Effect of Vitamin E on the Exhaustive Exercise-Induced Damage in the Skeletal Muscle of Adult Male Albino Rat: A Functional, Histological and Ultrastructural Study

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Abstract

Background: Swimming is a minor traumatic exercise for animals and has been commonly used to elucidate the physiologic and molecular responses of the muscle to exercise stress. Short term exhausting exercise increases the formation of harmful reactive oxygen species (ROS). Vitamin E (α-tocopherol) is a fat soluble vitamin that can inhibit the generation of ROS in the body as it acts as an antioxidant.

Aim of the Study: The present study was designed to investigate the possible protective effect of vitamin E against the untrained exhaustive exercise-induced damage in the skeletal muscle, and on the plasma levels of proinflammatory cytokines in adult male albino rat.

Material and Methods: 24 adult male albino rats, age 3 months and about 90-120g body weight were purchased from Animal House, Faculty of Medicine, Assiut University. All animals were kept in stainless-steel cages at room temperature at a natural photoperiod with free access to standard rat chow and tap water. Animals were randomly divided into 3 groups (8 rats each): Group C (control group), Group S (submitted to exhaustive swimming stress group), and Group SE (submitted to exhaustive swimming stress plus vitamin E-received group). The animals from groups S and SE were submitted to bouts of swimming stress for 1 hour daily for a week. The rats from SE group received oral gavage administration of vitamin E (100mg/kg/day) before swimming stress.

Results: Exhaustive swimming stress in group S significantly elevated plasma tumor necrosis factor-alpha (TNF-α), interferon gamma (INF-γ) and C reactive protein (CRP). Light microscopy of gastrocnemius muscle specimens showed in group S hypervascularity, apparent increase in the number of interstitial nuclei, splitting of muscle fibers, and central location of nuclei compared to C group. The animals from groups S and SE were submitted to bouts of swimming stress for 1 hour daily for a week. The rats from SE group received oral gavage administration of vitamin E (100mg/kg/day) before swimming stress.

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Conclusion: Vitamin E supplementation has promising protective role against exercise-induced elevation of cytokines and muscular damage.

Key Words: Swimming – TNF-α – INF-γ – CRP – Vitamin E – Skeletal muscle structure – ENOS immunohistochemistry.

Introduction

MODERATE daily exercise is known to be beneficial to health, reducing risks of a number of age-related disorders [1]. However, earlier research studies showed that short term exhausting exercise increased the formation of presumably harmful reactive oxygen species (ROS) [2]. Swimming is a form of minor traumatic exercise for animals and has been commonly used to elucidate the physiological and molecular responses of the muscle to exercise stress [2,3]. Evidences suggest that regular exercise antagonizes inflammation through modulating cytokines [4]. Previous studies reported that regular exercise training reduces tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and interleukin-6 (IL-6) levels; and increases anti-inflammatory markers such as IL-4 and IL-10 [5]. However, the effect of regular well trained exercise versus acute untrained exercise on proinflammatory cytokines appears to be contradictory. Some researchers found rise of proinflammatory cytokines with acute bouts of exercise [2,6]. C-reactive protein is an acute-phase protein that is elevated in response to inflammation [7]. TNF-α [8] and interferon gam-
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ma (IFN-γ) [8] are proinflammatory cytokines that have antiviral and immune-regulatory functions. It has been reported by several studies that skeletal muscles during intense prolonged contraction generate free radicals that damage cellular constituents [9]. Nitric oxide (NO) is one of the primary free radicals generated in the cells [10]. Vitamin E (α-tocopherol) is a fat soluble vitamin and thought to protect the body tissues by reducing or preventing oxidative damage [11]. Vitamin E regulates oxidation processes in the body as it acts as a strong antioxidant [12] protecting against membrane damage mediated by free radicals [13]. However, previous studies showed conflicting outcomes for the effect of α-tocopherol on the proinflammatory mediators [14].

**Aim of the study:**

The present study was designed to investigate the possible protective effect of vitamin E against the untrained exhaustive exercise-induced damage in the skeletal muscle, and on the plasma levels of inflammatory cytokines in adult male albino rat.

**Material and Methods**

**I- Scope, place and time of study:**

Scope of study included physiological (plasma levels of proinflammatory cytokines), structural (light and transmission electron microscopy), and immunohistochemical analysis, and the study was conducted between July and August 2014 in Assiut University, Egypt.

**II- Study design:**

The study was experimental design post-test only one control group. The animals were kept in the laboratory for one week for adaptation before they were randomly divided into one control and two experimental groups.

**III- Inclusion and exclusion criteria:**

For inclusion criteria, the rats were albino rats, males, adults of age 3 months for all rats at the beginning of the study and weighed 90-120 grams.

For exclusion criteria, the rats that died before the end of the experiment were excluded.

**IV- Animals:**

A total number of 24 male albino rats weighing 90-120 grams were randomly used in this study. The animals were obtained from the Animal House, Faculty of Medicine, Assiut University, Assiut, Egypt. They were kept for one week in the laboratory for adaptation before the experiment. The animals were isolated in clean properly ventilated stainless-steel cages under normal conditions at room temperature, normal light/dark cycle with free access to food (standard rat chow) and tap water. Each cage contained four rats. All animals of the three groups were at the age of 3 months at the beginning of the experiment. The two experimental groups were forced to swim until exhaustion for 1 hour/day for a period of one week. The experiments were carried out according to the protocol approved by the Local Ethical Committee of the Faculty of Medicine, Assiut University in accordance with the ethical guidelines for scientific research in conscious animals.

**V- Animal grouping:**

The animals were randomly divided into three groups (one control and two experimental):

- **Group C (Normal control group, n=8):** Received oral gavage administration of 1ml of water for seven consecutive days.
- **Group S (Exhaustive exercise stress group, n=8):** Received oral gavage administration of 1ml of water and submitted to exhaustive swimming stress for seven consecutive days.
- **Group SE (Exhaustive exercise stress plus vitamin E-received group, n=8):** Received oral gavage administration of 1ml of vitamin E before submitted to exhaustive swimming stress for seven consecutive days.

**VI- Drug dosage:**

1- Vitamin E was given by oral gavage at a dose of 100mg/kg body weight/day [15] for seven consecutive days.

The vitamin E was manufactured by Safe Pharma for Pharco Company for Pharmaceuticals, Alexandria, Egypt. It is dispensed in the form of soft gelatinous capsules each containing 1000mg of α-tocopherol acetate.

**VII- Study variables:**

Independent variables included the doses of vitamin E. Dependent variables included the plasma samples and the gastrocnemius muscle tissues collected from the animals.

**VIII- Experimental analysis:**

Two methods of analysis were used in the present study:

A- Quantitative analysis; in which the putative effects of the exhaustive swimming alone and combined swimming plus vitamin E on the plasma levels of proinflammatory markers were noticed by studying the differences in these levels between
the control and the experimental groups. This was attained by measuring the following parameters:
1. Plasma TNF-α.
2. Plasma INF-γ.
3. Plasma CRP.

B- Structural analysis: in which the putative effects of the exhaustive swimming alone and combined swimming plus vitamin E on the structure of the gastrocnemius muscle were observed by studying the histological and morphological changes. This was attained by light and transmission electron microscope using:
1. Hematoxylin and Eosin staining for muscle sections.
2. Toluidine blue staining for semithin sections.
3. Uranyl acetate and lead citrate staining for ultrathin sections examined by transmission electron microscope.
4. Endothelial nitric oxide synthase (eNOS) immunostaining for muscle sections and studying the differences in its expression between the control and the experimental groups. This was attained by densitometry analysis.

IX- Experimental methodology:
Rats were forced to swim for one hour without a load until exhaustion daily for 7 consecutive days [16]. This period of time is able to achieve significant changes in antioxidant levels [17]. The swimming tank was cylindrical (50cm diameter, and 80cm deep) containing warm water maintained at 30 °C and regularly checked by a thermometer. When the animal stop swimming and sink for 10 seconds [18], it was removed from the water for a minute and returned back to continue swim for an hour. The depth of the tank prevented the animals from resting their tails on the bottom of the tank while swimming. Swimming was selected to avoid muscle trauma caused by other methods of forced exercise as prolonged running or exercise-stimulated electric shock [1].

Quantitative analysis:
The total body weights of the rats were recorded at the beginning and at the end of the experiment. Half an hour after the last session of swimming stress, the animals were anesthetized by ether inhalation and blood samples are collected from the eyes into heparinized tubes. The plasma were isolated and stored at −20 °C until use. Plasma c reactive protein (CRP), tumor necrosis factor α (TNF-α), gamma interferon (INF-γ) levels were detected using enzyme-linked immunosorbert assay (ELISA) kits following the manufacturer’s instructions [AVITEX® CRP Latex serology test for detection of C-reactive protein (OD073/OD023/OD023/E) was purchased from Omega Diagnostics Ltd, Alva, Scotland, UK; and rat TNF-alpha ELIZA Kit (K0331196), and rat IFN-gamma ELIZA Kit (K0331209) were purchased from Koma Biotech Inc., Yeongdeungpo-gu, Seoul, Korea].

Structural analysis:
For light microscopy and immunohistochemistry, after sacrifice tissue specimens of the middle part of gastrocnemius muscle were collected and fixed in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol, cleared, embedded in paraffin according to the previously described steps [19]. Paraffin sections were cut at 5 µm, then, sections were stained with Hematoxylin and Eosin (H & E), before being examined using light microscope for studying histological structure. For immunohistochemical staining for eNOS antigen, paraffin sections were mounted on silaine-coated slides. To block the endogenous peroxidase activity the sections were incubated in 3% H2O2 for 10 min. [20]. Antigen retrieval was done by heating slides in sodium citrate buffer (pH 6.0) at 95 °C in a microwave for 15 min and slowly cooling them to room temperature [21]. Slides were then incubated for 18h at 4 °C with the primary rabbit polyclonal antibody against endothelial Nitric Oxide Synthase (eNOS) [Thermo Scientific, South San Francisco, California, USA (dilution 1:100)]. The slides were washed and incubated with biotinylated goat anti-polysvalent secondary antibody (dilution 1:200) for 30min at room temperature [UltraVision Detection System anti-polysvalent HRP/DAB kit, Thermo Fisher scientific, USA]. Then sections were incubated with streptavidin peroxidase for 10min and finally with 0.05% dianinobenzidine (DAB) plus chromogen for 10min. eNOS-immunopositive cells appeared brown. Slides were examined using an Olympus CX41 optical microscope equipped with an Olympus EVOLT E-330 digital camera interfaced to a computer. Expression of eNOS protein was quantified for their mean relative densities in 8 random slides from each group using the densitometry analysis tool from ImageJ, Version 1.31c (National Institutes of Health, Bethesda, Maryland, USA) [22].

For transmission electron microscopy, gastrocnemius muscle specimens were fixed in 5% cacodylated-buffered glutaraldehyde for 24h at 4 °C and postfixed in 1% osmium tetroxide for 1 hour 4°C [23]. About 2mm-thick specimens were processed and embedded in Epon-araldite mixture. Semithin sections of 1 µm thickness were cut with a glass knife in KLB, Bromma Ultramicrotome,
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and stained with toluidine blue and examined. Ultrathin sections (450-500Å) from muscle selected areas were cut with ultramicrotome, mounted on copper grids, stained with uranyl acetate and lead citrate [23], examined with a JEOL transmission electron microscope model JEM 100 CXII (JEOL, Tokyo, Japan), and photographed in the Assiut University Electron Microscope Unit.

X- Statistics:
GraphPad Prism 5 (Graph Pad Software Inc., La Jolla, CA, USA) was used for data analysis. Data were presented as mean ± standard error of the mean (SEM). Data were compared among groups using One-Way Analysis of Variance (ANOVA) followed by Bonferroni Multiple comparison posthoc test. A (p) value of less than 0.05 was considered to represent a statistically significant difference.

Results

Quantitative results:
Effect of exhaustive swimming stress versus swimming stress and vitamin E on the plasma level of proinflammatory cytokines in rats: One hour bout of swimming stress until exhaustion for seven consecutive days significantly raised INF-gamma (p<0.005) Fig. (1), TNF-alpha (p<0.005) Fig. (2), and CRP (p<0.01) Fig. (3A) in group S compared to group C. Administration of Vitamin E prior to swimming bouts in group SE significantly decreased INF-gamma plasma level (p<0.05) Fig. (1) and TNF-alpha plasma level (p<0.01) Fig. (2) compared to group S. However vitamin E failed to decrease CRP plasma level in group SE compared to both group C and group S with insignificant difference (p >0.05) Fig. (3A). A significant rise in INF-gamma (p<0.01) Fig. (1) and in TNF-alpha (p<0.005) Fig. (2) in group SE versus group C was also found.

Effect of exhaustive swimming stress versus swimming stress and vitamin E on total body weight (TBW): Insignificant difference in the initial TBW of the studied groups C, S, and SE was found. At the end of the experiment there was insignificant difference in % change in TBW between group C and group S Fig. (3B). Administration of vitamin E caused significant rise in % change in TBW in group SE compared to both group C and group S Fig. (3B).

Structural results:
Control group C animals:
Light microscopic examination of H & E-stained longitudinal sections (LS) of the gastrocnemius muscle of the control rats showed normal histological architecture in the form of unbranched elongated muscle fibers with peripherally located nuclei, acidophilic cytoplasm and striated appearance Fig. (4). Toluidine blue-stained transverse sections (TS) showed polyhedral muscle fibers with flattened peripheral nuclei and blood capillaries located in the endomysium Fig. (5).

Transmission electron microscopic examination revealed normal ultrastructure of skeletal muscle in the form of several myofibrils running longitudinally parallel to the long axis of the muscle fiber cell, with oval elongated peripheral nucleus against the sarcolemma. Within each myofibril dark electron-dense A bands, and light electron-lucent I bands were seen Fig. (6). The light I bands were bisected by Z lines, and paired mitochondria were found around Z lines and around the nucleus. Each repeating part of the myofibril between adjacent Z lines was identified as a sarcomere.

Immunohistochemical staining of sections of the gastrocnemius muscle of the control rats showed normal minimal immunoreactivity for eNOS in the sarcoplasm of muscle fibers Fig. (7).

Exhaustive swimming stress group S animals:
Light microscopic examination of H & E-stained LS of the gastrocnemius muscle of group S rats showed histological changes in the form of splitting and branching of the muscle fibers with peripheral and central locations of the nuclei, acidophilic cytoplasm and striated appearance. Also, apparent increase in the number of connective tissue nuclei was found between the myofibers Fig. (8). Toluidine blue-stained TS showed polyhedral muscle fibers with central and peripheral nuclei. Several dilated congested blood capillaries were located in the endomysium, with increased interstitial nuclei Fig. (9).

Transmission electron microscopic examination of the gastrocnemius muscle of group S rats revealed marked skeletal muscle cell damage with destructed myofibrils and loss of some myofilaments, and areas of degeneration and vacuolation, with oval nucleus beneath the sarcolemma, and irregular wavy arrangement of the Z lines. Also, apparent irregular shape and small size of mitochondria were present in group S compared to group C. Myosatellite cells with dark nuclei were present close to the sites of vacuolar degeneration. There were also many dilated congested capillaries full of red blood cells present around the areas of vacuolar degeneration Fig. (10).

Immunohistochemical staining of sections of the gastrocnemius muscle of group S rats showed
positive moderate eNOS immunoreactivity localized in the sarcoplasm of the muscle fibers Fig. (11).

**Exhaustive swimming stress and vitamin E received group SE animals:**

Light microscopic examination of H & E-stained LS of the gastrocnemius muscle of group SE rats showed amelioration of the histological changes induced by exhaustive swimming stress with more or less similarity to group C. The muscle fibers were elongated parallel and unbranched with majority of peripherally located nuclei and a few central nuclei. The cytoplasm was acidophilic with striated appearance Fig. (12). Toluidine blue-stained TS showed that muscle fibers were apparently hypertrophied, whereas others were small and polygonal. The myonuclei were central and peripheral in location. Several collapsed blood capillaries were located in the endomysium Fig. (13).

Transmission electron microscopic examination of the gastrocnemius muscle of group SE rats showed amelioration of the degenerative changes induced by exhaustive swimming stress: Several myofibrils were running longitudinally parallel to the long axis of the muscle fiber cell, with elongated peripheral nucleus against the sarcolema. Internucleation of the myonucleus was found close to an area of damage and loss of some myofilaments Fig. (14) which was less loss than that in group S. The Z lines were regularly arranged in most of myofibrils similar to group C, and paired mitochondria were found around Z lines and around the nucleus, with apparent increase in the number of mitochondria. Myosatellite cells with dark nuclei were also present Fig. (14).

Immunohistochemical staining of sections of the gastrocnemius muscle of group SE rats showed strong positive dark brown colour for eNOS immunoreactivity localized in the sarcoplasm of the muscle fibers Fig. (15) which was more dense than that of groups C and S. Densitometry analysis of eNOS immunohistochemistry images of the rat gastrocnemius muscle showed levels of eNOS expression were higher in group SE than others. These differences were statistically significant in the analyzed sections of the gastrocnemius muscle \( p<0.05, n=8 \) Fig. (16).
Fig. (4): A photomicrograph of a longitudinal section (LS) of rat gastrocnemius muscle from control (C) group showing elongated unbranched parallel myofibers (F) with acidophilic cytoplasm and apparent transverse striations. Their nuclei (arrows) are peripherally located, oval flattened vesicular nuclei just beneath the sarcolemma. H & E, X1000.

Fig. (5): A photomicrograph of a semithin transverse section (TS) of rat gastrocnemius muscle from group C showing polygonal shape of the muscle fibers (F). Some fibers are sectioned along their broadest dimension; some at their narrowest dimension; and others at some intermediate dimension. Their nuclei (arrows) are flattened and peripherally located. Several capillaries are seen as ring-like structures (asterisks) present in the endomysium, and some capillaries contain red blood cells in the lumen. The muscle fibers are arranged into bundles separated by connective tissue, perimysium (P). Toluidine blue, X1000.

Fig. (6): Transmission electron micrograph of LS of rat gastrocnemius muscle from group C showing a peripheral oval flattened nucleus (N) beneath the sarcolemma (sl) of a muscle fiber. Regular arrangement of myofibrils is seen with dark (A), and light bands (I) bisected by Z lines (Z), and paired mitochondria (M) around Z lines and around the nucleus. Each repeating part of the myofibril between adjacent Z lines is a sarcomere. Note the presence of sarcoplasmic reticulum (SR) in the region of the A-I band junction, and a capillary (asterisk) contains red blood cell (arrow). Uranyl acetate and Lead citrate, X2900.

Fig. (7): Photomicrographs of LS (A) and TS (B) of rat gastrocnemius muscle from group C showing minimal endothelial nitric oxide synthase (eNOS) immunoreactivity in the sarcoplasm of the myocytes. The myofibers (F) have peripherally located nuclei (arrows). eNOS immunostain, X1000.
Fig. (10): Transmission electron micrograph of LS of rat gastrocnemius muscle from group S showing a peripheral oval nucleus (N) beneath the sarcolemma (sl) of a muscle fiber. Areas of destructed myofibrils and loss of some myofilaments, and areas of degeneration (D) are present creating vacuolar spaces, with many dilated capillaries (asterisks) containing several red blood cells (RBC). Myofibrils show disturbed irregular arrangement of Z lines (Z), and apparent irregular shape and small size of mitochondria (M) present in group S compared to group C. Muscle satellite cell nucleus (NS) can be distinguished by its dark abundant heterochromatin reflecting its mitotic quiescence. Muscle satellite cell present on myofibers between the sarcolemma and the external basal lamina. Note: Sarcoplasmic reticulum (SR), Uranyl acetate and Lead citrate, X2900.

Fig. (11): Photomicrographs of LS (A) and TS (B) of rat gastrocnemius muscle from group S showing moderate eNOS immunoreactivity in the sarcoplasm of the myocytes. The myofibers (F) have peripherally located nuclei (arrows), and some have central nuclei (arrowheads). Note: Positive staining indicated by brown colour. eNOS immunostain, X1000.
Fig. (12): A photomicrograph of LS of rat gastrocnemius muscle from group SE showing elongated unbranched myofibers (F), acido-philic cytoplasm and a striated appearance. The myonuclei (arrows) are peripherally located, oval flattened vesicular nuclei just beneath the sarcolemma. Centrally located nuclei (arrowheads) can also be seen in some fibers. H & E, X1000.

Fig. (13): A photomicrograph of semithin TS of rat gastrocnemius muscle from group SE showing myofibers polygonal in shape with apparent hypertrophy of some muscle fibers (F). The myonuclei (arrows) are peripherally located. Centrally located nuclei (arrowheads) can be seen in several fibers. Several collapsed capillaries are seen (asterisks) present in the endomysium. Toluidine blue, X1000.

Fig. (14): Transmission electron micrograph of LS of rat gastrocnemius muscle from group SE showing peripheral flattened nucleus (N) beneath the sarcolemma (sl) of a muscle fiber and a central nucleus (Nc) of another muscle fiber. Areas of loss some myofilaments and degeneration (D) are present. Myofibrils show majority of regular arrangement of Z lines (Z), with paired mitochondria (M) around Z lines, with apparent increase in the number of mitochondria. Muscle satellite cell nucleus (NS) appears dark due to its abundant heterochromatin. Note: Sarcoplasmic reticulum (SR). Uranyl acetate and Lead citrate, X2900.

Fig. (15): Photomicrographs of LS (A) and TS (B) of the rat gastrocnemius muscle from group SE showing marked intensity of eNOS immunoreactivity in the sarcoplasm of the myocytes. The myofibers (F) have peripherally located nuclei (arrows), and some have central nuclei (arrowheads). Note: Strong positive staining indicated by dark brown colour. eNOS immunostain, X1000.

Fig. (16): Immunohistochemical analysis comparing the eNOS expressions in the rat gastrocnemius muscle of the control and the experimental groups. Based on relative densitometry analysis of immunohistochemistry images of the rat muscle, levels of eNOS expression were much higher in group SE than other groups. All values are expressed as mean ± SEM, one way ANOVA with bonferoni's multiple comparison test was done. These differences were statistically significant in the analyzed sections of gastrocnemius muscle of group SE compared to group C and group S (p<0.05, n=8).
Discussion

Muscular exercise produces physical stress that challenges the body homeostasis [6]. The body deals with physical activity as any pathological condition that causes acute subclinical inflammatory state as a protective process important for repair of damaged tissues and fighting foreign bodies [6].

The present study showed that in group S a bout of exhaustive swimming stress for one hour/day repeated daily for a week caused significant rise of proinflammatory cytokines; TNF-α, IFN-γ, and CRP. These results are supported by previous studies reporting that the circulating basal levels of TNF-α were raised by acute extenuating exercise [2,6]. Significant activation of TNF-α and other cytokines were reported after unaccustomed muscle contractions increase the oxidative stress resulting in microdamage to sarcomeres [24,25]. Other studies demonstrated that TNF-α and IFN-γ through activation of NF-kB caused skeletal muscle degeneration and inhibited muscle repair [26]. It was reported that TNF-α caused elevation of IL-6 and CRP through inducing their synthesis [27]. The rise in CRP level after exhaustive swimming exercise is supported by another study that reported a maintained rise in CRP and leukocyte count starting after 30min of marathon swimming and continued for 8 days after recovery [28]. Also, it was reported that higher cytokine levels as increased TNF-alpha were generally associated with lower muscle mass and lower muscle strength in well-functioning older men and women [29].

This study also revealed that vitamin E administration before the exhaustive swimming exercise significantly decreased circulating levels of TNF-α and INF-γ. The ability of vitamin E to lower INF-γ and TNF-α levels was supported by the work of Belisle and his colleagues who observed a strong relationship between vitamin E supplementation and IFN-γ as well as TNF-α that is dependent on individual’s immune response at the onset of supplementation [30]. Studies done in Finland and Italy demonstrated that α-tocopherol form of vitamin E exerts anti-inflammatory actions and is effective in reducing asthma [31]. An in vitro study showed that pretreatment of endothelial cells overnight with α-tocopherol inhibited leukocyte migration [32]. In the present study, vitamin E supplementation caused insignificant change in the level of CRP in SE group compared to S group. This result is supported by the work of Block and his group who reported that treatment with vitamin C but not E caused significant decrease in circulating levels of CRP among healthy nonsmokers with CRP higher than 1.0mg/L [33]. Taken together, these suggest that vitamin E have dual antioxidant action direct as well as indirect through its anti-inflammatory effect.

Moreover, vitamin E supplementation in group SE significantly increased the total body weight compared to both C and S groups. This result is supported by a study done by Azman and his group reporting that vitamin E played an important role in the weight gain and excessive vitamin E intake might cause obesity through increasing fat mass in female rats [34]. Another study reporting lower death rate and higher body weight among lambs whose mother ewes were fed additional vitamin E for 3 weeks before lambing [35].

This study also revealed that untrained exhaustive swimming stress in group S rats caused structural changes in the gastrocnemius muscle characterized by increased vascularity, apparent increased number of interstitial nuclei and muscle satellite cells, splitting of muscle fibers, central nuclear position, destructed myofibrils and loss of some myofilaments, and areas of degeneration and vacuolization, irregular wavy arrangement of Z lines, and apparent irregular shape and small size of mitochondria were present in group S compared to group C. Consistent with the findings in the present study, other researchers reported an increase of blood capillary density, capillary-to-muscle fiber ratio with moderate swimming exercise [36]. The disordered alignment of the myofilaments with wavy Z lines induced by swimming stress was previously demonstrated [37]. In line with the results of the current study, earlier studies revealed that exhaustive exercises cause skeletal muscle protein oxidative damage and degradation, loss of sarcoplasmic reticulum integrity, and abnormal morphological changes of the mitochondria due to mitochondrial damage and vacuolar degeneration [37,38]. The mitochondria act as the primary ROS production factory in the cells, thus gets damaged by the oxidative stress [39]. The increased number of interstitial nuclei shown in this study could be attributed to leucocytic infiltration due to inflammation induced by exhausting exercise. Previous research emphasized that neutrophil infiltration of rat skeletal muscle interstitium occurred following untrained exhaustive swimming exercise suggest a protective role against muscle injury [40,41]. Previous researchers reported that the activation of muscle satellite cells is an important step for the muscle attempt to repair and regenerate after injury since the satellite cells contribute to new myofiber formation [41]. Also, the muscle fiber
splitting or branching demonstrated in the results of this study is known as a characteristic feature of muscle regeneration [41]. The central nuclear position found in group S muscles of this study could be explained by the muscle repair process causing expansion of muscle cells and their division to regenerate new muscle fibers that have characteristic centrally located myonuclei, and were observed in discrete portions of regenerating fibers close to the site of muscle damage [41].

Administration of vitamin E in group SE before the exposure to exhaustive swimming stress returned the structural alignment of sarcomeres to normal with apparent increase in the size of myofibers and in the number of mitochondria and satellite cells. The ability of satellite cells to proliferate maintains the stem cells and increases the number of myogenic cells that cause formation of new myofiber and regain a functional contractile apparatus has been previously reported [42]. The satellite cells contribute to new myofiber formation, and in the final stage of muscle regeneration the myogenic cells fuse together, thus newly formed myofibers increase in size and mitochondrial content, and the myonuclei move to the periphery of the muscle fiber [41]. In line with the findings in this study, previous research data have shown the ability of vitamin E to keep functional as well as the structural integrity of skeletal muscle cells including normal alignment of sarcomeres and promoting plasma membrane repair [43]. Also, in agreement with these results a previous ultrastructural study reported that vitamin E protected the mitochondria and the myofilaments of the rat skeletal muscles against damage induced by repeated exhausting exercises [37]. Vitamin E is a lipid soluble vitamin that exerts antioxidant properties, by scavenging ROS and boosting cellular antioxidative capacity to reduce oxidative damage [44]. Supplementation of vitamin E was reported to protect against oxidative stress and inflammation [45]. The beneficial effect of vitamin E on muscle structure is supported by previous studies that proved the role of antioxidants in control of gene expression, regulation of cell signaling pathways, and modulation of muscle force production of skeletal muscles [39,46,47]. Also, it has been suggested that vitamin E administration in aged people improves immune response by modulating cytokine production [30]. Taken together, these suggest that vitamin E supplementation before severe exercise helps to maintain healthy contractile units.

The emerging role of nitric oxide (NO) in the maintenance of cell physiology has highlighted the importance of this interesting molecule in cytoptosis since it contributes to vasodilatation and prevents leukocyte adhesion to the endothelium [48]. The endothelial nitric oxide synthase (eNOS) catalyses the conversion of L-arginine to L-citruline and finally produces NO. In this context, the present study attempted to localize the distribution and expression of eNOS immunohistochemically in the gastrocnemius muscle of the control and experimental groups. The results of this study showed significant increase in eNOS expression in the sarcoplasm of gastrocnemius muscle cells of group S compared to the control group C which is in agreement with previous research studies showed that acute treadmill exercise increased eNOS expression in the gastrocnemius muscle [49]. Increased eNOS expression in the muscle cells was found to be beneficial to stimulate regeneration and could be used in cell therapy for regenerating the infracted site [48]. Also, eNOS overexpression in an ischemic rat hind limb significantly increased skeletal muscle blood flow, muscle oxygen tension, and arteriogenesis with increased size and number of collateral arteries [50]. In addition, the results of the present work also revealed significant increase in the expression of eNOS in the sarcoplasm of gastrocnemius muscle cells of group SE compared to group S and the control group C. In line with this, Ülker and his group reported an increased activity of eNOS and production of NO that is associated with decreased superoxide formation induced by high concentrations of vitamin E (100 micromol/L) in rat aorta [51]. Vitamin E is an antioxidant that can inhibit the formation of reactive oxygen species (ROS) [52], therefore terminating lipid peroxidation and stabilizing the molecular composition of cellular membranes, and thus preventing the harmful effects of ROS on cells [53]. Moreover, previous studies revealed that increased eNOS expression has protective effect through producing NO that may serve as an oxidant scavenger, thereby minimizing the deleterious effects of superoxide and other ROS, inhibits synthesis of inflammatory cytokines, and participates in regulation of vascular smooth muscle cells proliferation [54,55]. Therefore, this study suggests that vitamin E through increasing the expression of eNOS stimulated increased vascularity and produced considerable amount of NO that stimulated skeletal muscle regeneration, and lowered the levels of proinflammatory cytokines in group SE animals.

Conclusion:

Supplementation of vitamin E before doing unaccustomed exhaustive swimming exercise can be beneficial prophylactic measure against elevated
proinflammatory markers and skeletal muscle damage. People who are not trained and get involved in exhaustive muscular exercise are advised to use vitamin E to protect them against muscular damage.

Conflict of interest:
There is no conflict of interest to declare.

References


الملخص العربي

الغلافية: ممارسة الرياضة تعزز طول العمر وتؤدي إلى التقليل من المواد المحترقة. ومع ذلك، فإن ممارسة الرياضة المتميزة أو المركبة، على المدى القصير، يزيد من تشكيل الأكسجين النشط من الفضلات. تستخدم مضادات الأكسدة على نطاق واسع كمكملات لمن يدري من ذلك ما إذا كانت قادرة على إيجاد التأثير السلبي لانخفاض النشاط من ممارسة السباحة الحادة غير معروف. هناك مجموعة متزايدة من الأدلة على أن ممارسة الرياضة بصفة عامة لها أثارها المفيدة جزيئية من خلال تثبيتها على السيرينات النشطة.

الهدف من هذه الدراسة: تقييم تأثير ممارسة السباحة المنتزعة على مرضى مرضى الآلتمات النشط في الدم وتكيف الجسم الشعري ووظيفية لعضلة الساقين الفريدة في ذكر الجرذان البالغ مع اختبار تأثيرات فيتامين D يوميا قبل القيام بممارسة تمرين السباحة.

الطريقة: تم تقسيم الحيوانات إلى 3 مجموعات: المجموعة S (تمارس السباحة يوميا لمدة سنة على مدى سنة أعمار جيم)، المجموعة C (تمارس السباحة ENOS) و C (تعتبر فيتامين D يوميا 1 ملليا (100 مجم/كم) عن طريق أنبوبية التغذية بالقليل ثم تمارس السباحة يوميا لمدة سنة وفقا لفترة زمنية متعددة).

النتائج: وجدتا أن الضغط الوعائي والخصائص النتائج عن ممارسة السباحة المنتزعة من غير سابق تمرير لمدة سنة كاملة على مدى سنة أعمار جيم. بالإضافة إلى ذلك، رصدنا تأثيرات الأعضاء في المجموعة S (تمارس السباحة) على مستوى الاضطرابات في دم من عامل نخر الدم، والإنسولين ونافوتين جاما، والبروتين المنفصل جيد. كما أن بروتين فيتامين E لمدة سنة من أعراض النشاط الدموي السريع في المجموعة، بالإضافة إلى النشاط في اليوسون الفيبر، سوءا. كما وجدنا أن أعراض فيتامين D لمدة سنة الخروج من السمك، والبروتينات المنفصلة في المجموعة، أحياناحارث في معالجة الفيتيدين في نظام العظام، وحالة ضعف العظام، وحالة ضعف العظام. بدأ في الخروج من السمك، وحالة ضعف العظام، وحالة ضعف العظام، بدأ في الخروج من السمك، وحالة ضعف العظام، وحالة ضعف العظام.

الخلاصة: استخدام فيتامين D كمكمل غذائي قبل ممارسة السباحة المنتزعة المحددة دون سابق تمرير يؤدي إلى تحفيز جاذبية ضد التغيرات الضذية الناجحة عن ارتفاع نسبة السيتوكتينات: عامل نخر الدم، والإنسولين ونافوتين جاما. وعلاقة على ذلك فان فيتامين D له دور واسع في معالجة الضرر الذي يحدث في أنسجة العضلات نتيجة لممارسة هذا النشاط الرياضي المراهق الحاد.