

## Effects of Endurance Training on A<sub>12</sub> Acetyl Cholinesterase Activity in Fast and Slow-Twitch Skeletal Muscles of Male Wistar Rats

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| Article information  | Abstract  |
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| <p>Article history:<br/>Received: 26 May 2012<br/>Accepted: 27 June 2012<br/>Available online: 9 Apr 2013<br/>ZJRMS 2013; 15(10): 28-31</p> <p>Keywords:<br/>Endurance Training<br/>Acetyl cholinesterase<br/>FHL<br/>SOL<br/>Neuromuscular junction</p> <p>*Corresponding author at:<br/>Department of Physical<br/>Education and Sport Sciences,<br/>Faculty of Humanities Zanjan,<br/>University of Zanjan, Zanjan,<br/>Iran.<br/>E-mail:<br/>Ali_Gorzi@yahoo.com</p> | <p><b>Background:</b> Endurance training improves the activity of G<sub>4</sub> type acetylcholine esterase (AChE) in muscle fibres. The purpose of this study was to investigate the effects of 8 weeks of endurance training (ET) on activity of A<sub>12</sub> type of AChE in Flexor Hallucis Longus (FHL) and Soleus (SOL) muscles of rats.</p> <p><b>Materials and Methods:</b> 16 male wistar rats (age: 10 weeks and weight: 172.17±10.080 gr), were randomly divided in 2 groups (control; N=8 and ET; N=8). Training group carried out 8 weeks (5 session/week) of endurance training on animal treadmill with speed of 10 m/min for 30 min at the first week which was gradually increased to 30 m/min for 60 min (70-80% of VO<sub>2</sub>max) at the last week. Forty eight hours after last session of training, FHL and Sol muscles of animals were moved out under sterilized situation by cutting on postero-lateral side of hind limb. For separating AChE subunits, homogenization and electrophoresis (0.06 non-denaturing polyacrilamide) methods were used. AChE activity was measured by Elisa kit.</p> <p><b>Results:</b> The activity of this protein significantly (<math>p=0.017</math>) increased in SOL muscle of ET group by 119%, but did not changed in FHL. In both groups (ET and Con), FHL muscle had significantly (ET: <math>p=0.028</math> and Con <math>p=0.01</math>) higher basic levels of AChE activity compared to SOL muscle. This significant increase in AChE of SOL might be indicative of responsiveness of AChE of this muscle following endurance training for improving acetylcholine (ACh) cycle in neuromuscular junction.</p> <p><b>Conclusion:</b> Endurance training might increase the A<sub>12</sub> type AChE activity to improve the ACh cycle as part of the adaptation of neuromuscular junction to increased level of physical activity.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p> |

### Introduction

Exercise training induces vast range of the cellular, molecular and tissue adaptations, especially in nerve and muscle cells. However, these adaptations and the underlying mechanisms have not yet been identified. Neuromuscular communications take place in the form of the electrochemical changes [1]. In which when impulse reaches the axon terminal, opening of the sodium channels depolarize the cell membrane. Depolarization then activates the voltage gated calcium channels and facilitates calcium ions influx to the axon terminals. Following this process, release of acetylcholine takes place due to calcium regulated exocytose [2]. Acetylcholine is discharged into synaptic cleft, immediately adjacent to the active zone (calcium channels) [3, 4]. Then, AChE destroys ACh in synaptic cleft and choline is actively transported back into axon terminal, where it is reused in the synthesis of ACh [5]. Main form of the acetylcholine esterase, which is associated with the hydrolysis of ACh in neuromuscular junction (NMJ), is A<sub>12</sub> [6-8]. A<sub>12</sub> is attached to the membrane by a collagen tail [6-8] and it is almost exclusively located in synaptic cleft (especially in fast

twitch fibers) and is the main form of the AChE in mammalian NMJs [9, 10]. Acetylcholine esterase is transported from cell body to axon terminal by the axoplasmic transport [1]. Studies have shown that the muscle fiber can produce this enzyme, but muscle denervation experiments indicate that this production is under control of the trophic effects of motoneuron [11]. Based on the previous findings, in fast twitch muscle fibers of rats, activity of A<sub>12</sub> and G<sub>4</sub> type of AChE is higher than that of slow twitch muscles and the amount of AChE in synaptic cleft is proportional to the release of ACh and level of increased muscular activity [11-13]. Studies have shown that endurance training can increase neuromuscular surface area, synaptic folds, acetylcholine receptors number and may improve the release of ACh and activity of acetylcholine esterase subtypes [10]. Moreover, resistance training increases muscle tension which might be due to neuromuscular facilitation [14-16]. In this regards, recently researchers tend to define muscle strength as amount of acetylcholine released per unit of neuromuscular junction surface [10]. On the other hand, reduction of acetylcholine release from nerve terminals

during prolonged and heavy exercise training, leads to fatigue at neuromuscular junction and increase the activity of G<sub>4</sub> type of acetylcholine esterase have been reported by some researches [10, 17, 18]. Thus, this study aims to investigate the effects of an incremental type of endurance training on the activity of A<sub>12</sub> type of AchE in fast and slow twitch muscle fibers.

## Materials and Methods

Sixteen male Wistar rats with 5 weeks of age and body weight of  $172.17 \pm 10.08$  g, provided from Razi institute (Iran) were divided to control and experimental groups (N=8 for each) which both of them did pre and post tests. Animals were housed in standard cages (4 rats in each cage) for 4 weeks to reach puberty, which followed by 1 week of familiarization with training protocol. Room temperature ( $22 \pm 1.4^\circ\text{C}$ ) and light-dark cycle (12:12) were controlled and water and food were available for rats ad libitum.

Endurance training protocol [19] consisted of 8 weeks (5 session/week) of running on a motorized rodent treadmill (Pishro andishe sanat comp, Iran). Training program started with speed of 10 m/min for 30 min at first week and increased up to 30 m/min for 60 min (equal to 70-80% of VO<sub>2</sub> max) at last week (Table 1). Animals were weighed prior to each training session. During familiarization period, weak electrical shock was used when animals avoided running. Control group experienced the same experimental condition as the treatment group, with the exception of running.

Forty eight hours after last session of training, animals were anesthetized with ketamine (30-50 mg/kg of BW) and xylazine (3-5 mg/kg of BW) and Sol and FHL muscles were moved out under sterilized situation by a posterior-lateral cut on hind limb. The tissues then immediately were frozen by liquid nitrogen ( $-196^\circ\text{C}$ ) and stored at  $-80^\circ\text{C}$  until laboratory assessment. AchE subunits were separated by electrophoresis (0.06 non-denaturing Polyacrilamide) and specific Elisa kit (Japan) were used for assessment of activity of AchE. Tissues were homogenized and PBS (Phosphate Buffer Saline) was used for homogenization in combination with aprotinin as antiprotease (1 ml). They were then centrifuged at 6000 rpm for 15 min and supernatant and pellet were obtained. Supernatant was used for measuring the activity of A<sub>12</sub> type of acetyl cholinesterase. The experiment was conducted in Tarbiat Modarres University with collaboration of Endocrine and Metabolism Research Centre of Shahid Beheshti University. After ensuring from normality of data with Kolmogorov Smirnov, independent *t*-test was used for comparison between groups and between fiber types. All statistical analysis was conducted using SPSS-18 software and  $p < 0.05$  was considered as significant.

## Results

In this study, endurance training protocol resulted in adaptations of rats (improvement in speed up to 30

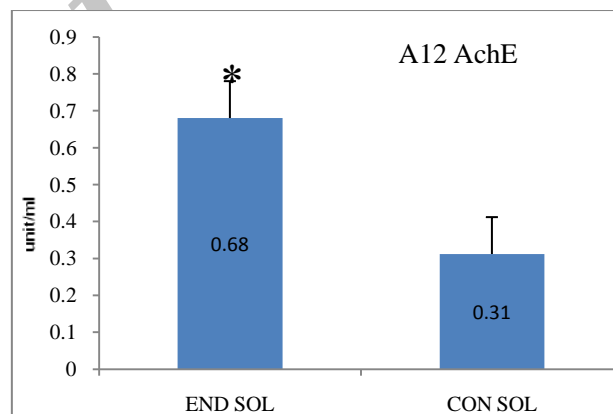
m/min, equal to 70-80% of vo<sub>2</sub> max, for 60 min at last week).

The Kolmogorov Smirnov test showed that the distribution of data for the activity of A<sub>12</sub> AchE, was normal (FHL: 0.919 and soleus: 0.930).

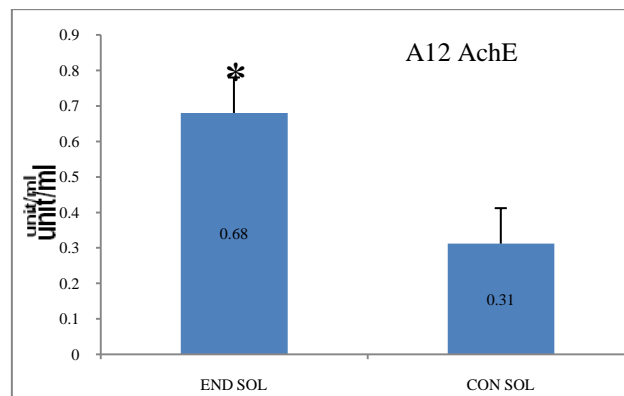
Independent *t*-test showed that the activity of this enzyme significantly ( $p=0.017$ ) increased in soleus muscle of endurance group (endurance:  $0.68 \pm 0.28$  vs. control:  $0.31 \pm 0.13$ ; 119% increases, figure 1). Also, independent *t*-test showed that, there was no significant difference in activity of A<sub>12</sub> type of AchE in FHL muscle between two groups (endurance:  $1.17 \pm 0.37$  vs. control:  $1.01 \pm 0.29$ ; 15% increases, Fig. 2).

Independent *t*-test also showed that the activity of total AchE in soleus (endurance:  $1.70 \pm 1.09$  vs. control:  $1.03 \pm 0.44$ ) and FHL (endurance:  $2.99 \pm 0.65$  vs. control:  $2.30 \pm 0.64$ ) muscles were insignificantly higher than control group.

It was also found that according to independent *t*-test, in both endurance and control groups, the activity of A<sub>12</sub> type of AchE in FHL muscle was significantly higher than that of soleus muscle [endurance, FHL:  $1.17 \pm 0.37$  vs. soleus:  $0.68 \pm 0.28$  ( $p=0.01$ ) and control, FHL:  $1.01 \pm 0.29$  vs. soleus:  $0.31 \pm 0.13$  ( $p=0.028$ )].



**Figure 1.** The effect of endurance training on activity of A<sub>12</sub> type of AchE in soleus muscle (\*: significant differences compared with control group,  $p=0.017$ )



**Figure 2.** The effect of endurance training on activity of A<sub>12</sub> type of AchE in FHL muscle

**Table 1.** Endurance training protocol

| Weeks          | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|----------------|----|----|----|----|----|----|----|----|
| Duration (min) | 30 | 40 | 45 | 50 | 55 | 60 | 60 | 60 |
| Speed (m/min)  | 10 | 20 | 20 | 25 | 25 | 30 | 30 | 30 |

## Discussion

Increased activity of A<sub>12</sub> type of AchE in muscle which was active in endurance training (soleus) may be indicative of the increased capacity of AchE storage in the neuromuscular junction which could delay the occurrence of fatigue. Findings of this study showed that activity of A<sub>12</sub> type of AchE in FHL muscle was not significantly different between training and control groups, but in soleus muscle, the activity of this enzyme in training group was significantly higher than control group. Moreover, in endurance group, the activity of this enzyme in FHL muscle was approximately 15% higher than control group which is thought provoking. This considerable responsiveness of soleus muscle is the sign of more involvement of this muscle in endurance type of activity, which is confirmed by other similar studies. It seems that, endurance training load of this study was enough to induce the increased activity of A<sub>12</sub> type of AchE in soleus muscle.

Gharakhanlou et al. had reported that calcitonin gene-related peptide (CGRP) changes in skeletal muscle following 12 weeks of resistance training, was related to the intensity of training [20]. These researchers reported that the effect of combined training (resistance and endurance) on CGRP levels was higher than that of one of resistance or endurance training programs [20].

Hubatsh and Jasmin [21], Gisiger et al. [22] and Fernandez et al. [23] had reported increased activity of G<sub>4</sub> type of AchE after endurance training and Drolotcher et al. [24] had shown increased Ach release after endurance training, which are in agreement with our findings.

In this study, in endurance trained group, the activity of total AchE was significantly higher than control group. Also, in endurance trained group, the activity of total AchE was higher. These findings support the

responsiveness of the A<sub>12</sub> subunit and can probably reflect an increase in other subunits (e.g. G<sub>4</sub>). Our data showed that, in both endurance and control groups, the activity of A<sub>12</sub> type of AchE in FHL muscle was significantly higher than that of soleus muscle. This difference indicates the faster nerve impulse transduction in neuromuscular junction of the fast fibers.

In conclusion, the results of this study are in consistence with the findings of most previous studies [22, 25] reporting increased activity of G<sub>4</sub> type of AchE after different exercise mode, particularly after endurance exercise [14, 18]. This study showed that, endurance training can improve activity of A<sub>12</sub> type of AchE, which may be a probable mechanism for enhanced endurance performance and delayed fatigue. It seems that, since the activity of AchE is higher in fast twitch fibers and these fibers are primarily used in intensive intermittent activities, if we had used intensive intermittent activities instead of continues endurance training, it could improve AchE activity in FHL muscle and even could lead to further AchE activity improvement in the soleus muscle. However, conclusive results in this area require further study about the effects of the different training intensity, duration and mode (e.g. intermittent).

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## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

## Conflict of Interest

The authors declare no conflict of interest.

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