

# **Effect of Omega 3 and regular exercise on the muscle performance: Special prevalence of histamine and nitric oxide production**

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## **ABSTRACT**

**Background:** Skeletal muscle fatigue due to unaccustomed hard and/or prolonged physical exercise may be caused by increase of lactic acid, inflammatory mediators and oxidative stress. Stimulation of H<sub>1</sub> receptors by histamine and the ensuing production of nitric oxide trigger the vasodilating effect of histamine on arterioles in exercise to supply O<sub>2</sub> and nutrients and removal of CO<sub>2</sub> and waste products.

**Objective:** Investigating the effect of omega 3 alone or with regular exercise on improving muscle performance and delaying muscle fatigue. **Subjects and methods:** Forty apparently healthy subjects were studied. Depending on their previous physical conditioning status, subjects were divided into four equal groups: Two sedentary groups (group 1a without omega 3 therapy, and group 1b with omega 3), and two athletes groups (group 2a without omega 3 therapy, and group 2b with omega 3). Exercise tolerance was evaluated for each subject by using a free swimming competition (100 meters). Pulmonary ventilation, vital capacity, respiratory rate and heart rate were estimated before and after competition. Total antioxidant, lipid peroxides, total nitrate and nitrites, lactic acid and histamine levels were determined

**Results:** Omega-treated and/or regular exercise volunteers showed significantly increase of pulmonary ventilation, vital capacity, total antioxidant, nitric oxide (NO) and histamine levels, and significantly decrease of qualifying time competition, respiratory, resting and maximal heart rates, plasma lipid peroxides, and lactic acid compared with corresponding values of volunteers without Omega 3 or regular exercise. The combination of omega 3 plus regular exercise was more effective than each one alone. **Conclusions:** The results obtained in the present study provided an evidence that daily administration of omega 3 and/or regular exercise improved muscle performance and delayed fatigue through release of histamine and NO during exercise.

**Keywords:** Omega 3- regular exercise - muscle performance - fatigue - histamine -NO.

## INTRODUCTION

Skeletal muscle fatigue due to unaccustomed hard and/or prolonged physical exercise is usually associated with both pain and stiffness in skeletal muscles. Despite exercise-induced increase of lactic acid, pro inflammatory substances and free radicals are well known mediators of muscle fatigue **(Gina et al., 2016)**, yet the exact underlying mechanisms have not been established . However, histamine, a classical and important mediator of inflammation, is increasing in blood during hard physical exercise and may play role of muscle fatigue. It exerts its effects on target cells in various tissues by binding to its four histamine receptors, i.e. HR<sub>1</sub>, HR<sub>2</sub>, HR<sub>3</sub>, and HR<sub>4</sub> **(Nent et al., 2013)**. It is supposed to be released from mast cells during muscle damage, and it may then induce pain and/or vasodilation in the muscle itself **(Philip, 2010)**. The vasodilating effect of histamine on arterioles is largely induced by stimulation of HR<sub>1</sub> with subsequent production of nitric oxide (NO) **(Fukie et al., 2012)**. Physical activity such as walking, aerobic exercise, running and sports are associated with variable changes in hemodynamic, metabolic, hormonal, neurological and cardiopulmonary functions **(Pedersen and Saltin, 2015)**. Omega-3 fatty acids ( $\omega$ -3  $\alpha$ -linolenic acid) are long chain, polyunsaturated fatty acids (PUFA) of plant and marine origin. Because these essential fatty acids (EFAs) cannot be synthesized in the human body, they must be supplemented from dietary sources. Both  $\omega$ -3  $\alpha$ -linolenic acid and  $\omega$ -6 linoleic acid are essential fatty acids in humans.  $\omega$ -3 and  $\omega$ -6 PUFAs compete for the same metabolic enzymes.  $\omega$ -6 linoleic acid induces intracellular Ca<sup>2+</sup> mobilization and histamine release more than  $\alpha$ -linolenic acid **(Myung et al., 2014)**.

The aim of this study was to assess the possible effect of omega 3 supplementation alone or with regular exercise in improvement of muscle performance and delaying fatigue in athletes and non-athletes.

## **SUBJECTS AND METHODS**

**Subjects:** Forty apparently healthy volunteer of undergraduate students and the sport education students of Sohag University were studied. They were selected for age (19- 21 years), sex (males), weight (65-79 kg) and height (169-182 cm). All subjects completed a detailed medical history questionnaire and underwent a physical examination (weight, height, heart rate, arterial blood pressure and respiratory rate). Exclusion criteria were the presence of cardiac diseases, pulmonary diseases, renal impairment, hypertension, visual or auditory impairment or diabetes documented from medical history and / or examination. Additionally, medical history indicated that participants did not use medications, e.g. anti-inflammatory or cardiovascular drugs, or nutritional supplements, e.g. antioxidants. Before taking part in the experiment, all participants signed consent forms and were fully informed about the protocol. The ethical guidelines were followed as **Harriss and Atkinson (2009)**.

### **Methods**

**The study groups:** Subjects were divided into four equal groups: Sedentary group without omega 3 (group 1) was composed of healthy volunteers with sedentary lifestyles from the undergraduate students of Sohag University and was considered as a control group, and athletes group (group 2) was composed of physically

active volunteers from the sport education students of Sohag University. The volunteers in each group were further subdivided into 2 subgroups: Subgroup (a) without omega 3 treatment and subgroup (b) with omega 3 (one capsule of 300 mg three times daily for 3 months (**Atashak et al., 2013**)).

**Swimming exercise program:** Volunteers were instructed not to perform any activity during the 48 hours period preceding the experiment. Exercise tolerance was evaluated for each subject by using a free swimming competition (100 meters) after a warming up period of five minutes. Volunteers were instructed and encouraged to exercise until exhaustion. Five minutes before and after competition, respiratory rates were estimated, pulmonary ventilation and vital capacities were estimated by spirometer. Also, heart rates (resting and maximal) were estimated using the formula **HR max = 208 – (0.7 x age)** (**Stephen and Jean, 2015**).

**Biochemical study:** Venous blood samples (5 ml) were obtained, in heparinized tubes, from all groups immediately after exercise. Plasma was separated by centrifugation at 3000 cycles / min. at 37°C and kept in deep freeze at –70°C for estimation of different biochemical parameters. Enzyme-linked immunosorbent assays (ELISA) were performed for measuring of total antioxidant concentration (Cat. No. M1916) (**Allard et al., 1998**), lipid peroxides (**Okhawa et al., 1979**), nitrite (**Green et al., (1982)**), lactic acid (**Tony et al., 2015**), and histamine (**Hyung et al., 2016**).

**Chemicals and Drugs:** Omega 3 plus capsules were purchased from SEDICO Pharmaceuticals Company (Cairo, Egypt). Lactic acid kit was purchased from Bio Merieux France. Total antioxidant

kit was obtained from Cell Biolabs, Inc (San Diego, CA 92126, USA). Histamine human kit was purchased from Rocky Mountain Diagnostics (Colorado, USA).

**Statistical analysis** was done using the computer software program SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL., USA). Data were expressed as means  $\pm$  SE. Statistical significance for data was determined using one-way analysis of variance (ANOVA), followed by a post-hoc Tukey test to make multiple comparisons between groups.  $P < 0.05$  was considered statistically significant.

## RESULTS

Omega3 treated and/ or regular exercise volunteers showed significantly higher pulmonary ventilation and vital capacity levels, and lower respiratory, resting and maximal heart rates, compared with corresponding values of volunteers without treatment or regular exercise. The effects of regular exercise were more evident in group (2a) than in omega 3 therapy (group 1b) as there was a significant difference between them. The combination of omega 3 plus regular exercise was superior of the others (Table 1).

**Table (1): Effect of omega 3 on physiological parameters in sedentary and athletic groups.**

<b>Groups</b>	<b>Group(1 a)</b>	<b>Group (1 b)</b>	<b>Group (2 a)</b>	<b>Group (2 b)</b>
<b>Parameters</b>	<b>Sedentary without omega 3</b>	<b>Sedentary with omega 3</b>	<b>Athletes without omega 3</b>	<b>Athletes with omega 3</b>
<b>Respiratory rate (cycles/min.)</b>	45±7.1	42.2±5.2*	35.2±3.9*	30.8±5.5*
<b>Pulmonary ventilation (L/min.)</b>	50.1±3.6	60.4±4.7*	68.4±4*	69.6±4.6*
<b>Resting HR (beats/min.)</b>	75.7±5.4	70.6±4.9*	56.6±4.2*	55.8±3.9*
<b>Maximal HR (beats/min.)</b>	187.9±18.2	170.9±16.6*	150.9±15.3*	123.2±19.3*#
<b>Vital capacity (L/sec)</b>	1.9±0.03	2.00±0.01	2.41±0.1*	2.6±0.2*

Values shown are mean ± SE (n=10 per group)

\* Significant from group 1a. # Significant from group 2a.

Table 2 showed a significant decrease in plasma of malonaldehyde and lactic acid in association with a significant increase in total antioxidant, NO and histamine levels compared with corresponding values of volunteers without treatment or regular exercise. The intensity of changes produced by regular exercise was of greater extent than produced by omega 3 treatment. The combination of omega 3 plus regular exercise was more effective than each one alone.

**Table (2): Effect of Omega 3 on biochemical parameters in sedentary and athletic groups.**

<b>Groups</b>	<b>Group(1 a)</b>	<b>Group (1 b)</b>	<b>Group (2 a)</b>	<b>Group (2 b)</b>
<b>Parameters</b>	<b>Sedentary without omega</b>	<b>Sedentary with omega</b>	<b>Athletes without omega</b>	<b>Athletes with omega</b>
<b>Total antioxidant (mmol/L)</b>	0.6±0.01	1.862±0.06780*	1.954±0.205*	2.488±0.109*#
<b>Malondialdehyde (nmol/L)</b>	15.882±1.0	9.326±0.1893*	7.184±0.669*	6.224±0.159*#
<b>Lactic acid (mg/dl)</b>	164.5±11.04	137.5±9.80*	97.5 ±5.585*	77.96±7.593*#
<b>NO<sub>x</sub> (μmol/l)</b>	8.52±0.37	14.88±0.7561*	21.59±4.003*	29.51±2.533*#
<b>Histamine (μg/dl)</b>	0.5± 0.03	1.32 ± 0.04*	2.67 ± 0.04*	3.91 ± 0.7*#

Values shown were mean ± SE (n=10 per group)

\* Significant from group 1a. # Significant from group 2a.

Omega-treated and/ or regular exercise volunteers showed significantly lower qualifying time level in 100-meter freestyle swimming competition compared with corresponding

values of volunteers without treatment or regular exercise. The effects of regular exercise were more evident in group (2a) than in omega 3 therapy (group 1b) as there was a significant difference between them. The combination of omega 3 plus regular exercise was superior of the others (Table 3).

**Table (3): Effect of omega 3 on the qualifying time in 100-meter freestyle swimming competition in sedentary and athletic groups.**

<b>Groups</b>	<b>Group (1 a)</b>	<b>Group (1 b)</b>	<b>Group (2 a)</b>	<b>Group (2 b)</b>
<b>Parameters</b>	<b>Sedentary without omega</b>	<b>Sedentary with omega</b>	<b>Athletes without omega</b>	<b>Athletes with omega</b>
<b>Qualifying Time</b>	7.21± 0.11	5.52± 0.11*	1.291±0.03 *	1.072± 0.01*#

Values shown are mean ± SE (n=10 per group)

\* Significant from group 1a. # Significant from group 2a.

## **DISCUSSION**

Good nutrition is a fundamental component of athletes' training and performance plan. Good nutrition ensures that an individual is increasing the fuels necessary for the energy production needs related to activity and recovery. However, our understanding of the effects of strenuous physiological training and nutritional variations in combination with exercise stress in youth athletes is not clear enough (**JohnEric et al., 2015**).

Regular exercise and/or Omega 3 reduced heart rate by reducing membrane electrical excitability of the cardiac myocyte by



lowering its resting membrane potential and the duration of the refractory period through inhibition of ion channels **(Jing, 2012)**.

Improved pulmonary function tests in this work following regular exercise and/or Omega 3 could be due to decreased airway resistance, increased airway caliber, and strengthened respiratory muscles as well as lung and thorax elasticity. It seems also possible that an increased adrenaline system activity during exercise training results in decreased lung revocability and induced vasodilatation of pulmonary vessels **(Bakhtiar et al., 2010)**.

Consistent findings of elevated oxidative stress and inflammatory responses in exercise-trained individuals have been observed with acute exercise **(Fisher-Wellman and Bloomer, 2009)**. It has been proposed that ischemia-reperfusion at the site of the active muscles is probably the mechanism for the production of ROS and oxidative stress **(Atashak et al., 2013)**. ROS can cause muscle fatigue by decreasing maximum  $\text{Ca}^{2+}$  activated force,  $\text{Ca}^{2+}$  sensitivity, and  $\text{Ca}^{2+}$  release, and this was demonstrated by experiments where administration of ROS scavengers/antioxidants delayed fatigue development **(Lamb and westerblad, 2011)**.

Omega 3 therapy and/ or regular exercise were observed to have protective effect against production of oxidative stress. Regular exercise reduced basal production of oxidants, reduction of radical leak during oxidative phosphorylation, increased the activity of antioxidant enzymes and increased oxidative damage repair systems to improved physiological function and enhanced resistance to oxidative stress **(Radak et al., 2007)**. Regular exercise also has a large impact on the availability and bioactivity of endothelial-derived nitric oxide. The stimulus for endothelial  $\text{NO}\bullet$

production is the increased flow through the vessels, which results in shear stress and increased activation of endothelial nitric oxide synthase (**Abdel-Sater, 2008**). Reduction of oxidative stress by omega-3 fatty acids could be related to their tight packing in complex membrane lipids and lipoproteins making the double bonds less available for free-radical damage (**Atashak et al., 2013**). Another potential mechanism is through increased production of antioxidant enzymes (**Șerban and Răzvan, 2016**). It is well known that, during exercise, there is an increased need for nutrients and O<sub>2</sub> to be supplied to skeletal muscles and for waste metabolites and CO<sub>2</sub> to be removed from them. The detailed mechanisms responsible for the support of such vascular functions remain unclear (**Fukie et al., 2012**). Histamine dilates precapillary arterioles and increases capillary permeability. Thus, histamine, if provided appropriately within or to skeletal muscles, may help to support the supply of O<sub>2</sub> and nutrients and the removal of CO<sub>2</sub> and waste products. Stimulation of histamine H<sub>1</sub> receptors and the ensuing production of nitric oxide triggers the vasodilating effect of histamine on arterioles (**Francesco et al., 2012**). Histamine receptors H<sub>1</sub> and H<sub>2</sub> have been suggested to enhance the availability of glucose to skeletal muscles by facilitating capillary permeability to the interstitium following exercise (**Pellinger et al., 2010**). Results in this study supported this hypothesis as a significant increase in histamine and NO levels were observed in Group 2 athletic with or without omega 3. The combination of omega 3 plus regular exercise was more effective than each one alone. These results were in accordance with **Aleksandra et al. (2015)** who found an increase in NO release in response to n-3

PUFA supplementation which may enhance exercise performance in cyclists. Regular exercise and/or omega 3 therapy improve endothelial functions that can be attributable to increased endothelial NO synthase (eNOS) protein levels within the endothelium and/or reduced oxidative stress. The reduced oxidative stress can help prevent the uncoupling of NO and therefore increasing NO bioavailability **(Ross et al., 2016)**. Omega 3 induced intracellular  $\text{Ca}^{2+}$  mobilization and histamine release. Histamine increased both NO production and calcium entry from extracellular fluid **(Lantoine et al., 1998)**. Results in the present study revealed that regular exercise and/or omega 3 supplementation caused delayed muscle fatigue by reducing lactic acid and oxidative stress. Lactic acid can increase intracellular acidity of muscles. This can lower the sensitivity of troponin C to  $\text{Ca}^{2+}$ . As reactive oxygen species (ROS) accumulate in contracting muscles, protein, lipid and DNA oxidation might inhibit force production, contributing to the development of acute fatigue **(Peter and Peter, 2015)**. Omega3 treated and/ or regular exercise volunteers showed significantly lower qualifying time level in 100-meters freestyle swimming competition compared with corresponding values of volunteers without treatment or regular exercise. These results can be explained by improvement of reactivity, attention and cognitive performances in addition to the improvement of mood state and the modifications of some neuro-electrical parameters by the omega 3 **(Jana and Zdenka, 2015)**. Omega 3 also improves performance in sports where perceptual motor activity and decision making are the keys to success **(Mickleborough, 2013)**.

**Conclusion:** Daily administration of omega 3, and/or regular exercise improved muscle performance and delayed fatigue through release of histamine and nitric oxide (NO) during exercise.

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## الملخص العربي:

تأثير أوميجا ٣ وممارسة التمارين الرياضية بانتظام على أداء العضلات: خصوصية انتشار وانتاج الهستامين وأكسيد النيتريك

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**خلفية البحث:** قد يكون سبب التعب العضلي الناتج عن ممارسة الرياضة البدنية غير المعتادة و / أو لفترات طويلة ناتجا عن زيادة في حمض اللاكتيك ووسطاء الالتهابات والإجهاد التأكسدي. ان للهستامين أثناء ممارسة الرياضة دور مهم في توسيع الاوعية الدموية وذلك لامداد العضلات بالاكسجين ومواد التغذية والتخلص من الفضلات وثنائي اكسيد الكربون ولعل هذا الدور ناتجا الى حد كبير عن تحفيز مستقبلات الهستامين (H1) وما يترتب على ذلك من انتاج اكسيد النيتريك.

**هدف البحث:** دراسة تأثير أوميجا ٣ منفردا أو مع ممارسة التمارين الرياضية بانتظام على تحسين أداء العضلات وتأخير الشعور بالاجهاد.

**طريقة البحث:** تم اجراء هذه الدراسة على عدد اربعين فردا ، تم تقسيمهم الى أربع مجموعات متساوية كالاتي:

- ١- أفراد غير رياضيين وبدون الاوميجا ٣.
  - ٢- أفراد غير رياضيين ويتم اعطاؤهم الاوميجا ٣ (٣٠٠ ملجم ثلاث مرات يوميا).
  - ٣- أفراد رياضيين وبدون الاوميجا ٣.
  - ٤- أفراد رياضيين ويتم اعطاؤهم الاوميجا ٣ (٣٠٠ ملجم ثلاث مرات يوميا).
- ثم تم تعريض جميع المجموعات الى سباحة ١٠٠ متر حرة وبعد ذلك تم تقدير التهوية الرئوية، والقدرة الحيوية، ومعدل التنفس ومعدل ضربات القلب قبل وبعد المنافسة ، وكذلك تم قياس إجمالي مضادات الأكسدة، بيروكسيدات الدهون، إجمالي النترات والنيترات، حامض اللبنيك ومستويات الهستامين بالدم وكانت النتائج كما يلي:

زيادة كبيرة في التهوية الرئوية، والقدرة الحيوية، ومضادات الأكسدة الكلية، وأكسيد النيتريك ، ومستويات الهستامين، وانخفاض كبير في زمن المنافسة، ومعدل التنفس، ومعدل ضربات القلب القصوى، بيروكسيدات دهون البلازما ، وحمض اللبنيك في المجموعات ٢، ٣، ٤ مقارنة مع المجموعة الاولى ، كذلك لوحظ ان الجمع بين ممارسة الرياضة بانتظام مع الاوميجا ٣ أكثر فاعلية من كل علي حدة.

**الخلاصة:** النتائج التي تم الحصول عليها في هذه الدراسة قدمت دليلا على أن العلاج اليومي بأوميجا ٣ و / أو ممارسة التمارين الرياضية بانتظام يحسن أداء العضلات ويؤخر التعب من خلال انتشار الهستامين والنيتريك اوكسيد أثناء ممارسة الرياضة