Response of Interleukin-6, C - Reactive protein and Glutamine to different intensities exercise

*Dr. Wafaa I ALRajhi
**Dr. Mohamed M Abd-ALZaher

Abstract

The object of this study was examine the effect of different exercise intensities protocol on response of **Interleukin-6 , C-Reactive protein and Glutamine** immediately after exercise and after 1 h recovery period . A nine male active from varying degrees in different sports participate in this study, The mean (Age 18.56 ±0.23yr, Body height 182.22±0.39 cm, Body mass Index 25.05 ±0.29 %, Body Fat 11.63 ±0.48). Subjects underwent a **different intensities exercise protocol** on treadmill for 24 min. During treadmill test, speed started 7 Km/h for 3 min this was followed by Seven additional consecutive runs of 3-min duration at treadmill speed of 4, 8, 7, 10, 12, 10, 12 Km/h. Oxygen uptake during exercise protocol on treadmill was measure by a gas analyzer (GmbH, Germany). Blood samples were collected from the study group immediately before and after exercise and after 60 min recovery period. Concentration of **Interleukin-6 (IL-6), C - reactive protein (CRP) and Glutamine (Glut)** were determined by using ELISA Kit. **The results** of this study show that: Measurements of cardio respiratory fitness on treadmill for maximum heart rate (HRmax) was 185.44±1.24 b/min, maximum Oxygen uptake (VO2max) was 2.35±0.12 L/min, VO2max per/ Kg was 32.14±2.44 $ml.kg^{-1}.min^{-1}$, Ventilation (VE) was 93.09±1.73 L/min and respiratory exchange ratio (RER) was 1.27±0.5. On the other hand, Levels **IL6, CRP and Glut** were significantly increased ($P<0.005$) immediately after exercise and remained elevated after 1 h recovery period except Glut was non significant, as

* Biology Department, Faculty of Science, Jazan University, Jazan city, KSA
** Health Courses, Department, Faculty of Physical Education, Helwan University, Egypt.

compared with that of before exercise. While all parameters, after I h recovery, exhibited significant retention (P<0.005), as compared with immediately after exercise protocol. **It was concluded that:** a significantly increases of **IL6, CRP and Glut** immediately after exercise seems to clear at different intensities exercise protocol. The intensity of exercise protocol at which effective on experimental parameters may be depends on some variables such as exercise duration, training status, Vo2max.

KEY WORDS: Exercise, cytokines, endotoxin stimulation, inflammation, interleukin-6, C-reactive protein, glutamine, recovery.

Introduction

Over the past 25 years a variety of studies have demonstrated that exercise induces considerable physiological change in the immune system (1) There are several lines of evidence suggesting that various forms of physical stressors can stimulate similar alterations in immune system (2).Exercise can have both positive and negative effects on the immune system (3). The interactions between exercise stress and the immune system provide a unique opportunity to link basic and clinical physiology and to evaluate the role of underlying stress and immune physiology mechanisms. (4). Moderate exercise has been reported to produce an anti-inflammatory environment and thus reduce the incidence of infection (5-11). Conversely, continuous, intense exercise causes a temporary suppression of many parameters of immune function, depending on the intensity and duration of exercise, may by increase oxidative stress (an overproduction of reactive oxygen species (ROS) compared to the body's ability to detoxify), inflammatory responses, as well as the risk for infection (5 ; 7 ; 12; 13). In general, acute exercise bouts of moderate duration (<60 min) and intensity (< 60% VO2max) are associated with fewer perturbations and less stress to the immune system than are prolonged, high-intensity sessions (14**)**. The functional capacity of the immune system is necessary to be determined in order to get useful information about the immune system status of athletes and its

impact on performance (5)

IL-6 is a pleiotropic hormone that has both pro- and anti-inflammatory cytokine actions. **(15)**. IL-6 is normally present at extremely low concentrations and is rapidly cleared from the blood and other body compartments. Although the pro-inflammatory cytokines (IL-1, IL-6) are released during and after exercise, the responses appear to be subtle and have not been consistently. It has been demonstrated that IL-6 has many biological roles such as: (1) induction of lipolysis; (2) suppression of TNF production; (3) stimulation of cortisol production (16) . Recently, IL-6 was introduced as the first myokine, defined as a cytokine that is produced and released by contracting skeletal muscle fibers, exerting its effects in other organs of the body. (10)

CRP is biomarkers of systemic inflammation**.** CRP production is part of the nonspecific acute-phase response which comprises the nonspecific physiological and biochemical responses of endothermic animals to most forms of tissue damage, infection, inflammation, and malignant neoplasia (17) .Exercise produces a short-term, inflammatory response, transient increase in serum CRP after strenuous exercise, produced by an exercise-induced acute phase response, mediated by the cytokine system and mainly IL-6 (18).

Glutamine is a conditionally essential amino acid that performs several roles in the human body, and is synthesized by skeletal muscle and other tissues (19). One role that has sparked recent attention involves immune function. Glutamine provides energy to immune cells through glutaminolysis, a process by which it becomes partially oxidized. Glutamine also has been linked to the regulation of T and B lymphocyte proliferation rates (20). Many studies have investigated the effect of exercise on plasma glutamine status. Change in plasma glutamine concentration can vary, based on the amount of time that has passed since the exercise session ended as well as the duration of the exercise bout. While it is common for exercisers to experience a decline in glutamine concentrations during

prolonged exercise (21; 22) shorter bouts can increase plasma glutamine concentrations (23; 24).

The aim of this study was to determine the effects of different intensities exercise protocol on **IL6, CRP and Glut** immediately after exercise and during 1h recovery period.

Subjects and Methods

Nine males active to varying degrees in different Sports were participate in this study, their descriptive characteristics are shown in table (1).

Table (1)

Showing Physical and physiological characteristic of the subjects

Variable	Before exercise	Immediately after exercise	(%C)	P-value
Age (years)	18.56 ±0.23*			
Body height (cm)	182.22 ±0.39*			
Body Mass Index (BMI)	25.05 ±0.29*			
Percent Body Fat (PBF %)	11.63 ±0.48*			
heart rate (HR b/min)		185.44± 1.24*	+ 114.8	<0.005
Vo2 max (L/min)		2.35±0.12*	+473.17	<0.005
Vo2 max (ml $.kg^{-1}.min^{-1}$)		32.14±2.44*	+446.6	<0.005
respiratory exchange ratio (RER)		1.27±0.05*	+41.11	NS
Ventilation (VE L/min)		93.09±1.73*	+528.56	<0.005

* means ± SE, %C=percentage of change as compared with that of before exercise ; $n = 9$

Experimental protocol The test was conducted in Human physical performance Laboratory at Helwan University (Egypt), Faculty of physical Education, and each subject completed a 10 – overnight fast and abstained from training or strenuous exercise for at least 36 h prior to test. On arrival at the laboratory a resting blood sample were drawn from the heated hand vein catheter immediately before and after exercise and after 60 min of recovery period to determined response of **IL6, CRP and Glut** for exercise protocol. Subjects then underwent a different intensity exercise protocol on treadmill for 24 min. During treadmill test, speed started at 7 Km/ h for 3 min this was followed by seven additional consecutive runs of 3-min duration at treadmill speed of 4, 8, 7, 10, 12, , 10, 12 Km/ h. Oxygen uptake when the subjects running on a motorized treadmill was measure by a gas analyzer (GmbH, Germany). The participants breathed through a respiratory valve with a mouthpiece. The results were registered both graphically and numerically in a computer (Compac descpro 286e). HR and RER were noted during exercise protocol and especially in the last period. TheVo_2 max, HR_{max}, V_E max, RER were determined over the final 1-min of the test.

Methods of Biochemical Determination: Concentration of **IL-6 & CRP** were determine by using ELISA Kit (GenWay Biotech, Inc) using the methods of Moscovitz et al (25) & Votila, et al (26) respictively**. Glut** content of serum was estimated by using glutamine enzymatic kit (Sigma-Aldrich,com) according to the method of Lund (27)

Statistical analysis: The data were analyzed using student's t-test statistical methods. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant. All the results were expressed as mean ± standard error of the mean.

Results

The cardio respiratory characteristic of the subjects are listed in Tables 1:The cardio respiratory data as shown in table 1 revealed that , HR max

was 185.44±1.24 b/min, Vo_2max was 2.35±0.12 L/min, Vo_2/body weight was 32.14±2.44 $ml.kg^{-1}.min^{-1}$, VE was 93.09±1.73 L/min and RER was 1.27±0.05

Biochemical results of the subjects are listed in Tables 2:

Table 2
Showing biochemical assayed of the subjects

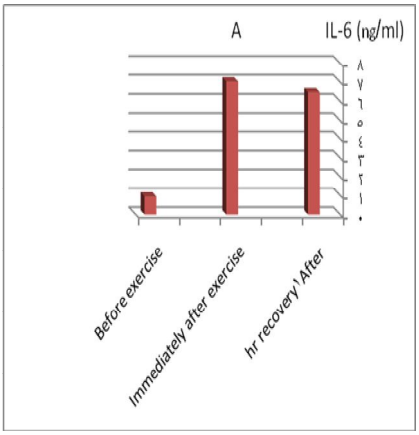
Variable	Statistical analysis	Before exercise	Immediately after exercise	After 1hr recovery
IL-6 (ng/ml)	Mean	0.96	7.04	6.49
	SE	±0.04	±0.03	±0.06
	%C*		+633.3	+562.1
	P-		<0.005	<0.005
	%C**			-7.8
	P-			<0.005
CRP (ng/ml)	Mean	3.6	7.1	4.7
	SE	±0.10	±0.08	±0.05
	%C*		+97.2	+30.6
	P-		<0.005	<0.005
	%C**			-33.8
	P-			<0.005
Glut (umol/ml)	Mean	564	588	562
	SE	±3.95	±2.4	±2.77
	%C*		4.26	0.36
	P-		<0.005	NS
	%C**			- 4.42
	P-			<0.005

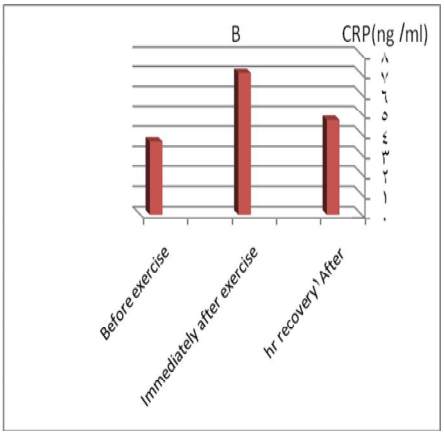
n=9; NS=Non-significant
%C*=percentage of change as compared with that of before exercise.
%C**=percentage of change as compared with that of immediately after exercise

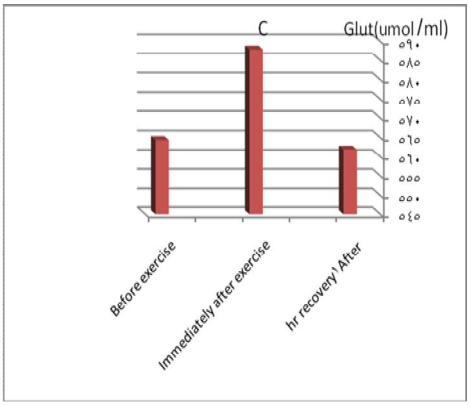
As shown in table 2 and fig1,the values of IL-6, CRP and Glut displayed significant elevation immediately after exercise protocol +633.3% ,+ 97.2% ,+ 4.26% respectively. Meanwhile, the elevation still present after1 h recovery in both IL-6 (+562.1%) and CRP (+30.6%) but Glut was non significant compared with before exercise. However, after 1h recovery, the three

parameter recorded significant decreased -7.8%, - 33.8%, - 4.42% respectively compared with immediately after exercise protocol.

FIG 1: Parameter assayed as in table 2: IL-6 (A), CRP (B) &Glut C)







Discussion

In the current study, The concentration of IL-6 increases up to 633.3% directly after exercise protocol ,which still present (+576.1%) after 1 h recovery as compared with before exercise. The current result is in agreement with previous findings of Ostrowski et al (28); Pedersen & Hoffman (1); Febbraio &Pedersen (29) . Numerous studies have consistently demonstrated that a single bout of exercise results in an elevation in circulating IL-6 (30; 31).

IL-6, an important cytokine involved in a number of biological processes, is

consistently elevated during periods of stress. The mechanisms responsible for the induction of IL-6 under these conditions remain uncertain (32).

In response to acute exercise, plasma IL-6 increases up to 100-fold from the basal level, mainly due to a major release of IL-6 from contracting skeletal muscle per se (33). Sterpetti et al (34) suggested that factors unrelated to muscle damage, such as endogenous catecholamines or endothelial shear stress are possible mechanisms contributing to the increase in IL-6 associated with exercise . It has also been suggested that the exercise induced increase in plasma IL-6 is related to the sympathoadrenal response to exercise (35).

Recent studies suggest that complex intramuscular signaling stimulates the exercised muscle to release IL-6 (36; 29), independently of muscle damage. Subsequently, muscle damage per se elicits a repair response, including macrophage entry into the muscle, causing further IL-6 production. This injury-induced IL-6 response is delayed and smaller than the IL-6 production related to muscle contraction. Furthermore, the increase in IL-6 is directly related to exercise intensity, duration, and mass of muscle recruited (29; **37**)).Furthermore, it has been demonstrated that , changes in calcium homeostasis, impaired glucose availability, and increased formation of ROS are all capable of activating transcription factors known to regulate IL-6 synthesis. Via its effects on liver, adipose tissue, hypothalamic-pituitary-adrenal (HPA) axis and leukocytes, IL-6 plays a major role in regulating the inflammatory process induced during exercise as part of an integrated metabolic regulatory network. (36-39). Therefore IL-6 is necessary for normal exercise capacity (40)

The role of muscle-derived IL-6 is under investigation, but it appears to act like a hormone, assisting glucose homeostasis and lipolysis during exercise, whereas it also may have immune regulatory effects by inhibiting tumour necrosis factor- alpha (TNF-alpha) production (36 ; 16 ; 41)

Furthermore, the search for ‘the exercise factor’ that mediates the health benefits of

exercise has revealed IL-6 as a strong muscle is silent at rest but rapidly transcribed during exercise, releasing IL-6 into the circulation in concentrations up to 100-fold (42). On the other hand , From the results obtained, the production of IL-6 increased significantly 1 hours after exercise, may attributed to the increased both percentage and absolute number of blood monocytes (43), or supported the hypothesis that the post-exercise cytokine production is related to skeletal muscle damage **(**44**)**

Is plausible that , in addition to IL-6 aid in maintaining metabolic homeostasis during periods of altered metabolic demand such as muscular exercise or insulin stimulation (via local and/or systemic effects) IL-6 may be used as a therapeutic drug in treating metabolic disorders and other diseases (29; 45). IL-6 is the main cytokine involved in the induction of acute phase response, which includes synthesis of certain proteins in the liver, one of which is C-reactive protein (CRP). From the results obtained, the increment recorded in CRP both directly after exercise protocol and after 1 h recovery (+97.2%, +30.6 %) respectively is in compatible with the current elevation of IL-6 during the period of observation. Kasapis & Thompson (18) reported that there is a short-term, transient increase in serum CRP after strenuous exercise, produced by an exercise-induced APR, mediated by the cytokine system and mainly IL-6. Pepys &Hirschfield (17) suggested that Plasma CRP is produced only by hepatocytes, predominantly under transcriptional control by the cytokine IL-6, although other sites of local CRP synthesis and possibly secretion have been when the stimulus for increased production completely ceases, the circulating CRP concentration falls rapidly, at almost the rate of plasma CRP clearance (17). Furthermore, the increase of IL-6 at the end of exercise is responsible for the increased CRP levels during recovery period (46).

Many studies have investigated the effect of exercise on plasma glutamine status. Change in plasma glutamine concentration can vary, based on the amount of

time that has passed since the exercise session ended as well as the duration of the exercise bout.

Our result revealed that Glut elevated significantly (+4.26%) directly after exercise protocol, as compared with before exercise. This finding is compatible with many previous studies. They documented that shorter exercise bouts can increase plasma glutamine concentrations (23; 24; 47; 48). During 10-20 min of exercise at 70 % of the maximal oxygen uptake, Bergstrom *et al.* (49) observed increased concentrations of glutamine. An interesting observation was made by Katz *et al.* (50). They found a 60% decrease in plasma glutamate concentration with maximal exercise, similar to that seen in other studies (21; 22; 51), but no significant change when glutamate was determined in whole blood. This was attributed to the fact that most of the glutamate is confined to the red blood cell; at rest, the whole-blood glutamate concentration is twice the plasma concentration.

Skeletal muscle is the major tissue involved in glutamine synthesis and storage; it is known to release glutamine into the blood and to influence plasma glutamine concentrations (53). There is evidence that glucocorticoids increase the rate of glutamine release from skeletal muscle in several species, including human (51) .However, the effect of exercise on the rate of glutamine release from muscle has not been defined (51). Much of the glutamine released by muscle is thought to be used by some key cells of the immune system. Newsholme et al (54) proposed that immune cells might use chemical messengers such as cytokines, released from different cells of the immune system, to communicate with skeletal muscle in relation to regulating the rate of glutamine release.

On other hand, 1h after exercise, Gluta concentration was restored to levels found in subject before exercise. This change was associated with lower muscle glutamine concentrations caused by impaired glutamine synthesis.

Conclusion: the release of IL-6, *CRP and Glut* immediately after exercise from working skeletal muscles is positively related to both intensities and duration of

exercise. However different intensities of exercise protocol may helps retention of significantly increased IL-6 , *CRP* immediately after exercise *and fast* recovery *of Glut* during 1 h recovery period **.**

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