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Treadmill exercise training enhances ATP-binding cassette protein-A1 (ABCA1) expression in male rats' heart and gastrocnemius muscles

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ABSTRACT

Background: Heart and gastrocnemius muscles function as highly oxidative and fast oxidative-glycolytic tissues and consume lipid and other metabolites at rest and during exercise. Exercise at low to moderate intensity increases fat mobilization and lipid metabolism, and several genes are involved in lipid and cholesterol metabolism (e.g. membrane-associated fatty acid transport proteins and ATP-binding cassette transporters [ABC] from adipose and other tissues).
Objectives: The purposes of the current study were to investigate the effect of chronic physical exercise training on (a) heart- and gastrocnemius-muscle ABCA1 expression and (b) plasma apolipoprotein-A (apo-A) and pre-β-HDL concentrations.
Materials and Methods: 10 adult male Wistar rats (12 to 14 weeks old, 200 to 220 g) were divided into control (n = 5) and training (n = 5) groups. The training group was exercised on a motor-driven treadmill at 25 m/min (0% grade) for 60 min/day, 5 days/week, for 12 weeks. Rats were sacrificed 48 h after the last exercise session. A portion of the heart and gastrocnemius muscles were excised, immediately cleaned, washed in ice-cold saline, and frozen in liquid nitrogen for extraction of ABCA1 mRNA. Unpaired t student tests were used to analyze the data.
Results: Higher and significant ABCA1 expression, plasma apo-A, and pre-β-HDL concentrations were found in the trained rats than in the control group.
Conclusions: The results indicate that exercise-training-induced ABCA1 expression in heart and gastrocnemius muscles was accompanied with higher plasma apo-A and pre-β-HDL concentrations. This might reinforce the initiation of reverse cholesterol transport process.

▸ *Implication for health policy/practice/research/medical education:*
The role of ABCA1 as a key element an initiator of reverse cholesterol transport (RCT) process and important factor in HDL formation has been well established. The results of the current article might be useful for cardiologist, researchers in lipid metabolism , dysfunction and obesity area, epidemiologist, pharmacologist, athletes and who want to have healthy life style.

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1. Background

The effects of regular exercise on blood-lipid and lipoprotein profiles, improvement of cardiovascular function, prevention of atherosclerosis, and enhancement of reverse cholesterol transport process and its elements are well established (1-5). The formation of high-density lipoprotein HDL as a heart disease predictor and antirisk factor as well as an antioxidant is also well known (6-8). The remodeling of HDL by plasma elements is a complex process that requires several factors, such as lipoprotein lipase (LPL), hepatic lipase (HL), lecithin-cholesterol ac-

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yltransferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transport protein (PLTP), and ATP-binding cassette transporters (ABC), particularly ABCA1 (8, 9). HDL assembly initially involves the cell surface ABCA1, a member of the ABC super family. ABCA1 transports cellular phospholipides (PL) and cholesterol to extracellular lipid-poor apolipoprotein-A (apo-A-1), and this is followed by the remodeling in the plasma compartment of HDL by specific plasma factors (8, 10, 11). ABCA1 is a ubiquitous protein expressed abundantly in the liver, brain macrophages, heart, skeletal muscles, and other tissues in humans and other animals (12-16). Data regarding ABCA1 expression and exercise are very sparse, and only very few studies have focused on the effects of physical activity on ABCA1 expression in leukocytes, vastus lateralis muscles, the liver, and the small intestine in human and animal subjects (1, 17, 18).

2.Objectives

It is well known that cardiac and gastrocnemius muscles are both metabolic organs with different functions and fuel consumption at rest. However, during exercise these muscles consume somewhat similar fuel sources. Thus, after our research on the expression of ABCA1 in the liver and small intestine as a main source of HDL secretion, I aimed to examine whether exercise-induced ABCA1 expression would be a key and initial element in the reverse cholesterol transport (RCT) process in these metabolic organs would be accompanied with possible changes in other plasma factors that are involved in plasma HDL remodeling. In addition, knowledge concerning the effects of exercise training on ABCA1 expression in heart and gastrocnemius muscle tissues is also lacking. Thus, this study was conducted to investigate the effects of a 12-week treadmill exercise-training regimen on ABCA1 expression on the heart and gastrocnemius muscle tissues and to determine whether a possible exercise-induced change in ABCA1 expression would be accompanied with changes in plasma apo-A and pre-β-HDL concentrations in male rats.

3. Materials and Methods

3.1. Animal Experiments

All experiments involving the animals were conducted according to the policy of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. The protocol was approved by the Ethics Committee of the School of Medical Sciences, Tarbiat Modares University (TMU), Tehran, Iran. Ten male Wistar rats (12 to 14 weeks old, 180 to 210 g) were acquired from Pasteur's Institute (Tehran, Iran) and kept in the Central Animal House, School of Medical Sciences, TMU. The animals were housed 5 per cage (46 L in volume, 24.5 × 15 × 8 in) with a 12 hour, 12 hour light–dark cycle. Temperature and humidity were maintained at 22°C ± 1.4°C and 55.6% ± 4.0%, respectively. The rats were provided food (a pellet rodent diet) and water ad libitum. The rats were randomly assigned to training groups (n = 5) and control (n = 5). Treadmill training began with familiarization of rats with the apparatus for 4 days by placing them on the motorized-driven treadmill that was located at the same place as animals were hosted. Strewment was changed every 4 days, and the same person handled and exercised the rats throughout the study.

3.2. Exercise training protocol

First, the animals were familiarized with the rat treadmill apparatus each day for 4 days (the 14-lane motorized-driven treadmill was designed by the primary author; TMU, Tehran, Iran). After a progressive-overload period for 10 days, the exercise group was trained for the next 12 weeks using the same training methods previously described.5,18 (1,17). The rats ran on a treadmill at 25 m/min for 60 minutes, 5 d/wk for the designated number of weeks. The animals were killed 48 hours after the last exercise session. Food but not water was removed from the rat cages 4 hours before tissue harvesting.

3.3. Tissue biopsies

Thirty-seven hours after the last training session, rats were anesthetized with intraperitoneal administration of a mixture of ketamine (30 to 50 mg/kg body weight) and xylazine (3 to 5 mg/kg body weight). Afterwards, the heart and gastrocnemius muscles were excised, cleaned, divided into 2 pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen for extraction of ABCA1 mRNA. The frozen heart and gastrocnemius muscles were stored at −80°C until the analyses were performed.

3.4. ABCA1 expression

The quick-frozen heart and gastrocnemius muscle samples were powdered with a cold mortar and pestle, and approximately 50 mg was used to isolate the RNA. Total RNA was extracted by the guanidine thiocyanate method.19 Relative expression levels of ABCA1 mRNA in the heart and gastrocnemius were determined using a semiquantitative PCR method, which has been described in detail elsewhere (1, 8). All reactions were repeated a minimum of three times to ensure repeatability. All PCR products were electrophoresed on an agarose gel and bands were visualized by ethidium bromide staining and quantified by computer integrated densitometry (Eastman Kodak, New Haven, CT, USA). Levels of mRNA were expressed as a ratio of signal intensity for the ß-actin gene. Optical density at a 260/280 ratio (spectrometric ratio, Eppendorf equipment) was employed as a tool for the assessment of the quality of the extracted RNA, and the cDNA was synthesized only for the sample and

had a 260/280 ratio between 1.85 and 2.

3.5. Plasma apo-A and Pre-β-HDL concentrations

Plasma pre-β-HDL was separated according to Khabazian *et al.* (1) agarose electrophoresis method, and the assays of apo-A1 in the pre-β-HDL band were conducted using a Rat Apo-A1 Enzyme immunoassay kit (Wuhan USCN Sciences Co. Ltd., Wuhan, China).

3.6. Statistics

The Kolmogorov-Smirnov test was used to determine the normality of the distribution, and variables were found to be normally distributed. All data are expressed as means ± SE. All variables were compared with unpaired t tests. All statistical analyses were performed in SPSS (version 13). All *P* values < .05 were considered significant.

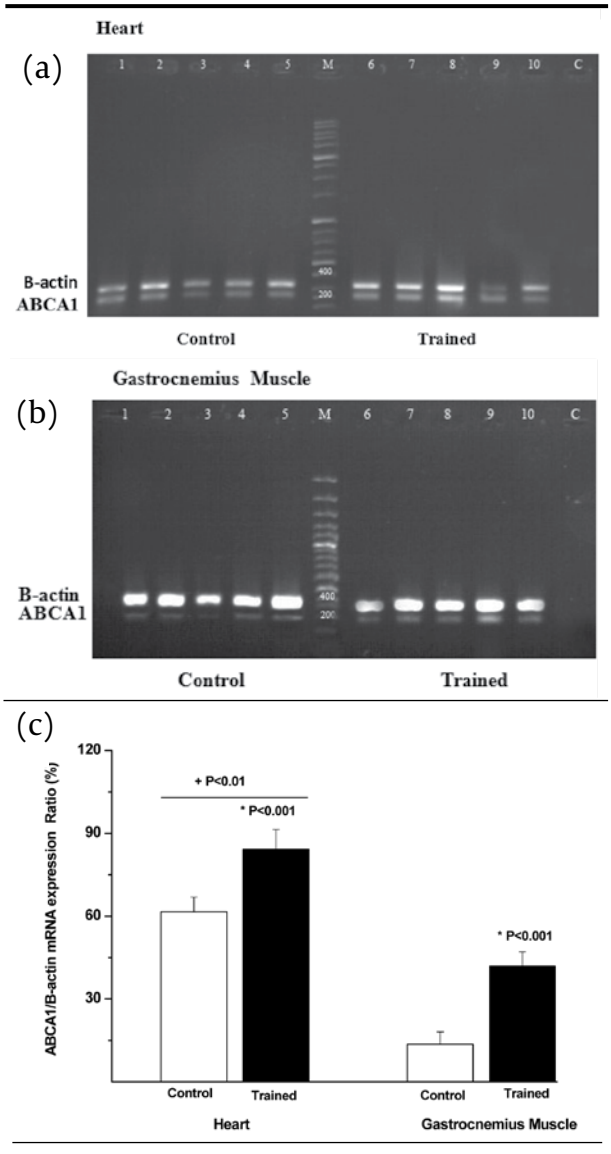

Figure 1. Semiquantitative RT-PCT of heart (a) and gastrocnemius (b) muscles' ABCA1 and β-actin mRNA expression in control and trained wild male rats and ABCA1/ B-actin mRNA expression ratio (%) in the heart and gastrocnemius muscles in control and trained wild-type male rats (c).

Trained vs. control rats (5 rats per group) and Heart vs. gastrocnemius muscle. Each column represents each group (5 rats per group). Data are expressed as means ± SE.

4. Results

Heart ABCA1 expression was significantly (P < .001) higher in the trained rats than in the control rats *(Figure 1a)*. A higher and significant ABCA1 expression was also observed in the trained rats' gastrocnemius muscles *(Figure 1a & 1b)*. The data also revealed that ABCA1 expression was significantly higher in the control and trained rats' heart muscles when compared with the control and trained rats' gastrocnemius muscles *(Figure 1c)*. Resting plasma apolipoprotein-A (apo-A; *Figure 2a*) and pre-β-HDL concentrations were significantly higher in the trained rats than in the control group *(Figure 2b)*.

5. Discussion

The major findings of the present study are that exercise training significantly increased the expression of ABCA1 in rats heart and gastrocnemius muscles, which were accompanied by significant changes in plasma apo-A and pre-β-HDL concentrations in trained male rats. The current study is the first to determine the effects of long-term exercise (12 weeks) on heart and gastrocnemius-muscle ABCA1 expression. Considering the level of ABCA1 expression in the heart and gastrocnemius muscles, the present data confirm previous findings in some cattle and tree-shrew tissues (12, 16) and did not support other reports by investigators who used real-time PCR techniques and human and bovine tissues (12, 13, 15). For instance, Willington *et al.* (15) used reverse transcriptase-polymerase chain reaction (RT-PCR), and on the basis of the relative abundance of ABCA1 mRNA in murine tissues, the investigators divided tissues into three groups. The highest ABCA1 mRNA expressions were observed in the liver, kidney, adrenal, heart, bladder, testis, and brain tissues. Moderate expression was observed in the lung, adipose, esophagus, stomach, and small-intestine tissues. Large intestine, skeletal muscle, thymus, and spleen tissues consistently revealed relatively low levels of ABCA1 mRNA (15). Zeng *et al.* (16) reported that the patterns of real-time PCR for testing the relative expression of ABCA1 mRNA in various tissues of three tree shrews indicated that the highest expression was observed in the lung tissue, followed by the liver, kidney, and spleen tissues. Medium expression levels were observed in the cardiac muscle and gall bladder, with low concentrations in the adipose tissue, testis, brain, and intestine, and no expression was observed in the skeletal muscles. Regarding changes in ABCA1 expression, apo-A, and pre-β-HDL with exercise training, the current findings are consistent with the three existing studies (1, 8, 19) in this area.

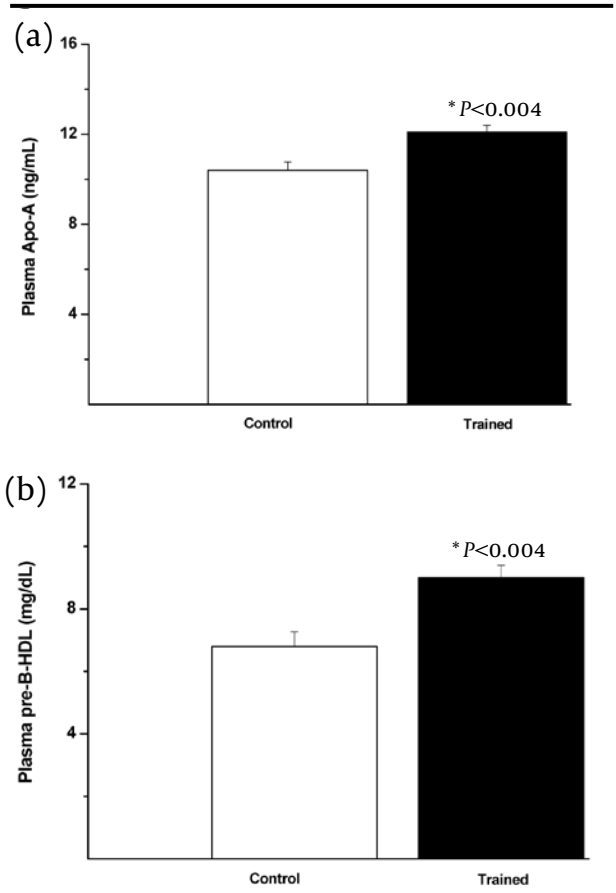

Figure 2. (a) Plasma apo-A concentration in control and trained wild male rats, and (b) pre-β-HDL concentration in control and trained wild male rats.

* Trained vs. control rats (5 rats per group)
Data expressed as means ± SE.

Wellington *et al.* (15) classified tissue distribution of murine ABCA1 mRNA by using the ratio of ABCA1 mRNA to 18s rRNA (using the maximal concentration of 18s rRNA competimer) as determined by RT-PCR and then by densitometric quantitation of band intensity on an ethidium bromide-stained agarose gel and also used Western blot techniques. They divided the relative ABCA1 abundance into three groups—high, moderate, and low—and reported that heart and muscle were classified into the high and low groups, respectively. Ghanbari-Niaki *et al.* (17) reported that treadmill running for 6 weeks enhanced ABCA1 expression in rat livers. A similar result was reported in rat livers and small intestines by Khabazian *et al.* (1). Ghanbari-Niaki *et al.* (17) reported that a single session of circuit resistance exercise at different intensities increased peripheral blood lymphocyte ABCA1 expression in young female college students. The mechanism(s) by which the exercise training can influence ABCA1 mRNA expression in heart and gastrocnemius muscles is poorly understood. The literature has established that PPAR is a nuclear receptor similar to the liver X receptor (LXR) and retinoid X receptor (RXR) that regulates the expression of genes controlling lipid and glucose metabolism (20) Three PPAR isoforms (α, β/δ, γ) are widely expressed in metabolic tissues including the heart, liver, skeletal muscle, and kidneys (21, 22). PPAR agonists' four fibrates (fenofibrate, bezafibrate, gemfibrozil, and LY518674) have been shown to regulate ABCA1 expression and HDL biogenesis (23, 24). On the other hand, the effect of exercise training on PPAR mRNA expression has been taken into consideration by several researchers (25-28). Fatone *et al.* (25) reported that 2 sessions per week of combined aerobic exercise (at 55 to 70% of maximal oxygen uptake) and resistance circuit training (at 60 to 80% of 1 repetition maximum) resulted in a significant increase in PPAR-α after 6 and 12 months, whereas PPAR-γ increased only after 6 months. Spangenburg *et al.* (28) suggested that acute exercise and training for 12 weeks resulted in a greater degree of PPAR mRNA expression in the gastrocnemius and plantaris muscles, but PPAR-γ mRNA levels were lowest in the skeletal muscles. Butcher *et al.* (29) reported that low-intensity activity (walking 10,000 steps 3 times/week) resulted in significant changes in LXR receptor-gene expression in human leukocytes. Changes in plasma and tissue adiponectin concentrations and expression following an exercise training program was considered to be an effective factor for regulation of ABCA1 expression (1, 30, 31). It also possible that the heart and gastrocnemius muscles, as the most important tissues in the overall uptake of both apo-A-I and cholesteryl ethers, as well as lipoprotein lipase activity, might be involved in cholesterol metabolism (32-35). In summary, this is the first study to demonstrate the effect of exercise training on ABCA1 expression of the heart and gastrocnemius muscles. The present study also clearly shows that treadmill exercise induced a more pronounced increase in gastrocnemius ABCA1 mRNA expression than in heart tissue. The expression of ABCA1 in heart and gastrocnemius muscle might have protective effects on the tissue itself and reinforce the liver and small intestine by initiating the RCT process and HDL formation in the plasma. Further investigation of the effects of exercise training on the expression and concentration of PPAR isoforms in heart and skeletal muscle is warranted.

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Conflict of interest

None declared.

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