Research interests:** exercise immunology, obesity, physical fitness  
**E-mail:** damirchi@guilan.ac.ir

**Name:** Hamid Arazi  
**Employment:** Guilan university  
**Degree:** Ph.D  
**Research interests:** exercise immunology, physical fitness  
**E-mail:** hamid_arazi2003@yahoo.com