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The study of effect of resistance training on plasma S1P level and gene expression of S1P1,2,3 receptors in male Wistar rat

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Aim. **The purpose of present study was to study the effect of 8 weeks resistance training on plasma Sphingosine-1-phosphate (S1P) level and gene expression of S1P receptors in skeletal muscles of male Wistar rat.**
Methods. **In this study 24 (8 week-old) male Wistar rats (190-250 gr) were divided randomly to a control (N.=12) and a training (N.=12) group. Resistance ladder was 1 meter height with 2 cm grid ladder. The content of plasma S1P and relative mRNA expression of S1P receptors were determined by high pressure liquid chromatography (HPLC) and Real-time PCR, respectively.**
Results. **Resistance training increased the content of plasma S1P of exercised group compared to control group (P=0.001). Furthermore, Resistance exercise training increased the gene expression of S1P1 (P=0.001), S1P2 (P=0.000) and S1P3 receptors (P=0.021) in exercised flexor hallucis longus (FHL) compared to control group. In soleus (SOL) muscle, resistance training increased the gene expression of S1P1 (P=0.000), S1P2 (P=0.603) and S1P3 receptors (P=0.009).**
Conclusion. **The key conclusion is that resistance training strongly caused to increase in plasma S1P content and its receptors in skeletal muscles of rat that might indicate to the involvement of S1P signalling in the molecular events controlling adaptations of resistance trained muscles which needs to be elucidated in future studies.**

KEY WORDS: Resistance training - Receptors, lysosphingolipid - Muscle, skeletal.

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Skeletal muscles have the capacity to adapt to altered functional requirements. S1P is a platelet-derived sphingolipid [1] that is abundant in plasma,[2] but recent studies have suggested that red blood cells (RBCs) and other hematopoietic cells are major cellular sources of plasma S1P. It has been shown that the amount of S1P secreted into blood flow increases by platelet activation.[3] Following stimulation of cells with a variety of growth factors (platelet-derived growth factor (PDGF), epidermal growth factor (EGF), Hepatic growth factor (HGF), insulin-like growth factor-1 (IGF-I) and cytokines, SK1 (the enzyme that phosphorylates sphingosine to produce S1P) is activated and translocated from the cytoplasm to the plasma membrane where it appears to be the major source of S1P secreted from cells under these conditions.[4-8]

Early in 1992, Igarashi's group suggested

that S1P could act as an extracellular mediator through putative transmembrane G protein–coupled receptors named as S1P1 (EDG-1), S1P2 (EDG-5), S1P3 (EDG-3), S1P4 (EDG-6), and S1P5 (EDG-8),[9, 10] which have been identified in mammals. S1P receptors couple to different heterotrimeric G proteins including $G_{i,q,12/13}$ [11] and activate phospholipase C (PLC), Ca^{2+}, extracellular signal-regulated kinases 1/2 (ERK1/2), mitogen-activated protein kinase (MAPK), adenylate cyclase (AC), phosphoinositide 3-kinase (PI3K), small GTPases Rac and Rho, protein kinases Akt, c-Jun N-terminal kinase (JNK), phospholipase D (PLD) and other downstream mediators.[11-13] Northern blot analysis suggests the presence of S1P1, S1P2, and S1P3 receptors in rat soleus skeletal muscle, with S1P1 expressed at the highest level.[14]

S1P has some physiological actions on skeletal muscles and other tissues. Recently it was demonstrated that exogenous S1P exerts a protective action during muscle fatigue.[14, 15] SK1 overexpressing cells not only have higher growth rates but are also protected from apoptosis.[16] S1P has an important role in muscular regeneration through regulation of satellite stem cells.[4, 5] Notably, the activity of SK1 and the levels of endogenous S1P were significantly higher in the injured fibers and associated satellite cells, stressing the relevance of SK1/S1P axis in skeletal muscle protection and repair.[17] *In vivo* infusion of the denervated muscles with either S1P or sphingosine has increased the expression level of the two myogenic transcription factors myoD and myogenin.[14] Furthermore, Danieli-Betto *et al.* (2010) showed that exogenous addition of S1P stimulated the growth of regenerating myofibers. These modifications clearly suggest the involvement of S1P signaling in the molecular events controlling the early stages of muscle regrowth of injured muscles. This study indicates the S1P as a novel myogenic factor and the involvement of sphingolipid pathways in muscle growth.[18] S1P acts as strong inducer of the proliferation and myogenic differentiation programs.[19]

Danieli-Betto *et al.* (2005) have shown that acute prolonged exercise increases the content of S1P in the soleus and the red gastrocnemius skeletal muscle fibers.[15] Błachnio-Zabielska *et al.* (2008) illustrated that in the soleus there were no changes in the content of S1P until 90th min of running and at the point of exhaustion it was two folds higher than in the sedentary animals.[20] To the best of our knowledge, the adaptations of plasma S1P and its receptors to prolonged resistance exercise training have not been investigated yet, thus the aim of present research was to study the effect of resistance training on plasma S1P level and gene expression of $S1P_{1,2,3}$ receptors in fast and slow twitch skeletal muscles of male Wistar rat.

Materials and methods

Animals

The investigation was approved by the Ethical Committee for Animal Experiments at Tarbiat Modares University of Iran. In this study 24 eight-week-old male Wistar rats (190-250 g) were obtained from a licensed laboratory animal vender (Pasture institute, Tehran, Iran). All animals were maintained in pairs in an environmentally controlled room at 22 °C under a fixed 12:12-h photoperiod cycle and allowed normal cage activity. The animals were fed standard rat chow and water was available. After a week of acclimation to the animal facility, the rats were assigned randomly to control group (N.=12) and training group (N.=12). The control group rats remained in their cages for the duration of the experiment. Body mass measurements of both groups were monitored weekly and served as a general indicator of the health of the animals.

Expreimental protocol

Resistance ladder was 1 meter height with 2 cm grid ladder with an 85 degree incline. After one week familiarizing with resistance exercise training, exercise started by using

weights (made from lead fishing sinkers) that were attached to the upper portion of base of rat's tail (1~2 cm from the proximal end) with adhesion. The second week began with a load equal to 50% of their individual body mass and was measured and attached to their tails. The load was then increased by 200% of their body mass at the end of 8th week. A successful repetition was when that the animal climbed from the bottom of the rack to the top, within 8 seconds. The rats were placed at the bottom of the ladder and motivated to climb the ladder. The only encouragement necessary during the training period was an occasional hand prod at the base of the animal's tail. We did not use food reward and unnatural incentives such as cold water, forced air or electrical stimulation. The number of climbs (repetitions) performed was 20 repetitions/session. When a rat reached the top of ladder it was placed at the base of the ladder to perform another repetition. After finishing one set (5 repetitions) it was permitted to rest for 2 minutes. The rats performed each set of 5 repetitions with 15s between each repetition. The rats trained 3 days/week (Saturday, Monday, and Wednesday) for 8 weeks. The rats performed a warm-up consisting of two sets of five un-weighted repetitions, with a 3 minutes rest between sets. At the end of each session, the rats completed a cool-down consisting of five un-weighted repetitions with 3 minutes rest between sets.

Surgical procedures

All surgical procedures were performed in a single session. Rats were anesthetized by an intraperitoneal injection of Ketamine (75 mg/kg) and Xylazine (20 mg/kg) after the 8 weeks of resistance training and 48 h after the last exercise bout to avoid the acute effects of training. The flexor hallucis longus (FHL) as fast-twitch muscle and soleus (SOL) as a slow twitch muscle were removed. The muscles were cleaned of any visible adipose tissue, nerves and fascias. The blood samples were centrifuged for 15 min at 4°C and 8000 g to remove the blood cells. Plasma and muscles were frozen in liquid nitrogen and stored in -80 °C for later analysis.

Measurement of content of S1P

S1P and C17-S1P (a 17 carbon analog of S1P as internal standard) were purchased from Avanti Polar Lipids (Alabaster, AL). *O*-phthalaldehyde (OPA) (suitable for HPLC fluorimetric detection) and Alkaline Phosphatase obtained from Sigma-Aldrich. Other solutions such ethanol, Methanol, HPLC-grade water, acetonitrile and β-Mercaptoethanol/2-Mercaptoethanol (2ME) were purchased from Merck (Hohmburg, Germany). Lipid standards were dissolved in methanol and were stored at -20 °C.

The content of S1P present in the chloroform layer was determined by means of high pressure liquid chromatography (HPLC) with fluorescent detection system. According to Min *et al.* (2002) C17-S1P as an internal standard was added to the samples before homogenization and ultrasonication in Ice-cooled. Sphingoid bases were converted to their o-phthalaldehyde derivatives and analyzed on a HPLC system (ProStar, Varian, Inc.), equipped with a fluorescence detector and C18 reversed-phase column (Aligent 1200 series NanoLC). The isocratic eluent composition of acetonitrile: water (9:1 v/v) and a flow rate of 1 ml/min were used. [21]

Gene expression

Total cellular RNA was isolated by homogenizing ~50 mg FHL and soleus muscles by hestle. Tissues were ground into liquid nitrogen and frozen powder was incubated in 1ml Trizol reagent. RNA was quantified spectrophotometrically at a wavelength of 260 nm, 280nm. RNA integrity was assessed by RNA agarose gel electrophoresis followed by visualization of the 18S and 28S ribosomal RNA bands under U.V light. The RNA samples were stored at -80°C until later analysis. 2μg of total RNA was reverse transcribed to synthesize cDNA using a Kit (Fer-

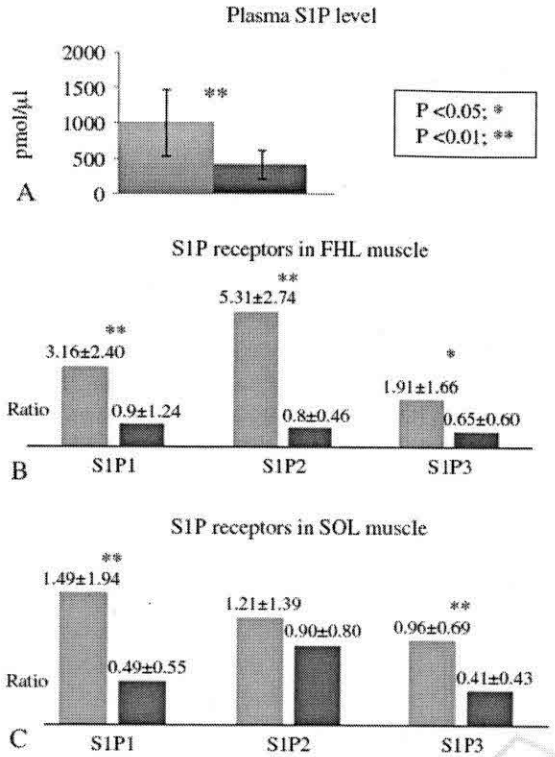

Figure 1.—Effect of 8 weeks of exercise training on plasma S1P content (A), gene expression of S1P1,2,3 receptors in FHL muscle (B), and gene expression of S1P1,2,3 receptors in SOL muscle (C). Values are means ± SD (N.=12 in each group).

mentase). Determination of relative mRNA expression was performed by real-time RT-PCR using a Real-Time PCR Detection System (Applied Biosystems StepOnePlus™ Real-Time PCR System [48 well]). The forward (F) and reverse (R) primer sequences for 18S, S1P1, S1P2, S1P3 were the following:

18S -F: GTTGGTTTTCGGAACTGAGGC, R: GTCGGCATCGTTTATGGTCG (204bp), S1P1 (NM-017301) - F: TCATCGTCCGGCATTACAACTA, R: GAGTGAGCTTGTAGGTGGTG (273bp), S1P2 (NM-017192)-F: CGGAGGCACTGACTAATCAGATT, R: TCCCAGCACTCAGGACACAGTTA (278pb), S1P3 (XM-225216)-F: ACGCGCGCATCTACTTCCT, R: TGGATCTCTCGGAGTTGTGGTT (69bp).

The total volume of the reaction tube was 20 µl; 10 µL SYBR Green Master Mix (primer Design, UK), 1.4 µl forward and reverses primers, 5 µL nuclease-free water and 3.6 µL cDNA template. The amplification profile involved a denaturation step at 95 °C for 15 s, primer annealing at 60 °C for 15 s and 30 s at 72 °C with 40 cycles, and extension at 72 °C for 60 s. To help control for differences in amplification efficiency during thermocycling, all PCR reactions were prepared from the same stock solution. All samples were run in duplicate. Relative fold changes in mRNA were determined by the delta-delta cycle threshold method after normalizing to the internal control gene; 18s was used as a control to normalize the mRNA content of the target genes in each sample; 18s mRNA expression was similar for all tissues, treatment groups, and time points in this study (data not shown).

Statistical analysis

The data was analyzed statistically using the parametric independent t-test. $p<0.05$ was considered significant. The results are presented as means ± standard deviation.

Results

There was no significant difference in pre-training rat body weights in exercise and control, 223.25±11.95 gr and 224.41±15.77 gr, respectively (P=0.840). Furthermore, there wans not significant difference in post-training weights in exercise and control, 285.83±18.50 gr and 280.58±16.20 gr, respectively (P=0.467). Resistance exercise training increased the total content of S1P in plasma (P=0.001) in comparison to control group (Figure 1A). Figure 1B shows that resistance training increases gene expression of S1P1 receptor (P=0.001), S1P2 receptor (P=0.000) and S1P3 receptor (P=0.021) in exercise group compared to control group in FHL muscle. Furthermore, regarding the SOL muscle, resistance training increased gene expression of S1P1 receptor (P=0.000), S1P2 receptor (P=0.603) and S1P3 receptor (P=0.009) in exercise group compared to control group in SOL muscle (Figure 1C).

Discussion

The study demonstrated for the first time that S1P content increased in plasma and gene expression of $S1P_{1,2,3}$ receptors is changed in fast (FHL) and slow (SOL) twitch skeletal muscles after 8 weeks resistance training in male Wistar rat. The results obtained clearly show that resistance exercise training markedly affects the S1P plasma levels in rats.[3] It is evidence-based that physical exercise alters platelet count and platelet functionality [22] through increased plasma levels of norepinephrine and epinephrine, altered response of platelet α_2-adrenergic receptors, and impaired sensitivity of platelets to nitric oxide.[23] Significant increases were shown in adjusted platelet count and plateletcrit in response to resistance exercise.[24] The studies could be interpreted as indicating that elevated IGF and cytokines levels may be a reason of SK1 activation and S1P production.[7, 25] In another study, Formigli *et al.* (2010) illustrate that activity of SK1 and the levels of endogenous S1P were significantly higher in the injured fibers and associated satellite cells. Together, these findings are in favor for a role of S1P in skeletal muscle healing and regeneration.[17] S1P produced intracellularly may liberate at sites of tissue injury and stimulate cellular repair responses.[18] However, no data in the literature are available regarding S1P and resistance training to be compared with these results. But we have compared these results with few studies in which acute and prolonged endurance exercises have been used. It has been shown that the increase the content of S1P in the soleus and the red gastrocnemius skeletal muscle fibers.[20] In a recent study, Błachnio-Zabielska *et al.* (2011) showed that the content of S1P did not change in muscles after five weeks of treadmill endurance training.[26] But, in this study we showed that following 8 weeks resistance training S1P plasma levels of rats increased. In another study, Baranowski and colleague (2011) illustrated that a single bout of endurance training enhanced production and release of S1P by erythrocytes.[27] that it was similar to present study.

Zanin *et al.* (2008) have illustrated that the gene expression of $S1P_{1,3}$ receptors significantly diminishes during denervation in rat soleus muscle.[14] As denervation caused to inactivity and this led to decrease in gene expression of S1P receptors, it might be the case that resistance training which activated the muscles has increased gene expression of these receptors, too. It seems that these findings are in same line with results of present study as one may speculate that S1P might be involved in the process of skeletal muscle adaptation to resistance training.

Conclusions

It is concluded that resistance training strongly affects the synthesis of systemic S1P and gene expression of S1P receptors in skeletal muscles which might be indicative to the involvement of S1P signaling in the molecular events controlling adaptations of resistance trained muscles. In this study, growth factors, cytokines, plateletes and red blood cells were not measured. Future studies should elucidate the effects of all types of exercise training of S1P signalling in human and animal groups.

Riassunto

Effetto dell'allenamento della resistenza sul livello di S1P plasmatica e sull'espressione genica dei recettori S1P1, 2, 3 in ratti Wistar di sesso maschile

Obiettivo. Obiettivo del presente studio è stato quello di esaminare l'effetto di 8 settimane di allenamento della resistenza sul livello plasmatico della sfingosina 1-fosfato (S1P) e sull'espressione genica dei recettori della S1P nei muscoli scheletrici di ratti Wistar di sesso maschile.

Metodi. Nel presente studio, 24 ratti Wistar di sesso maschile (190-250 grammi, 8 settimane di vita) sono stati assegnati in maniera casuale a un gruppo di controllo (N.=12) e a un gruppo sottoposto ad allenamento della resistenza (N.=12). La scala della resistenza misurava 1 metro di altezza con una griglia di 2 cm. Il contenuto di S1P plasmatica e la relativa espressione dell'mRNA dei recettori S1P sono stati determinati rispettivamente mediante cromatografia liquida ad alta pressione (*high pressure liquid chromatography*, HPLC) e PCR in tempo reale.

Risultati. L'allenamento della resistenza ha au-

mentato il contenuto di S1P plasmatica nel gruppo sottoposto ad allenamento rispetto al gruppo di controllo (P=0,001). Inoltre, l'allenamento della resistenza ha aumentato l'espressione genica dei recettori S1P1 (P=0,001), S1P2 (P=0,000) e S1P3 (P=0,021) nel muscolo flessore lungo dell'alluce dei ratti sottoposti ad allenamento della resistenza rispetto al gruppo di controllo. Nel muscolo soleo, l'allenamento della resistenza ha aumentato l'espressione genica dei recettori S1P1 (P=0,000), S1P2 (P=0,603) e S1P3 (P=0,009).

Conclusioni. La conclusione principale è che l'allenamento della resistenza ha causato un forte aumento del contenuto plasmatico di S1P e dei suoi recettori nei muscoli scheletrici dei ratti; ciò potrebbe indicare un coinvolgimento della segnalazione della S1P negli eventi molecolari che controllano gli adattamenti dei muscoli allenati alla resistenza il quale necessita di essere chiarito in futuri studi.

Parole chiave: Esercizio di resistenza - Lisosfingolipidi, ricettori - Muscoli scheletrici.

References

1. Igarashi J, Erwin PA, Dantas APV, Chen H, Michel T. VEGF induces S1P1 receptors in endothelial cells: Implications for cross-talk between sphingolipid and growth factor receptors. Proc Natl Acad Sci U S A 2003;100:10664.
2. Takuwa Y, Okamoto Y, Yoshioka K, Takuwa N. Sphingosine-1-phosphate signaling and biological activities in the cardiovascular system. Biochim Biophys Acta 2008;1781:483-8.
3. Dyatlovitskaya EV, Kandyba AG. Bioeffector Sphingolipids as Stimulators of Cell Growth and Survival. Russ J Bioorg Chem 2004;30:201-6.
4. Nagata Y, Partridge TA, Matsuda R, Zammit PS. Entry of muscle satellite cells into the cell cycle requires sphingolipid signaling. J Cell Biol 2006;174:245.
5. Nagata Y, Kobayashi H, Umeda M, Ohta N, Kawashima S, Zammit PS Sphingomyelin levels in the plasma membrane correlate with the activation state of muscle satellite cells. J Histochem Cytochem 2006;54:375.
6. Bencini C, Squecco R, Piperio C, Formigli L, Meacci E, Nosi D *et al.* Effects of sphingosine 1-phosphate on excitation–contraction coupling in mammalian skeletal muscle. J Muscle Res Cell Motil 2003;24:539-54.
7. Kim RH, Takabe K, Milstien S, Spiegel S. Export and functions of sphingosine-1-phosphate. Biochim Biophys Acta 2009;1791:692-6.
8. Rapizzi E, Taddei ML, Fiaschi T, Donati C, Bruni P, Chiarugi P. Sphingosine 1-phosphate increases glucose uptake through trans-activation of insulin receptor. Cell Mol Life Sci 2009;66:3207-18.
9. Sanchez T, Hla T. Structural and functional characteristics of S1P receptors. J Cell Biochem 2004;92:913-22.
10. Venkataraman K, Lee YM, Michaud J, Thangada S, Ai Y, Bonkovsky HL *et al.* Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. Circ Res 2008;102:669.
11. Graler MH. Targeting sphingosine 1-phosphate (S1P) levels and S1P receptor functions for therapeutic immune interventions. Cell Physiol Biochem 2010;26:79-86.
12. Pebay A, Bonder CS, Pitson SM. Stem cell regulation by lysophospholipids. Prostaglandins Other Lipid Mediat 2007;84:83-97.
13. Serra M, Saba JD. Sphingosine 1-phosphate lyase, a key regulator of sphingosine 1-phosphate signaling and function. Adv Enzyme Regul 2010;50:349.
14. Zanin M, Germinario E, Dalla Libera L, Sandonà D, Sabbadini RA, Betto R *et al.* Trophic action of sphingosine 1-phosphate in denervated rat soleus muscle. Am J Physiol Cell Physiol 2008;294:C36.
15. Danieli-Betto D, Germinario E, Esposito A, Megighian A, Midrio M, Ravara B *et al.* Sphingosine 1-phosphate protects mouse extensor digitorum longus skeletal muscle during fatigue. Am J Physiol Cell Physiol 2005;288:C1367.
16. Lahiri S, Futerman AH. The metabolism and function of sphingolipids and glycosphingolipids. Cell Mol Life Sci 2007;64:2270-84.
17. Sassoli C, Formigli L, Bini F, Tani A, Squecco R, Battistini C *et al.* Skeletal muscle repair/regeneration after eccentric contraction-induced damage: effects of S1P. J Cell Mol Med 2011;15:2498-511.
18. Danieli-Betto D, Peron S, Germinario E, Zanin M, Sorci G, Franzoso S *et al.* Sphingosine 1-phosphate signaling is involved in skeletal muscle regeneration. Am J Physiol Cell Physiol 2010;298:C550-8.
19. Donati C, Bruni P. Sphingosine 1-phosphate regulates cytoskeleton dynamics: implications in its biological response. Biochimica et Biophysica Acta (BBA)-Biomembranes 2006;1758:2037-48.
20. B achnio Zabielska A, Baranowski M, Zabielski P, Górski J. Effect of exercise duration on the key pathways of ceramide metabolism in rat skeletal muscles. J Cell Biochem 2008;105:776-84.
21. Min JK, Yoo HS, Lee EY, Lee WJ, Lee YM. Simultaneous quantitative analysis of sphingoid base 1-phosphates in biological samples by O-phthalaldehyde precolumn derivatization after dephosphorylation with alkaline phosphatase* 1. Anal Biochem 2002;303:167-175.
22. Hilberg T, Glaser D, Koksch M, Schmidt V, Sossdorf M, Gabriel HHW. Differentiation of platelet-leukocyte conjugate formation by short term exercise. Clin Hemorheol Microcirc 2004;31:217-26.
23. Coppola A, Coppola L, dalla Mora L, Limongelli FM, Grassia A, Mastrolorenzo L *et al.* Vigorous exercise acutely changes platelet and B-lymphocyte CD39 expression. J Appl Physiol 2005;98:1414.
24. Ahmadizad S, El-Sayed MS, MacLaren DPM. Responses of platelet activation and function to a single bout of resistance exercise and recovery. Clin Hemorheol Microcirc 2006;35:159-68.
25. Ishii I, Fukushima N, Ye X, Chun J. Lysophospholipid receptors: signaling and biology. Ann Rev Biochem 2004;73:321-354.
26. BÅ‚achnio-Zabielska A, Zabielski P, Baranowski M, Gorski J. Aerobic training in rats increases skeletal muscle sphingomyelinase and serine palmitoyltransferase activity, while decreasing ceramidase activity. Lipids 2011;1-10.
27. Baranowski M, Charmas M, Długołęcka B, Górski J. Exercise increases plasma levels of sphingoid baseâ€ 1 phosphates in humans. Acta Physiol (Oxf) 2011;203:373-80.

Conflicts of interest.—There is no conflict for present study.

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