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ORIGINAL ARTICLE

Continuous Swimming Training Arises a Remarkable Effect on Some Longevity Biomarkers in Rat Skeletal Muscles

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ABSTRACT

Background. SIRT3 is one of the members of the Sirtuin deacetylase protein family which is linked to the longevity of human being and is used as an important therapeutic and diagnostic marker in illness and aging. **Objectives.** The aim of this study was studying the effect of continuous swimming training on some biomarkers of longevity in slow-twitch (SOL) and fast-twitch (EDL) muscles of adult male rats. **Methods.** 20 healthy male, 5 months old rats were divided into two groups: control (n = 10) and exercise (n = 10) groups. Continuous swimming training was performed 5 days a week for 8 weeks, including 30 minutes of workout without adding weights in the first week to 60 minutes of workout, adding weights by 3% of the body weight in the eighth week. Twenty four hours after the last exercise session, SOL and EDL muscles were removed and the changes in variables (PGC-1α, SIRT3, and GSH: GSSG) were measured by ELISA method. **Results.** The results showed that conducting 8 weeks of continuous swimming training significantly increased PGC-1α and SIRT3 levels in slow-twitch (SOL) and fast-twitch (EDL) muscles ($p < 0.05$). There was no significant difference in the aging index (GSH:GSSG) of the SOL and EDL groups compared to the control group ($p > 0.05$). **Conclusion.** According to the findings of the present study, the implementation of continuous swimming exercises can improve the PGC-1α and SIRT3 proteins, which are biogenesis mitochondrial and life span biomarkers in slow- and fast-twitch muscles.

KEYWORDS: ***Continuous Training, Swimming, Longevity, PGC-1α, SIRT3***

INTRODUCTION

The mitochondrial dispersion is not the same in different types of muscle fibers with different metabolic capacities and can be found more in slow-twitch fibers, which is one of the most important reasons for the oxidation of these fibers (1). Mitochondria is also the main site for the creation of free radicals (ROS) (2). Free radicals are highly reactive, unstable and toxic molecules, and since unpaired electrons have the ability to damage many biological molecules, such as nucleic acids, proteins and lipids, they cause defects in the structure and function of these molecules that are themselves the cause of various types of degenerative diseases (3). Free radicals are continuously produced in the cell as by-product of metabolic reactions. To counteract and neutralize the effects of these reactive substances, organisms have various mechanisms, including antioxidant defense systems, which can include proteins and enzymes such as Catalase (CAT), Peroxidase (POD), Superoxide Dismutase (SOD) (4). Lipids are one of the most important molecules invaded by free radicals, and their peroxidation process ultimately reduces cell life and eventually leads to death (5). Physical

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activity is an inseparable part of human life. Various adaptations occur in the body after exercise, most notably mitochondrial biogenesis (increasing the size and content of mitochondria) and increasing antioxidant enzymes (6). This event is vital for regulating and modifying the processes such as cell metabolism and energy production, calcium signaling, production of oxygen reactive species (ROS) and apoptosis. Mitochondrial biogenesis is a complex process that requires synthesis mechanisms and expression of many genes. The process of regulating the expression of mitochondrial biogenesis genes follows the general principles of molecular biology, and typically requires activator, regulator or inducer (7, 8). One of these nuclear inducers, which play a pivotal role in the mitochondrial biogenesis, metabolism and suppression of ROS is PGC-1 which is considered as a major transcriptional coagulant (9, 10). Caloric restriction or exercise causes the expression of PGC-1α (11, 12). It has been observed that mice without PGC-1α have many deficiencies, including obesity, neuroprotection, cardiomyopathy and high sensitivity to ROS (13). PGC-1α is likely to result in the transcription of the CREP (CAMP response element-binding protein) factor. This protein then increases the free radicals' toxicity enzymes such as GPX١ and Mn-SOD. The molecular mechanism involved in activating these antioxidants is that PGC-1α is combined with an estrogen-related receptor alpha (ERRα) and activates SIRT3 in the mitochondrial matrix (14).

Interestingly, PGC-1α plays an important role in controlling SIRT3 gene expression. SIRT3 is a family of proteins of deacetylase NAD^+-dependent ADP-ribosyltransferase, present in the mitochondrial membrane (15, 16). The findings have revealed that the increase in Sirt3 leads to increase in glutathione peroxidase levels (GPX1) and as a result of glutathione peroxidase (GPX1) action, GSH is oxidized to GSSG which in turn increases the ratio of NAD^+ to NADPH and stimulates the expression of SIRT3 (17). In various studies, the reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio is considered as an indicator of oxidative stress and aging (17, 18). Although the effects of endurance exercises on mitochondrial biogenesis have been studied, the simultaneous effects of these exercises on the molecular changes of PGC-1α and SIRT3 and their effects on aging index (GSH: GSSG) and also their comparison in two types of slow-twitch and fast-twitch muscle tissues is less investigated. Therefore, considering the importance of delay the aging process and degenerative diseases caused by it in order to improve the quality of life, the researchers, with the design and implementation of this research, answered the question to what extent the Continuous Swimming Training can stimulate the mitochondrial biogenesis and increase the SIRT3 activity and finally suppresses ROS and improves the aging index in two types of slow-twitch muscle (SOL) and fast-twitch (EDL) muscles of adult male rats.

MATERIALS AND METHODS

Animals. Twenty adult male rats (5 month old) with a weight range of 258 ± 7 gr were purchased from Razi Institute and during the study they were kept in special polycarbonate rat cages, in an environment with a mean temperature of 22 ±1.4°C and 55 ± 4% humidity and a light-dark cycle of 12:12 hours. One week after adaptation of the rats with the laboratory environment, the rats were randomly divided into two groups: Continuous training (CT) (n = 10) and Control group (C) (n = 10), regarding the weight matching. The exercise group (CT) performed 8 weeks of specified exercise and during this time, the control group did not exercise at all. At all stages of the study, the rats had free access to the water. All stages of retention, training, and killing the rats were carried out based on Ethics Committee of Shahid Chamran University of Ahvaz with ethical code for animal experiment research of: EE/97.24.3.17660/scu.ac.ir.

Research Design. The swimming practices were done in a glass pool with 540×530×660 dimensions, (19) filled with 31 ±2°C water (20). In the first 5 days before the beginning of the training, to adapt the animals to the new environment and eliminate their stress, before training, all rats (training group and control group) were kept in shallow water (5 cm and 31 ±2°C) for 20 minutes (21). Continuous swimming training was performed 5 sessions a week (Monday to Friday) for 8 weeks, from 8 am to 11 am (20). In each training session, the intensity of the exercise was adjusted based on the body mass of the rats, by hanging small

weights to the tail (22, 23). Training load ranged from 0% to 3% of the body mass of the rats based on the endurance training protocol with medium and low intensity (Table 1). After each training session, the rats were dried and returned to standard bioterium conditions (20).

Table 1. Continuous Swimming Training Protocol (20)

Week	Number of repeats per day (5 days a week)	Workout time (min)	Training load (body weight percentage)
1	1	30	0
2	1	40	0
3	1	30	1
4	1	40	1
5	1	40	2
6	1	50	2
7	1	50	3
8	1	60	3

The load intensity was attached to the animal's tail and was individually adjusted in each exercise session according to animal body mass. The progression of load intensity, volume of continuous training was adapted from a standard protocol.

Preparation of the Tissue. To prepare the tissue samples, a combination of Ketamine (60-40 mg/kg body weight) and Xylene (15-5 mg/kg body weight) were injected intraperitoneally and following the rats anesthesia, SOL and EDL muscles were removed in order to measure the amount of mitochondrial biogenesis proteins (PGC-1α, SIRT3) and GSH and GSSG (reduced and oxidized glutathione). Cell extract was prepared in accordance with a modified protocol as previously described (24). In summary, tissues were placed in porcelain mortar, frizzed in liquid nitrogen, and then powdered with porcelain pestle. Powdered muscle samples were lysed with homogeneous buffer (Tris-HCL 500 µL, pH 8, EDTA 0/003, NaCl 0.08 g, sodium oxalate 0/025 g, SDS 0/01 g, 1 protease inhibitor cocktail tablets, 10 µl NP40 1%). In order to homogenize the tissue, the lysis buffer was added (five times of the weight of the samples) and was homogenized with homogenizer at 300 rpm for 30 seconds with 30 seconds intervals in order to prevent protein denaturation. The homogenized tissue was centrifuged at +4˚C for 10 minutes at 3600 rpm and the supernatant was separated (24). The supernatant protein concentration was calculated using Bradford method and an appropriate concentration of protein was calculated (25). The presence or absence of these proteins was evaluated by applying a specific ELISA sandwich method, using the PGC-1α, SIRT3 and GSH and GSSG specific antibodies. All procedure were don in accordance with manual data shit powered by manufacturing company (Crystal chem co. & Zellbio co.). By employing the ELISA titration method, which was performed, based on a systematic dilution series, the amount of increase or decrease of the expression of the interested targets was quantified and compared.

Statistical Analysis. The Kolmogorov–Smirnov test was used to determine data normalization. Statistical analysis was conducted with IBM SPSS statistics 23 software. Continuous data was described as the mean ± standard deviation (SD). Independent t student tests were used to compare differential levels of PGC-1α, SIRT3 proteins and GSH: GSSG in the groups.

p-value under 0.05 was considered to be statistically significant.

RESULTS

The weight changes of the control and continuous swimming training groups during the eight-week training period are shown in Table 2. The findings showed that the mean weight of continuous swimming training rats group at the end of the training period was lower than the control group (p=0.001) which reflects the effect of the exercise.

Table 2. Average and Standard Deviation of Group's Weight (mean ± SE)

Groups	Quantity (n)	Initial weight (gr)	Final weight (gr)	Percent change
Control	10	257.11 ± 6.13	342.22 ± 4.35	33.31
Continuous	10	260.54 ± 4.32	317.54 ± 7.85	21.84

The mean weight of continuous swimming training rats group at the end of the training period was significantly lower than the control group ($p \leq 0.05$)

The results of t student test of independent groups revealed that the level of PGC-1α protein in the SOL (p=0.016) and EDL (p=0.009) muscles increased significantly in the continuous swimming training group compared to the control group. (Discriptive results confirmed that the expression level of PGC-1α in SOL and EDL muscle tissues were increased 1.16 and 1.4 fold respectively) (Figure 1).

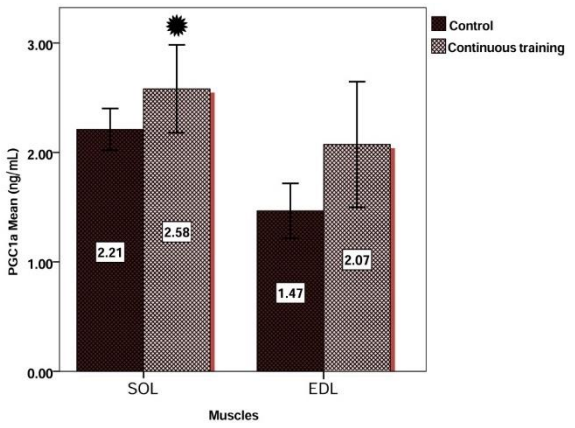

Figure 1. PGC1α Changes in SOL and EDL Muscles in Continuous Swimming Training Group and Control Group
*** Significantly greater than control group.**

In addition, the effect of continuous swimming training on SIRT3 protein expression levels in two muscle tissues was also investigated. Independent t-test showed that the level of SIRT3 in the SOL (p=.001) and EDL (p=.006) muscles of the rats in the training group was significantly increased compared to the control group. (Discriptive results confirmed that the expression level of SIRT3 in SOL and EDL muscle tissues were increased 1.4 and 1.3 fold respectively). (Figure 2)

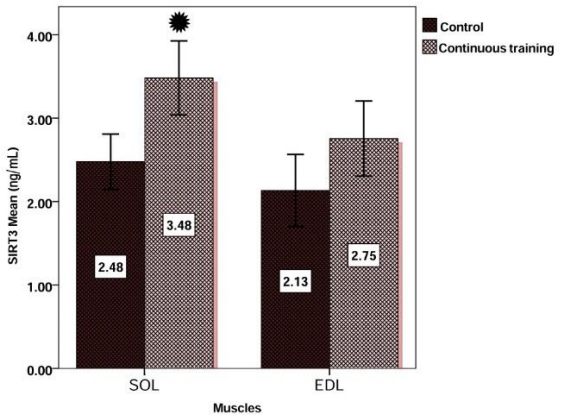

Figure 2. The Amount of SIRT3 Changes in the SOL and EDL Muscles in the Continuous Swimming Training Group and the Control Group
*** Significantly greater than control group.**

The results of t-test for independent groups showed a non-significant deference in the ratio of GSH: GSSG as an indicator of aging index in the SOL (p=0.882) and EDL (p=0.529) muscles of the experimental group rats as compared to the control group. This ratio remained constant in the SOL muscle while it decrease in the EDL muscle compared to the control group (Table 3) (Figure 3).

Table 3. Comparison of GSH and GSSG between Two studied Groups in EDL and SOL Muscle (mean ± SE)

Variables	Groups	N	Mean	±SD
GSH-EDL	**C**	10	0.0289	0.03822
GSH-EDL	**T**	10	0.0200	0.00632
GSH-SOL	**C**	10	0.0144	0.00726
GSH-SOL	**T**	10	0.0145	0.00688
GSSG-SOL	**C**	10	0.0289	0.00333
GSSG-SOL	**T**	10	0.0300	0.00000
GSSG-EDL	**C**	10	0.0300	0.01225
GSSG-EDL	**Ct**	10	0.0309	0.01514

GSH: Reduced Glutathione. GSSG: Oxidized Glutathione. EDL: Extensor Digitorum Longus (fast twitch). SOL: Soleus Muscle (Slow Twitch). C: Control Group. T (Test Group): Continuous Training Group. GSH-EDL; P=0.509, GSH- SOL; P=0.975, GSSG-SOL; P=0.347, GSSG- EDL; P=0.884

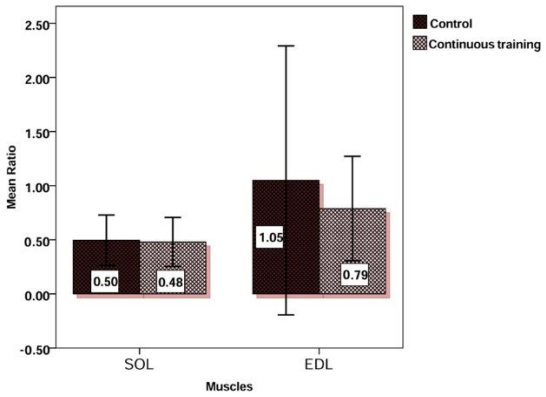

Figure 3. The Amount of GSH: GSSG Changes in SOL and EDL Muscles in the Continuous Swimming Training and Control Group

DISCUSSION

Aging is an involuntary process that gradually reduces the effectiveness of many physiological actions and increases the likelihood of disease and death. Two of the most prominent biochemical aging theories are mitochondrial theory and the theory of free radicals that point to changes in energy metabolism, free radicals, lifetime, and mitochondrial health (26-28). In previous studies, it has been shown that PGC-1α increases the expression of mitochondrial transcription factor A (mtTFA) by simultaneously activating NRF-1 and NRF-2 which it increases the mitochondrial biogenesis (29). Research results indicate that PGC-1α stimulates expression of SIRT3 at mRNA levels (30). Sirutins are NAD-dependent enzymes that are involved in a wide range of physiological and pathophysiological conditions, including diabetes, cancer, life span and neurodegeneration through the deacetylation of multiple substrates (31). Scientific evidence shows a pathway that regulates mitochondrial

biogenesis and ROS regulation which is performed by PGC-1α, ERRα, and SIRT3 (32). Research has shown that regarding the ability of SIRT3 to increase oxidative mitochondrial capacity and protection against apoptosis, SIRT3 is the only Sirtuin associated with human life span (32, 33). The results of this study showed a significant increase in the expression of PGC-1α and SIRT3 proteins in SOL and EDL muscles. This result becomes even more important when the oxidative stress and aging index of GSH: GSSG is also studied in SOL and EDL muscles. The results of statistical descriptive analyzes indicate that the ability of the fast twitch muscle (EDL) in the face of the endurance training protocol for the effective conversion of GSSG to GSH has not been as successful. Whereas the slow twitch muscle (SOL) in the form of this training strategy has been able to create a proper and balanced interaction between the two GSSG and GSH molecules. The reflection of these phenomena can be seen well in the longevity molecular index (SIRT3), so that the experiments showed that the SIRT3 expression in the SOL increased to EDL. This process can indicate the ability of the antioxidant system of this type of muscle to maintain the balance of oxidative stress as a result of more adaptation of these types of warps to endurance exercises. Increasing the expression of SIRT3 in the muscles increases the process of deacelytation and activation of Isocitrate dehydrogenase (IDH) and consequently increases NADPH levels, which in turn can increase the activity of glutathione reductase and Thereby increasing GSH regeneration from GSSG (17). The findings of this study, such as those found in SOL and EDL muscles, are completely consistent with the mentioned theory and are justifiable. Due to the involvement of SOL muscle in the long-term activities, the expression of the protein associated with the SIRT3 life span and the antioxidant system of these muscles improved and neutralized the free radicals produced by the aerobic oxidation of mitochondria.

CONCLUSION

In general view, according to the results of this study, it can be concluded that the continuous swimming training, increases the biogenesis of mitochondrial biomarkers (PGC-1α and SIRT3) in skeletal muscles and this process is expected to in turn increase cellular longevity especially in slow twitch muscles. However, in term of the capacity of this study, the results could be cited and consistent with the principles of molecular biology, but to develop the idea and draw a comprehensive action plan for increasing cell longevity, extensive research on various organs with the presence of factors other is needed.

APPLICABLE REMARKS

According to the results of this study, people are recommended to use continuous exercise to improve mitochondrial biogenesis and delay cell aging.

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CONFLICT OF INTEREST

The authors have indicated that they have no conflict of interests regarding the content of this article.

REFRENCES

1. Robergs RA, Roberts S. Fundamental principles of exercise physiology: for fitness, performance, and health: McGraw-Hill College; 2000.
2. Brand MD, Buckingham JA, Esteves TC, Green K, Lambert AJ, Miwa S, et al., editors. Mitochondrial superoxide and aging: uncoupling-protein activity and superoxide production. Biochemical Society Symposia; 2004: Portland Press Limited.
3. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem*. 2015;**30**(1):11-26. **doi:** 10.1007/s12291-014-0446-0 **pmid:** 25646037
4. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J*. 2012;**5**(1):9-19. **doi:** 10.1097/WOX.0b013e3182439613 **pmid:** 23268465

5. Pryor WA, Stanley JP, Blair E. Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. *Lipids*. 1976;**11**(5):370-379. **doi:** 10.1007/bf02532843 **pmid:** 1271974
6. Taylor EB, Lamb JD, Hurst RW, Chesser DG, Ellingson WJ, Greenwood LJ, et al. Endurance training increases skeletal muscle LKB1 and PGC-1alpha protein abundance: effects of time and intensity. *Am J Physiol Endocrinol Metab*. 2005;**289**(6):E960-968. **doi:** 10.1152/ajpendo.00237.2005 **pmid:** 16014350
7. Russell AP, Feilchenfeldt J, Schreiber S, Praz M, Crettenand A, Gobelet C, et al. Endurance training in humans leads to fiber type-specific increases in levels of peroxisome proliferator-activated receptor-gamma coactivator-1 and peroxisome proliferator-activated receptor-alpha in skeletal muscle. *Diabetes*. 2003;**52**(12):2874-2881. **doi:** 10.2337/diabetes.52.12.2874 **pmid:** 14633846
8. Coffey VG, Hawley JA. The molecular bases of training adaptation. *Sports Med*. 2007;**37**(9):737-763. **doi:** 10.2165/00007256-200737090-00001 **pmid:** 17722947
9. Rose AJ, Hargreaves M. Exercise increases Ca2+-calmodulin-dependent protein kinase II activity in human skeletal muscle. *J Physiol*. 2003;**553**(Pt 1):303-309. **doi:** 10.1113/jphysiol.2003.054171 **pmid:** 14565989
10. Little JP, Safdar A, Wilkin GP, Tarnopolsky MA, Gibala MJ. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *J Physiol*. 2010;**588**(Pt 6):1011-1022. **doi:** 10.1113/jphysiol.2009.181743 **pmid:** 20100740
11. Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, et al. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature*. 2001;**413**(6852):179-183. **doi:** 10.1038/35093131 **pmid:** 11557984
12. Palacios OM, Carmona JJ, Michan S, Chen KY, Manabe Y, Ward JL, 3rd, et al. Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging (Albany NY)*. 2009;**1**(9):771-783. **doi:** 10.18632/aging.100075 **pmid:** 20157566
13. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell*. 2006;**127**(2):397-408. **doi:** 10.1016/j.cell.2006.09.024 **pmid:** 17055439
14. Steinbacher P, Eckl P. Impact of oxidative stress on exercising skeletal muscle. *Biomolecules*. 2015;**5**(2):356-377. **doi:** 10.3390/biom5020356 **pmid:** 25866921
15. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature*. 2000;**403**(6771):795-800. **doi:** 10.1038/35001622 **pmid:** 10693811
16. Sahlin K, Shabalina IG, Mattsson CM, Bakkman L, Fernstrom M, Rozhdestvenskaya Z, et al. Ultraendurance exercise increases the production of reactive oxygen species in isolated mitochondria from human skeletal muscle. *J Appl Physiol (1985)*. 2010;**108**(4):780-787. **doi:** 10.1152/japplphysiol.00966.2009 **pmid:** 20110545
17. Kincaid B, Bossy-Wetzel E. Forever young: SIRT3 a shield against mitochondrial meltdown, aging, and neurodegeneration. *Front Aging Neurosci*. 2013;**5**:48. **doi:** 10.3389/fnagi.2013.00048 **pmid:** 24046746
18. Mockett RJ, Bayne AC, Kwong LK, Orr WC, Sohal RS. Ectopic expression of catalase in Drosophila mitochondria increases stress resistance but not longevity. *Free Radic Biol Med*. 2003;**34**(2):207-217. **pmid:** 12521602
19. Oh T, Tanaka S, Naka T, Igawa S. Effects of high-intensity swimming training on the bones of ovariectomized rats. *J Exerc Nutrition Biochem*. 2016;**20**(3):39-45. **doi:** 10.20463/jenb.2016.09.20.3.6 **pmid:** 27757386
20. da Rocha GL, Crisp AH, de Oliveira MR, da Silva CA, Silva JO, Duarte AC, et al. Effect of High Intensity Interval and Continuous Swimming Training on Body Mass Adiposity Level and Serum Parameters in High-Fat Diet Fed Rats. *ScientificWorldJournal*. 2016;**2016**:2194120. **doi:** 10.1155/2016/2194120 **pmid:** 26904718
21. Vieira JM, Carvalho FB, Gutierres JM, Soares MSP, Oliveira PS, Rubin MA, et al. Caffeine prevents high-intensity exercise-induced increase in enzymatic antioxidant and Na(+)-K(+)-ATPase activities and reduction of anxiolytic like-behaviour in rats. *Redox Rep*. 2017;**22**(6):493-500. **doi:** 10.1080/13510002.2017.1322739 **pmid:** 28482759

22. Terada S, Tabata I, Higuchi M. Effect of high-intensity intermittent swimming training on fatty acid oxidation enzyme activity in rat skeletal muscle. *Jpn J Physiol*. 2004;**54**(1):47-52. **pmid:** 15040848
23. Carnevali LC, Jr., Eder R, Lira FS, Lima WP, Goncalves DC, Zanchi NE, et al. Effects of high-intensity intermittent training on carnitine palmitoyl transferase activity in the gastrocnemius muscle of rats. *Braz J Med Biol Res*. 2012;**45**(8):777-783. **doi:** 10.1590/s0100-879x2012007500105 **pmid:** 22735180
24. Long YC, Barnes BR, Mahlapuu M, Steiler TL, Martinsson S, Leng Y, et al. Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. *Diabetologia*. 2005;**48**(11):2354-2364. **doi:** 10.1007/s00125-005-1962-5 **pmid:** 16237515
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;**72**:248-254. **doi:** 10.1006/abio.1976.9999 **pmid:** 942051
26. Viña J, Borrás C, Miquel J. Theories of ageing. *Iubmb Life*. 2007;**59**(4-5):249-254.
27. Romano AD, Serviddio G, de Matthaeis A, Bellanti F, Vendemiale G. Oxidative stress and aging. *J Nephrol*. 2010;**23 Suppl 15**:S29-36. **pmid:** 20872368
28. Behnaz F, Soltanpoor P, Teymourian H, Tadayon N, Mohseni GR, Ghasemi M. Sympatholytic and Anti-Inflammatory Effects of Ropivacaine and Bupivacaine After Infraclavicular Block in Arterio Venous Fistula Surgery. *Anesth Pain Med*. 2019;**9**(1):e85704. **doi:** 10.5812/aapm.85704 **pmid:** 30881912
29. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*. 1999;**98**(1):115-124. **doi:** 10.1016/S0092-8674(00)80611-X **pmid:** 10412986
30. Kamei Y, Ohizumi H, Fujitani Y, Nemoto T, Tanaka T, Takahashi N, et al. PPARgamma coactivator 1beta/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity. *Proc Natl Acad Sci U S A*. 2003;**100**(21):12378-12383. **doi:** 10.1073/pnas.2135217100 **pmid:** 14530391
31. Anastasiou D, Krek W. SIRT1: linking adaptive cellular responses to aging-associated changes in organismal physiology. *Physiology (Bethesda)*. 2006;**21**:404-410. **doi:** 10.1152/physiol.00031.2006 **pmid:** 17119153
32. Kong X, Wang R, Xue Y, Liu X, Zhang H, Chen Y, et al. Sirtuin 3, a new target of PGC-1alpha, plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS One*. 2010;**5**(7):e11707. **doi:** 10.1371/journal.pone.0011707 **pmid:** 20661474
33. Hey-Mogensen M, Goncalves RL, Orr AL, Brand MD. Production of superoxide/H2O2 by dihydroorotate dehydrogenase in rat skeletal muscle mitochondria. *Free Radic Biol Med*. 2014;**72**:149-155. **doi:** 10.1016/j.freeradbiomed.2014.04.007 **pmid:** 24746616