Effect of Muscular Exercise and Antioxidants on Myokines in Male Albino Rats

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Abstract: Background: The interactions between exercise and the immunological changes provide a unique opportunity to link basic and clinical physiology and to evaluate the role of underlying changes during exercise and immuno-physiological mechanisms. **Objectives:** To delineate the cytokine response to exercise training. In addition, it was designed to investigate the existence of any beneficial additive effects of chronic exercise training and antioxidants intake. **Material and methods:** In the present study, a total number of 40 healthy male albino rats were used. The animals were divided into four groups. **Results:** The results of this study showed that exercise caused significant elevation of the tumor necrosis factors (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), malondialdehyde (MDA) and total antioxidants associated with significant decreased level of interleukin-10 (IL-10). Antioxidants supplementation caused significant decreased levels of TNF, IL-1, IL-6, MDA and total antioxidants associated with significant elevation of IL-10 when compared with control group. Exercise training and antioxidant supplementation caused significant elevation of TNF, IL-1, IL-6, MDA and total antioxidants associated with insignificant decreased level of IL-10 when compared with control group. **Recommendations:** To avoid any hazardous effect of exercise, antioxidant must be administered to exercised individuals. Also, further studies should be done to evaluate the effect of different modes, intensities and durations of exercise training on myokines.
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1. Introduction

Over the past 15 years, varieties of studies have demonstrated that exercise induces considerable physiological changes in the immune system. 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 immuno-physiological mechanisms **(Paul et al., 2014).**

Physical exercise can be regarded as a prototype of physical stress. Many clinical physical stressors e.g. surgery, trauma, burn, and sepsis induce a pattern of hormonal and immunological responses that have similarities to that of exercise **(Gordon et al., 2012).**

The humoral and cellular changes occurring after strenuous muscular activity resemble in some aspects the acute-phase response to trauma and inflammation **(Svenia and Christoph 2015).**

Exercise-induced cytokine production was linked to muscle damage. However, other studies did not confirm this and it is likely that IL-6 is produced because of muscle contractions and is involved in repair mechanisms in relation to muscle damage **(Heredia et al., 2013).** In addition, exercise was found to increase the levels of reactive oxygen species, both in the blood and within the working muscles **(Booth et al., 2012).** Cytokines play vital roles in host defense but, when present in excess or in the course of inappropriate autoimmune response they can have self-destructive effect. The proinflammatory cytokines groups contain tumor necrosis factor (TNF), interlukein-1 (IL-1), interlukein-6 (IL-6), IL-16 and IL-17 **(Boney et al., 2014).** TNF and IL-1 stimulate the production of IL-6, which has been classified as a proinflammatory and an anti-inflammatory cytokine. Currently, IL-6 is considered to have primarily anti-inflammatory effects. Infusion of IL-6 into humans results in fever but does not cause shock or a capillary-leakage–like syndrome as observed with the typical proinflammatory cytokines IL-1 and TNF **(Johnen et al., 2013).**

Mononuclear phagocytes are the major cellular source of TNF-α but many other nucleated cells are capable of producing it. Among the cells that were shown to produce TNF-α upon appropriate stimulation are T cells (T helper (Th) cells and T cytotoxic (Tc) cells), B cells, natural killer cells, vascular endothelial cells, keratotinocytes, smooth muscle cells, mast cells, neutrophils, astrocytes and glial cells **(Neta et al., 2002).**

Agents that stimulate the production of TNF-α often lead to the production of IL-1 as well. Among the most employed stimuli are various bacterial products, especially lipopolysaccharides (LPS), but also ultraviolet light, virus infection, protozoa and other microorganisms. The production of TNF can also upregulated by variety of cytokines and other endogenous mediators, including TNF itself, IL-1,

interferon, granulocyte-macrophages colony stimulating factor (GM-CSF), IL- 2, transforming growth factor, supstance P and platelet activating factor **(Neta et al., 2002).**

Mononuclear phagocytes are the major cell source of IL-1, but all other nucleated cells are capable of producing IL-1. Normal cells must be stimulated to produce IL-1. IL-1ra is also produced by many cell types, tissue macrophages and keratinocytes are good producers **(Dinarello, 2007)**.

IL-1 production by macrophages can be triggered by a wide variety of stimuli, including bacterial cell wall products, LPS, microbial superantigens, dipeptide antigens, endogenous cytokines, leukotrienes, activated complement components, immune complexes, ultraviolet irradiation, silica particles, viruses, parasites and other micro organisms. Agents that activate lymphocytes can stimulate macrophages to produce IL- 1 either by direct cell contact, which is genetically controlled by major histocompatibility complex (MHC) class II, or by producing cytokines, such as TNF-α, GM-CSF or IL-1 itself, which can stimulate macrophages and other cells to produce more IL-1 **(Dinarello, 2007).**

Biologic activities of IL-I are mostly the indirect result of induction of a cascade of other mediators. Its biologic activities largely overlap with those of TNF-α and IL-6. IL-1 is a pielotropic mediator of the host response to infections and injurious insults, and it coordinates the activities of other cells and cytokines. Many of the effects of IL-1 are mediated through its capacity to increase the production of other cytokines, such as granulocyte-colony stimulating factor (G-CSF), TNF-α, IL-6, IL-8 and homologous members of the chemokine family, platelet-derived growth factor (PDGF), and IL-11. Further amplification of the biologic effects of IL-1 is achieved by the ability of IL-1 to upregulate receptor expression for itself and for the receptors for IFN, IL-3, and GM-CSF, and the consequent synergistic action of some of these cytokines with IL-1 **(Muegge et al., 2012).**

IL-1 production within the thymic microenvironment induces expression of IL-2 receptor chain on early immature thymocytes. Thus, IL-1 can contribute to thymocyte maturation and CD4 differentiation. Despite this, thymic development in knock-out mice that lack the IL-1 type I receptor is normal, presumably based on redundancy of cytokine activities and compensatory pathway **(Yang et al., 2013).**

IL-1 can also promote B-cell maturation and differentiation. IL-1 augments B-cell proliferation, surface IgM expression, and antibody production. However, knock-out mice that lack the IL-1 type I receptor have normal serum immunoglobulin levels and a normal immune response. IL-1 acts on monocytes and neutrophils, inducing secretion of several cytokines, including IL-8 and IL-1 itself. IL-1 is reported to prolong the survival of neutrophils in vitro. IL-1 augments the antigen-presenting capacity of dendritic cells **(Canto and Auwerx, 2012)**.

IL-6 has long been regarded as a proinflammatory cytokine induced by lipopolysaccharides (LPS) along with TNF-α and IL-1. IL-6 is often used as a marker for systemic activation of proinflammatory cytokines. Like many other cytokines, IL-6 has both proinflammatory and anti-inflammatory properties. Although IL-6 is a potent inducer of the acute-phase protein response, it has anti-inflammatory properties as well **(Barton et al., 2006).**

IL-6 appears to play a central role in lipopolysacharide -induced fever and has growth-regulatory effects on a number of cells. Thus, IL-6 induces B and T cell differentiation and is involved in bone metabolism by regulating osteoclast and osteoblast development and function **(Tamura et al., 2011).**

Another role for the exercise-produced IL-6 may be to regulate processes within the producing muscles. IL-6 is known to have local regulatory functions in other tissues such as the nervous system and the skeleton **(Argiles et al. 2009).**

Interleukin-10 (IL-10) is an anti-inflammatory cytokine with a crucial role in preventing inflammatory and autoimmune pathologies **(O'Garra et al., 2008).**

IL-10-deficient mice develop inflammatory bowel disease following colonization of the gut with particular microorganisms and show other exaggerated inflammatory responses to microbial challenge. Although the absence of IL-10 leads to better clearance of some pathogens with no enhanced immuno pathology **(Ejrnaes et al., 2006),** during other infections the absence of IL-10 can be accompanied by an immunopathology that is detrimental to the host but does not necessarily affect the pathogen load **(Trinchieri, 2007).** This suggests that an absence of IL-10 is not always compensated by other regulatory mechanisms and thus that there is a non-redundant role for IL-10 in limiting inflammatory responses in vivo. Interleukin 10 (IL-10) is an important immunoregulatory cytokine mainly secreted by macrophages, but also by T helper 1 (Th1) and Th2 lymphocytes, dendritic cells, cytotoxic T cells, B lymphocytes, monocytes and mast cells **(Trifunović et al., 2015).**

Exercise training is well known to improve physical fitness and to combat chronic diseases such as diabetes as well as other aging associated disorders **(Anton et al., 2013).**

There are detectable physiological changes in the immune system that could be induced by exercise. The interactions between exercise stress and the immune system could be considered as a link between basic and clinical physiology and to evaluate the role of underlying stress and immunophysiological mechanisms **(Thompson et al., 2009).**

The local response to an infection or exercise involves the production of cytokines that are released at the site of inflammation. These cytokines facilitate an influx of lymphocytes, neutrophils, monocytes, and other cells, and these cells participate in the clearance of the antigen and the healing of the tissue (Silveira et al., 2010). The local inflammatory response is accompanied by a systemic response known as the acute phase response. This response includes the production of a large number of hepatocyte-derived acute phase proteins, such as C-reactive protein (CRP) **(Bailey et al., 2007).**

Injection of TNF- , IL-1, and IL-6 into laboratory animals or humans will produce most, if not all, aspects of the acute phase response. These cytokines are therefore usually referred to as "inflammatory" or "proinflammatory cytokines," although it may be more reasonable to classify IL-6 as an inflammation-responsive cytokine rather than a proinflammatory cytokine since IL-6 does not directly induce inflammation **(Thompson et al., 2009).**

Strenuous exercise induces an increase in the proinflammatory cytokines TNF-α and IL-1β and a dramatic increase in the inflammation responsive cytokine IL-6. This release is balanced by the release of cytokine inhibitors and the anti-inflammatory cytokine IL-10 **(Fehrenbach et al., 2006).**

Production of inflammatory cytokines in response to exercise may stimulate the production of prostaglandins. Further support comes from the high levels of plasma IL-6 found immediately after the completion of an exhaustive exercise bout. Thus the IL-6 production or release precedes neutrophil and macrophage accumulation in the muscle, the increase in prostaglandin E2, the increase in cytokines and the sensation of delayed onset muscle soreness **(Keller et al., 2013).**

Exercise is not characterized by a fully developed systemic proinflammatory response. This lack for systemic response may be due to only a transient cytokine release in response to exercise. Alternatively, this may reflect an adaptation to the cytokine response (e.g., increased ability to induce effective naturally occurring inhibitory cytokines and cytokine receptors) **(Pedresen and Hoffman-Goetz, 2011).**

Oxidative stress is defined as increase in steady state levels of reactive oxygen species, including superoxide anion (O2−•), hydrogen peroxide (H2O2•), and hydroxyl radical (OH•), which result either from increased production of precursors to reactive oxygen species and/or decreased free radical scavenger activity **(Droge et al., 2009).**

Free radicals can be generated in the human body either due to exposure to exogenous chemicals (e.g. Trichloro-carbon CCl3), electromagnetic radiation (Gamma rays) or generated endogenously during normal metabolic processes and from activated phagocytes (neutrophils, monocytes, macrophages, eosinophils) that generate large amounts of superoxide as part of the mechanism by which foreign organisms **(Halliwell, 2011).**

It was believed that the toxicity ascribed to the super oxide radical was caused by superoxide's direct interaction with biological targets but it is now clear that many tissue effects of superoxide result from the secondary formation of other oxygen radicals in addition to direct reactions of superoxide with biological targets such as lipids, catecholamines and DNA **(Macarthur et al., 2012).**

Oxygen free radicals have important direct and indirect actions in both the cortical and medullary renal micro circulation; they directly constrict renal micro circulation and indirectly affect renal vascular tone by mediating the effects of other vasoconstrictors in the renal cortex. In addition, the tonic production of oxygen radicals in renal medulla causes vasoconstriction, antidiuresis and antinatriuresis, and thereby it may contribute to the control of medullary blood flow and overall fluid and electrolyte balance **(Zou et al., 2010).**

The existence and development of cells in oxygen containing environment would not be possible without the presence of defense systems which protect them from oxidant induced damage. Thus, detoxication of reactive oxygen species (ROS) is one of the prerequisites of aerobic life and many defenses have evolved, providing an important antioxidant defense system **(Partharathy et al., 2009).**

An antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Antioxidants can act by scavenging biologically important reactive oxygen species by preventing their formation or repairing the damage that they do **(Halliwell, 2011).**

The best defined role for vitamin E is an antioxidant for unsaturated fatty aryl moieties of lipids within membranes, thus protecting fats within the cell membrane from breaking down **(Donald et al., 2010).** Vitamin E reacts as a chain-breaking antioxidant, neutralizing free radicals and interrupt production of free radicals at the initial stage, in addition to be a potent scavenger of singlet oxygen **(Harper et al., 2004).**

Vitamin E was found to be an inhibitor of platelet aggregation and may inhibit arachidonic acid peroxidation, which is required for formation of prostaglandins involved in platelet aggregation **(Marsano et al., 2015).**

Vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body, humans and other primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gluconolactone oxidase an enzyme required for the biosynthesis of vitamin C via the glucuronic acid pathway, thus vitamin C must obtained from diet **(Carr and Frei, 2009).**

Vitamin C has a variety of physiological processes that may be relevant to cardiovascular and renal disease; it was found that low vitamin C status in the population has been associated with higher rates of cardiovascular disease such as stroke and coronary disease **(Soutar et al., 2007).**

A small but rather creditable amount of data indicates that exercise at high intensity can cause increased free radical production in the skeletal muscle and myocardium. Vitamin E deficiency alone or in conjunction with exercise also increased free radical production, accompanied by a series of cellular disorders, such as lipid peroxidation, loss of sarcoplasmic reticulum latency, and mitochondrial uncoupling **(Miyazaki et al., 2011).**

A general awareness has developed of the importance of antioxidants in the diseased state. However, we still have insufficient knowledge about the interaction of each antioxidant and exercise, which is important in assessing the adequacy of protection against oxidative damage and the necessity of dietary manipulation and/or supplementation **(Vassilakopoulos et al., 2003).**

2. Materials and Methods

The present work was carried out on 40 male albino rats of local strain. Rats were brought from (Nile Pharmaceuticals Company) and were kept in suitable cages (20 × 32 × 20 cm for every 5 rats) at constant comfortable temperature with the natural light-dark cycle. Rats had free access to water and fed on rodent chow diet food all over the period of the work (2 months), and kept at room temperature. They were kept for 2 weeks for the adaptation to the new environment before the start of the experiment.

Rats were randomly divided into four equal groups (10 rats each):

Group 1 (Control group): received sesame oil orally daily for 2 months.

Group 2 (Exercise group): undergo swimming exercise (5d/wk for 2 months)**.**

Group 3 (Antioxidant group): received 1.08 mg of vitamin C daily orally and 0.27 mg of vitamin E daily orally for 2 months.

Group 4 (Exercise and antioxidant group): received the same doses of vitamin C and vitamin E for 2 months and undergo swimming exercise (5d/wk for 2 months).

At the end of the experimental period, rats were anesthetized by ether then blood samples were collected (3 ml of blood for each) from the retro-orbital plexus using heparinized capillary tube (0.75-1.0 mm internal diameter) inserted in the medial canthus medial to eye globe, then the following parameters were measured: (Interleukin 1 (IL-1), Interleukin 6 (IL-6), Tumor necrosis factor-α (TNF-α), Interleukin10 (IL-10), Malondialdehyde (MDA), Total antioxidants).

3. Results

Table (1): Tumor necrosis factor-α level in different studied groups. (n = 10)

Parameter \ Groups		Group 1	Group 2	Group 3	Group 4
TNF-α level (Pg / ml)		25.47±3.1	31.17±2.8	23.3±2.5	29.1±3.96
Versus control group (Group 1)	t- test		4.48	1.76	2.39
	P value		<0.05	<0.05	<0.05
Versus exercise group (Group 2)	t- test			6.28	1.39
	P value			<0.05	>0.05
Versus antioxidant group (Group 3)	t- test				4.07
	P value				<0.05

Table (1) shows that, Significant lower level of tumor necrosis factor in group 3 with significant higher levels of tumor necrosis factor in group 2 and group 4 as compared with the group 1. As regard comparison of group 2 with group 2 and group 4 the obtained data showed:

- Significant lower level of tumor necrosis factor in group 3 with insignificant lower level of tumor necrosis factor in group 4.
- Significant higher level of tumor necrosis factor of group 4 as compared with group 3.

Table (2): IL-6 level in different studied groups. (n = 10)

Groups / Parameter		Group 1	Group 2	Group 3	Group 4
IL-6 level (Pg / ml)		3.48±0.51	6.36±0.49	2.3±0.46	3.68±0.61
Versus control group (Group 1)	t- test		13.45	5.65	0.8
	P value		<0.05	<0.05	>0.05
Versus exercise group (Group 2)	t- test			19.77	11.29
	P value			<0.05	<0.05
Versus antioxidant group (Group 3)	t- test				5.89
	P value				<0.05

Table (2) shows that, Significant lower level of IL-6 in group 3 with significant higher levels of IL-6 in group 2 and group 4 as compared with the group 1. As regard comparison of group 2 with group 2 and group 4 the obtained data showed:

- Significant lower levels of IL-6 in group 3 and group 4.
- Significant higher level of IL-6 of group 4 as compared with group 3.

Table (3): IL-1 level in different studied groups. (n = 10)

Groups / Parameter		Group 1	Group 2	Group 3	Group 4
IL-1 level (Pg / ml)		2.38±0.47	4.34±0.47	2.08±0.47	3.5±0.46
Versus control group (Group 1)	t- test		9.72	1.56	5.6
	P value		<0.05	>0.05	<0.05
Versus exercise group (Group 2)	t- test			11.23	4.19
	P value			<0.05	<0.05
Versus antioxidant group (Group 3)	t- test				7.13
	P value				<0.05

Table (3) shows that, Insignificant lower level of IL-1 in group 3 with significant higher levels of IL-1 in group 2 and group 4 as compared with the group 1. As regard comparison of group 2 with group 2 and group 4 the obtained data showed:

- Significant lower levels of IL-1 in group 3 and group 4.
- Significant higher level of IL-1 of group 4 as compared with group 3.

Table (4): IL-10 level in different studied groups. (n = 10)

Groups / Parameter		Group 1	Group 2	Group 3	Group 4
IL-10 level (Pg / ml)		2.8±0.4	1.6±0.28	3.86±0.87	2.78±0.26
Versus control group (Group 1)	t- test		7.97	3.41	0.46
	P value		<0.05	<0.05	>0.05
Versus exercise group (Group 2)	t- test			8.19	10.18
	P value			<0.05	<0.05
Versus antioxidant group (Group 3)	t- test				3.92
	P value				<0.05

Table (4) shows that, Significant higher level of IL-10 in group 3 with significant lower level of IL-10 in group 2 and insignificant lower level in group 4 as compared with the group 1. As regard comparison of group 2 with group 2 and group 4 the obtained data showed:

- Significant higher levels of IL-10 in group 3 and group 4.
- Significant lower level of IL-10 of group 4 as compared with group 3.

Table (5): Malondialdehyde (MDA) level in different studied groups. (n = 10)

Groups / Parameter		Group 1	Group 2	Group 3	Group 4
MDA level (nmol / ml)		15.54±1.49	22.61±2.42	12.5±1.3	17.48±1.76
Versus control group (Group 1)	t- test		8.23	5.07	2.78
	P value		<0.05	<0.05	<0.05
Versus exercise group (Group 2)	t- test			12.19	5.68
	P value			<0.05	<0.05
Versus antioxidant group (Group 3)	t- test				7.53
	P value				<0.05

Table (5) shows that, Significant lower level of malondyaldehyde in group 3 with significant higher level of malondyaldehyde in group 2 and group 4 as compared with the group 1. As regard comparison of group 2 with group 2 and group 4 the obtained data showed:

- Significant lower level of malondyaldehyde in group 3 and group 4.
- Significant higher level of malondyaldehyde of group 4 as compared with group 3.

Table (6): Total antioxidants level in different studied groups. (n = 10)

Groups / Parameter		Group 1	Group 2	Group 3	Group 4
Total antioxidants level (Pg / ml)		1.46±0.19	1.94±0.17	1.99±0.24	2.4±0.28
Versus control group (Group 1)	t- test		6.13	5.76	9.34
	P value		<0.05	<0.05	<0.05
Versus exercise group (Group 2)	t- test			0.6	4.95
	P value			>0.05	<0.05
Versus antioxidant group (Group 3)	t- test				3.98
	P value				<0.05

Table (6) shows that, Significant higher level of total antioxidants in group 3 with significant higher levels of total antioxidants in group 2 and group 4 as compared with the group 1. As regard comparison of group 2 with group 2 and group 4 the obtained data showed:

- Insignificant higher level of total antioxidants in group 3 and significant higher level in group 4.
- Significant higher level of total antioxidants of group 4 as compared with group 3.

4. Discussion

Regular exercise is beneficial to our health. However, unaccustomed or exhaustive exercise can result in detrimental health effects such as muscle damage, inflammation and oxidative stress. Specifically, repetitive muscle contraction involves accumulation of reactive oxygen species (ROS) **(Zuo et al., 2015).** The overproduction of ROS induced by exhaustive exercise training or other stresses, along with compromised antioxidant defenses, can lead to oxidative stress and related tissue damage **(Zuo et al., 2012).**

It can elicit muscle injuries, which then lead to the activation of the neutrophils and macrophages via interferon-γ (IFN-γ), interleukin-1 (IL-1) and tumor necrosis factor (TNF) **(Steinbacher and Eckl, 2015).**

The present study aimed to evaluate the effects of muscular exercise and antioxidants on myokines in male albino rats.

In the present study, exercise leading to significant increased the tumor necrosis factors (TNF),

interleukin-1 (IL-1), interleukin-6 (IL-6), malondialdehyde (MDA) and total antioxidants associated with significant decreased the level of interleukin-10 (IL-10). These results agree with **Chen et al. (2007)** who reported that four weeks of exercise training increased the levels of proinflammatory cytokines (TNF and IL-1).

Exercise increases anti-inflammatory cytokine IL-6 plasma levels (Starkie et al., 2003). Elevated levels of IL-6 may be due to regeneration after injury of the muscle **(Pedersen and Febbraio, 2008 & Isanejad et al., 2015). Coelho *et al*., (2013)** demonstrated that aerobic exercise training was able to prevent, oxidative stress. The exercise-induced increase in the antioxidant defense system of muscles is most likely the underlying mechanism responsible for protecting muscle cells against oxidative damage.

Regular exercise and physical activity is associated with specific production of cytokines and myokines, including IL-1β, IL-6, TNF-α, IL-1 receptor antagonist and acute phase proteins by the skeletal muscle to maintain the metabolic homeostasis of lipids and proteins **(Golbidi et al., 2012).**

IL-6 mediates many aspects of the exercise-induced acute-phase response, including the up regulation of antioxidant defenses as response to oxidative stress. After strenuous physical exercise, IL-6 is synthesized by actively contracting muscle fibers and increase up to 100-fold in relation to other pro-inflammatory cytokines such as TNF-α and IL-1β. IL-6 also lead to increased proliferation of satellite cells and thereby regeneration of damaged myofibers **(Golbidi et al.0, 2012).**

The acute phase response activates the production of oxygen radicals together with various cytokines like interleukins and tumor necrosis factor-α. The most prominent cytokine produced during the contractions is IL-6 which is expressed within the muscle cells and released into the blood **(Nielsen and Pedersen, 2008).**

Tanskanen et al. (2010) showed that exercise led to an increase in oxygen radical absorbance (antioxidant) capacity and malondialdehyde levels. Over-trained athletes showed negative correlations between oxygen radical absorbance capacity at rest and protein carbonyls after exhaustive exercise indicating that increased oxidative stress may play a role in the pathophysiology of overtraining syndrome.

Plasma levels of total antioxidants increased significantly after the eccentric exercise probably due to cellular damage caused by eccentric exercise, release of intracellular muscle enzymes to the blood and high concentrations of Glutathione (GSH). These factors ultimately lead to plasma antioxidant concentration **(Lee and Clarkson, 2003).**

There are many factors related to the increased plasma level of total antioxidants after the exercise. One of them is to restore antioxidants from tissue-to-plasma and contrast between different antioxidants which lead to plasma total antioxidants improvement. Another factor is the increase in plasma GSH levels. Since plasma GSH is used as a determinate to evaluate the total capacity of plasma, the increased plasma levels may have stemmed from the increase in plasma GSH levels **(Norouziyan et al., 2014).**

In the present study, antioxidant supplementation leading to significant decreased the tumour necrosis factors (TNF), IL-6 and malondialdehyde (MDA) associated with insignificant decrease of IL-1. Also led to significant increase of IL-10 and total antioxidants when compared with control group.

These findings were in agree with **Vassilakopoulos *et al.* (2003)** who stated that antioxidant supplementation drastically could affects the plasma TNF-α, IL-1β and IL-6 induction secondary to exercise, so that plasma IL-1β becomes undetectable, the TNF-α response is abolished, and the IL-6 response is significantly blunted. These results suggest that oxidative stress is a strong stimulus for the exercise-induced IL-1β and TNF-α response. Antioxidants abolished the exercise-induced elevation of TNF-α, whereas IL-1β was below the control group level, which suggests that antioxidants affected both the constitutive (baseline) and the exercise-induced production of IL-1β.

The decreased MDA is an indicator of lipid per-oxidation. After the reduction of oxidative stress in body, antioxidant defense system in order to balance with combining resistance and endurance training in a several months' period activates and this increase needs consumption of anti-oxidant supplements to have more effects **(Delavar et al., 2017).**

In the present study, exercise training and antioxidant supplementation leading to significant increased the tumour necrosis factors (TNF), IL-1, malondialdehyde (MDA) and total antioxidants levels associated with insignificant increase of IL-6. Also led to insignificant decrease of IL-10 when compared with control group.

He et al. (2015) noticed that short-term combined vitamin C and E supplementation not only attenuated levels of creatine kinase (a muscle damage marker) and muscle soreness, but also enhanced muscle protection following the second bout of aerobic exercise.

After habitual physical activity, an enhancement of intrinsic antioxidant potential, and a reduction in lipid peroxidation occurs. Adaptation to oxidative stress in trained individuals is clearly evidenced by a decrease in DNA damage, by sustained levels of protein oxidation and by an increment of resistance

against chronic administration of hydrogen peroxide **(Edite et al., 2012).**

The beneficial effects of physical activity on health due to an increase in oxygen consumption and metabolic rate during exercise, the oxidative stress is generated by increasing the reactive oxygen species productions. Anti-oxidative system maintains the cellular inner balance at rest and during the moderate exercise **(Norouziyan et al., 2014).**

5. Conclusion

Exercise produces elevation of the basal level of proinflammatory cytokines. Monocytes had been initially suggested as a source of the exercise-induced plasma cytokines production. In addition, exercise was found to increase the levels of reactive oxygen species (ROS), both in the blood and within the working muscles. Antioxidant supplementation drastically could affects the plasma levels of IL-1, TNF-α and IL-6. These results suggest that oxidative stress is a strong stimulus for the exercise-induced IL-1β and TNF-α response. Antioxidants affected both the constitutive (baseline) and the exercise-induced production of IL-1β.

Recommendations

Based on the results of this study and the conclusions, we can recommend the following:

- To avoid any hazardous effect of exercise, antioxidant must be administered to exercised individuals.
- Also, further studies should be done to evaluate the effect of different modes, intensities and durations of exercise training on myokines.

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