

Effects of A 4-Week Aerobic Exercise on Lipid Profile and Expression of *LXR α* in Rat Liver

Fatemeh Kazeminasab, M.Sc.¹, Mohammad Marandi, Ph.D.¹, Kamran Ghaedi, Ph.D.^{2*}, Fahimeh Esfarjani, Ph.D.¹, Jamal Moshtaghian, Ph.D.²

1. Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran
2. Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

*Corresponding Address: Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
Email: kamranghaedi@sci.ui.ac.ir

Received: 23/Dec/2015, Accepted: 12/Jun/2016

Abstract

Objective: Liver X receptors (LXRs) are ligand-activated transcription factors of the nuclear hormonal receptor superfamily which modulate the expression of genes involved in cholesterol homeostasis. Hence, further unraveling of the molecular function of this gene may be helpful in preventing cardiovascular diseases.

Materials and Methods: This experimental intervention study included twelve adult Wistar male rats (12-14 weeks old, 200-220 g) which were divided into the control (n=6) and training (n=6) groups. The training group received exercise on a motor-driven treadmill at 28 meters/minute (0% grade) for 60 minutes a day, 5 days a week for 4 weeks. Rats were sacrificed 24 hours after the last session of exercise. A portion of the liver was excised, immediately washed in ice-cold saline and frozen in liquid nitrogen for extraction of total RNA. Plasma was collected for high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglycerides (TG) measurements. All variables were compared by independent t test.

Results: A significant increase in *LXR α* transcript level was observed in trained rats ($P<0.01$). Plasma HDL-C concentration was also significantly higher ($P<0.01$) in trained rats. There was a significant decrease in the concentrations of LDL-C ($P<0.01$) and TC ($P<0.02$), and the ratios of TC/HDL-C ($P<0.001$) and LDL/HDL-C ($P<0.002$) in trained rats. However, the TG concentration was unchanged ($P>0.05$).

Conclusion: We found that endurance training induces significant elevation in *LXR α* gene expression and plasma HDL-C concentration resulting in depletion of the cellular cholesterol. Therefore, it seems that a contributor to the positive effects of exercise in cardiovascular disease prevention is through the expression of *LXR α* , which is a key step in reverse cholesterol transport.

Keywords: Treadmill Exercise, Liver X Receptor α , High-Density Lipoprotein Cholesterol, Rat

Cell Journal (vakhteh), Vol 19, No 1, Apr-Jun (Spring) 2017, Pages: 45-49

Citation: Kazeminasab F, Marandi M, Ghaedi K, Esfarjani F, Moshtaghian J. Effects of a 4-week aerobic exercise on lipid profile and expression of *LXR α* in rat liver. Cell J. 2017; 19(1): 45-49.

Introduction

Coronary artery disease (CAD) is among the greatest causes of morbidity and mortality in most countries and is strongly correlated with increase in plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) concentrations (1-6). Reverse cholesterol transport (RCT) is a complex process resulting in the net movement of cholesterol. In this process, the formation and remodeling of HDL-C in plasma requires several

factors. The ATP-binding cassette transporter A1 (ABCA1), lecithin cholesterol acyltransferase (LCAT), lipoprotein lipase (LPL), hepatic lipase (HL), cholesteryl ester transport protein (CETP) and phospholipid transport protein (PLTP) are among these factors (2, 7, 8).

Liver exerts its lipid-regulating function through numerous receptors. One of these receptors which play a key role in cholesterol metabolism is the liver X receptor (LXR). LXRs are ligand-activated

transcription factors of the nuclear receptor superfamily that are thought to play a role in the regulation of liver lipid metabolism (9). The two LXR subtypes α and β form heterodimers with retinoid X receptor upon activation and bind to the LXR response element found in the promoter region of the target genes (9-12). Both are involved in regulating the expression of genes involved in cholesterol homeostasis (*ABCA1*, *ABCG1*) (10, 13, 14).

LXRs stimulate the reverse cholesterol transport through increasing the expression of *ABCA1* gene and enhancing the availability of extracellular cholesterol acceptors including apolipoprotein E (15, 16). Previous study has have shown that the natural and synthetic agonists of LXRs cause an increase in the expression of *ABCA1* and excretion of cholesterol from the cells. Hence, they may be potential therapeutic agents for preventing atherosclerosis (17).

To the best of our knowledge, effects of regular exercise on blood-lipid and lipoprotein profiles have been well established. Also, exercise has been shown to improve the capacity of cardiovascular function and enhance the reverse cholesterol transport process, resulting in up-regulation of plasma HDL (6, 7, 18, 19). Butcher et al. (20) reported that low intensity exercises (1000-step walking) in 3 sessions per week caused an increase in *LXRα* gene expression in human leukocytes. However, the knowledge about the effect of exercise on the expression of *LXRα* in liver is not established. We thus aimed to investigate the expression of *LXRα* in rat liver along with HDL-C, LDL-C, TG and TC concentrations after 4 weeks of treadmill exercise training.

Materials and Methods

This animal-based experimental intervention study was approved by the Research Committee of the University of Isfahan (Office of Research Affairs) according to the policy of the Ethics Committee of University of Isfahan.

Animals

Twelve male Wistar rats with an estimated weight of 200-220 g were kept under normal light conditions (12 hours light dark cycle), temperature ($23 \pm 1^\circ\text{C}$) and moisture of ($50 \pm 3\%$) in special

cages. The rats were fed a pellet rodent diet ad libitum and had free access to water. The whole process was carried out by the same person. After two weeks of work in the laboratory to minimize the effect of human intervention, animals were randomly assigned to the control (n=6) and training (n=6) groups.

Exercise training protocol

The training program began with adapting rats with the apparatus for 7 days by placing them on a motor-driven treadmill (School of Medicine, Isfahan University of Medical Sciences). The training protocol started with the rats receiving exercise on the treadmill at 16 meters/minute for 15 minutes. One week after the initial stage, the time and speed of running was increased steadily to 60 minutes per day at 23 meters/minute. After this stage, the rats of the training group were put into a progressive exercise. They were put again on a treadmill to run for 60 minutes per day, 5 days a week. During the first week, the speed was set to 20 meters/minute, while for the second and third and fourth weeks it was adjusted to 25, 27 and 29 meters/minute respectively. The angle of inclination was 0° during the whole study period. This condition corresponded to a moderate intensity with about 65% of maximal oxygen consumption (21, 22).

Liver biopsy and blood samples

Twenty-four hours after the last exercise session (fourth week), the rats were anesthetized intraperitoneally with a mixture of ketamine (30-50 mg/kg of body weight) and xylazine (3-5 mg/kg of body weight). After confirming unconsciousness by observing no foot reaction to a physical stimulant, 3 mL of the blood was taken from the right ventricle of each rat and immediately poured into a test tube. The blood samples were centrifuged for 15 minutes at 4000 rpm to separate the blood serum. The obtained sera were kept in a deep freezer (-80°C) for future measurements.

After collecting the blood samples, the abdominal part of the rats were cut and a portion of the liver was excised and washed in ice-cold saline. The

samples were then immediately frozen in liquid nitrogen for total RNA extraction. The frozen liver tissues were kept at -80°C for further experiments.

Lipid and lipoprotein measurements

The concentration of TC, TG and HDL-C were measured in a calibrated biochemical analyzer (Hitachi 902 Automatic analyzer, Japan) in the following manner. TC and TG were measured by assessing the amount of H_2O_2 produced (23). For measurement of the HDL-C content, chemical precipitation of lipoproteins containing apoprotein B was performed using dextran sulfate- Mg^{2+} . HDL-C was then measured by coupling the product of cholesterol oxidase reaction with an indicator reaction as described (24). The amount of LDL-C was calculated based on the values for TC, TG and HDL-C (25).

LXR α transcript level quantification

To extract RNA, 50 mg of the frozen liver tissue was homogenized. Total RNA was isolated by the RNA-Plus kit (CinnaGen, Iran) according to the manufacturer's instructions. Next, the extracted RNA solution was decontaminated from any DNA using RNase free DNaseI (Fermentas, Lithuania). Two μg of RNA from each sample was used for synthesizing the first strand cDNA using cDNA synthesis kit (Fermentas, Lithuania) by utilizing oligodT primers. Real-time (SYBRGreen) polymerase chain reaction (PCR) was carried out in a thermal cycler (Bio-Rad, CA, USA) as suggested by the protocol (TaKaRa, Japan). The PCR mixture contained 10 μL Rotor-Gene SYBR Green PCR Master Mix (TaKaRa, Japan), 3 pM of each primer and 25 ng cDNA in a final volume of 20 μL .

The *LXR α* -specific real-time PCR primers were
 F: 5'-CCTGATGTTTCTCCTGACTC-3'
 R: 5'-TGACTCCAACCCTATCCTTA-3'
 In case of the *Rn β -actin*
 F: 5'-GGAGAAGATTTGGCACCACAC-3'
 R: 5'-GGATGGCTACGTACATGGCTG-3'

All primers were purchased from Metabion (Germany).

Relative mRNA concentrations were calculated from take-off point of reactions (Ct) using the

software provided by the manufacturer and normalized to *β -actin* expression level. All measurements were done in duplicates and data were analyzed according to the $\Delta\Delta\text{Ct}$ method as our previous study (1).

Statistical analysis

All results were expressed as mean \pm SEM (standard error of mean) obtained from three independent replicated observations. Changes in all variables were analyzed by independent t test. All statistical analysis was performed by SPSS (Version 13). $P < 0.01$ were considered significant.

Results

LXR α expression in liver, plasma lipoprotein (HDL-C, LDL-C) and lipid (TC and TG) profiles were examined in rats. *LXR α* expression was significantly increased 2.8 fold ($P < 0.01$) in trained rats (Fig.1). Plasma HDL-C was also significantly higher in trained rats ($P < 0.01$). On the other hand, plasma LDL-C was significantly decreased ($P < 0.01$) in trained rats (Table 1). Plasma TC concentration ($P < 0.02$), and TC/HDL-C ($P < 0.001$) and LDL/HDL-C ($P < 0.002$) ratios significantly decreased in trained rats following the 4-week exercise. However, we observed no significant change in TG concentration ($P < 0.6$) (Table 1).

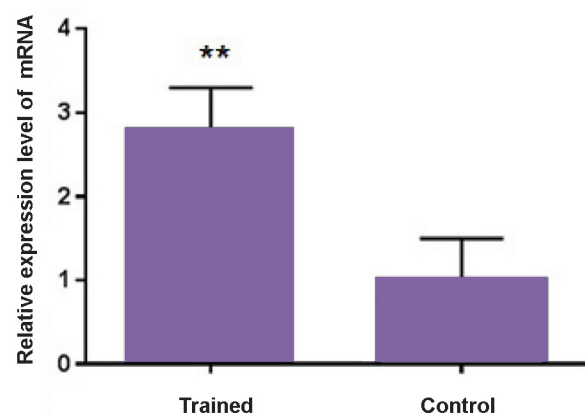


Fig.1: The relative expression level of *LXR α* normalized to *β -actin* gene in trained and control groups. **, Significant difference between trained and control samples at $P < 0.01$.

Table 1: Comparison of lipid and lipoprotein profiles, and TC/HDL-C and LDL/HDL-C ratios between control and trained Wistar male rats

Variables	Training group (n=6)	Control group (n=6)	P value
HDL-C (mg/dL)	39.83 ± 1.4	35.16 ± 0.7	0.01
LDL-C (mg/dL)	6.33 ± 0.8	9.83 ± 0.79	0.01
TG (mg/dL)	50.5 ± 2.3	51.83 ± 2.27	0.6
TC (mg/dL)	47.66 ± 2.18	61 ± 4.36	0.02
TC-HDL-C ratio	1.09 ± 0.06	1.72 ± 0.1	0.001
LDL-HDL-C ratio	0.14 ± 0.01	0.28 ± 0.02	0.002

Data are expressed as mean ± SEM.

HDL-C; High-density lipoprotein cholesterol, LDL-C; Low-density lipoprotein cholesterol, TG; Triglyceride, and TC; Total cholesterol.

Discussion

The major finding of the present study is the significant increase in *LXRα* expression level as a result of performing endurance training. Despite the importance of *LXRα* in cholesterol metabolism, studies have rarely investigated the effect of exercise and exercise training on *LXRα* transcript level. A similar study which analyzed the effect of physical activity on *LXR* reported that low intensity activity (walking 10,000 steps, 3 times a week) resulted in a significant upregulation in *LXR* expression in human leukocytes (20). Baranowski et al. (26) demonstrated that the activation of *LXRα* increased fatty acid utilization during exercise and prevented fatigue caused by glucose insufficiency.

According to Khabazian et al. (27), hepatic *ABCA1* expression was increased in liver after a 6-week exercise on a treadmill with a speed of 26 meters/minute (0° slope) for 60 minutes a day, 5 days a week. Similarly, Ghanbari-Niaki et al. (2) reported increased *ABCA1* expression in rats that had exercised on a treadmill for 5 days per week for 6 weeks with a speed of 25 meters/minute and 90 minutes per session. More recently, Kazeminasab et al. (1) showed that liver *LXRα* and *ABCA1* expression was up-regulated in rats trained for a prolonged period of exercise during 8 weeks. All studies therefore consistently suggest that regular endurance exercise can cause an enhancement in liver *ABCA1* gene expression in rats.

The higher plasma HDL-C levels observed in this study could be explained by exercise training-induced changes in several parameters

of HDL-C metabolism and HDL-C remodeling factors including LCAT, HL, CETP, LPL (28, 29) and *ABCA1* (7). Our results are not in line with those by previous studies which reported reduced HDL levels after an exercise activity (29, 30). Although a few studies have shown that exercise activity has no significant effect on HDL levels, the majority of the studies have reported an increase in HDL level after performing exercise activities (31, 32).

We also observed a significant reduction in LDL-C and TC following an exercise session which were in good agreement with previous studies (33, 34) but not all (27, 35).

Conclusion

We show that treadmill running induces higher plasma HDL-C levels due to the higher *ABCA1* expression in liver, a key element in the RCT process which is in turn due to the up-regulation of *LXRα*. Further investigation is required to clarify the effect of different intensities and various modes of exercise on liver *LXRα* expression.

Acknowledgments

This study was funded by a grant in aid of research from the Chancellorship Research and Technology Office of The University of Isfahan (Grant no.90/97891). None of the authors has any conflicts of interest to disclose.

References

1. Kazeminasab F, Marandi M, Ghaedi K, Esfarjani F, Moshtagian J. Endurance training enhances *LXRα* gene expression in Wistar male rats. *Eur J Appl Physiol.* 2013;

- 113(9): 2285-2290.
2. Ghanbari-Niaki A, Khabazian BM, Hossaini-Kakhak SA, Rahbarizadeh F, Hedayati M. Treadmill exercise enhance ABCA1 in rat liver. *Biochem Biophys Res Commun.* 2007; 361(4): 841-846.
 3. Abbott RD, Wilson PW, Kannel WB, Castelli WP. High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction. The framingham study. *Arteriosclerosis.* 1988; 8(3): 207-211.
 4. Boden WE. High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the veterans affairs high-density lipoprotein intervention trial. *Am J Cardiol.* 2000; 86(12A): 19L-22L.
 5. Morgan J, Carey C, Lincoff A, Capuzzi D. High-density lipoprotein subfractions and risk of coronary artery disease. *Curr Atheroscler Rep.* 2004; 6(5): 359-365.
 6. Khabazian BM, Ghanbari-Niaki A, Safarzadeh-Golpordeh-sari Ar, Ebrahimi M, Rahbarizadeh F, Abednazari H. Endurance training enhances ABCA1 expression in rat small intestine. *Eur J Appl Physiol.* 2009; 107(3): 351-358.
 7. Ghanbari-Niaki A. Treadmill exercise training enhances ATP-binding cassette protein-A1(ABCA1) expression in male rats' heart and gastrocnemius muscles. *Int J Endocrinol Metab.* 2010; 8(4): 206-210.
 8. Lee JY, Parks JS. ATP-binding cassette transporter A1 and its role in HDL formation. *Curr Opin Lipidol.* 2005; 16(1): 19-25.
 9. Baranowski M. Biological role of liver X receptors. *J Physiol Pharmacol.* 2008; 59 Suppl 7: 31-55.
 10. Wójcicka G, Jamroz-Wiśniewska A, Horoszewicz K, Bełtowski J. Liver X receptors (LXRs). Part I: structure, function, regulation of activity, and role in lipid metabolism. *Postepy Hig Med Dosw (Online).* 2007; 61: 736-759.
 11. Zhao C, Dahlman-Wright K. Liver X receptor in cholesterol metabolism. *J Endocrinol.* 2010; 204(3): 233-240.
 12. Jamroz-Wiśniewska A, Wójcicka G, Horoszewicz K, Bełtowski J. Liver X receptors (LXRs). Part II: non-lipid effects, role in pathology, and therapeutic implications. *Postepy Hig Med Dosw (Online).* 2007; 61: 760-785.
 13. Bełtowski J, Semczuk A. Liver X receptor (LXR) and the reproductive system—a potential novel target for therapeutic intervention. *Pharmacol Rep.* 2010; 62(1): 15-27.
 14. Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, et al. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem.* 1997; 272(6): 3137-3140.
 15. Sato M, Kawata Y, Erami K, Ikeda I, Imaizumi K. LXR agonist increases the lymph HDL transport in rats by promoting reciprocally intestinal ABCA1 and apo A-I mRNA levels. *Lipids.* 2008; 43(2): 125-131.
 16. Wouters K, Shiri-Sverdlov R, van Gorp PJ, van Bilsen M, Hofker MH. Understanding hyperlipidemia and atherosclerosis: lessons from genetically modified apoe and ldlr mice. *Clin Chem Lab Med.* 2005; 43(5): 470-479.
 17. Zhao SP, Yu BL, Xie XZ, Dong SZ, Dong J. Dual effects of oxidized low-density lipoprotein on LXR-ABCA1-apoA-I pathway in 3T3-L1 cells. *Int J Cardiol.* 2008; 128(1): 42-47.
 18. Long X, Bratz IN, Alloosh M, Edwards JM, Sturek M. Short-term exercise training prevents micro- and macrovascular disease following coronary stenting. *J Appl Physiol (1985).* 2010; 108(6): 1766-1774.
 19. Wilund KR, Feeney LA, Tomayko EJ, Weiss EP, Hagberg JM. Effects of endurance exercise training on markers of cholesterol absorption and synthesis. *Physiol Res.* 2009; 58(4): 545-552.
 20. Butcher LR, Thomas A, Backx K, Roberts A, Webb R, Morris K. Low-intensity exercise exerts beneficial effects on plasma lipids via PPARgamma. *Med Sci Sports Exerc.* 2008; 40(7): 1263-1270.
 21. Powers SK, Criswell D, Lawler J, Martin D, Lieu FK, Ji LL, et al. Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium. *Am J Physiol.* 1993; 265(6 Pt 2): H2094-2098.
 22. Vincent HK, Powers SK, Stewart DJ, Demirel HA, Shanely RA, Naito H. Short term exercise training improves diaphragm antioxidant capacity and endurance. *Eur J Appl Physiol.* 2000; 81(1-2): 67-74.
 23. Parakh AC, Gank DH. Free and total cholesterol. In: Bauer JD, editor. *Clinical laboratory methods.* Toronto: Mosby Company; 1982; 546-549.
 24. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg²⁺-precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem.* 1982; 28: 1379-1388.
 25. Demacker PN, Hijmans AG, Breninkmeijer BJ, Jansen AP, van 't Laar A. Five methods for determining low-density lipoprotein cholesterol compared. *Clin Chem.* 1984; 30(11): 1797-1800.
 26. Baranowski M, Zabielski P, Błachnio-Zabielska AU, Hara-siuk D, Górski J. LXR activation prevents exhaustive exercise-induced hypoglycaemia and spares muscle glycogen but does not enhance running endurance in untrained rats. *Acta Physiol (Oxf).* 2011; 201(3): 373-379.
 27. Khabazian BM, Ghanbari Niaki A, Rahbarizadeh F, Hossaini Kakhak SER, Jabari Noghbi M. The effect of 6 weeks endurance training of hepatic ABCA1 in male wistar rats. *Research on Sport Sciences.* 2008; 6(18): 101-114.
 28. Kantor MA, Cullinane EM, Herbert PN, Thompson PD. Acute increase in lipoprotein lipase following prolonged exercise. *Metabolism.* 1984; 33(5): 454-457.
 29. Seelbach JD, Kris-Etherton PM. The effect of vigorous treadmill exercise on plasma lipoproteins and hepatic lipoprotein production in Zucker rats. *Atherosclerosis.* 1985; 57(1): 53-64.
 30. Pels AE 3rd, White TP, Block WD. Effects of exercise training on plasma lipids and lipoproteins in rats. *J Appl Physiol (1985).* 1985; 58(2): 612-618.
 31. Couillard C, Després JP, Lamarche B, Bergeron J, Gagnon J, Leon AS, et al. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the health, risk factors, exercise training and genetics (HERITAGE) family study. *Arterioscler Thromb Vasc Biol.* 2001; 21(7): 1226-1232.
 32. Dabidi Roshan V. Effect of training on high sensitive C-reactive protein and blood lipids responses in rats. *Middle-East J Sci Res.* 2011; 9(1): 115-122.
 33. Karanth J, Jeevaratnam K. Effect of dietary lipid, carnitin and exercise on lipid profile in rat blood, liver and muscle. *Indian J Exp Biol.* 2009; 47(9): 748-753.
 34. Petridou A, Nikolaidis MG, Matsakas A, Schulz T, Michna H, Mougios V. Effect of exercise training on the fatty acid composition of lipid classes in rat liver, skeletal muscle, and adipose tissue. *Eur J Appl Physiol.* 2005; 94(1-2): 84-92.
 35. Laaksonen DE, Atalay M, Niskanen LK, Mustonen J, Sen CK, Lakka TA, et al. Aerobic exercise and the lipid profile in type 1 diabetic men: a randomized controlled trial. *Med Sci Sports Exerc.* 2000; 32(9): 1541-1548.