The Effects of Eight Weeks Interval Endurance Robe Training on Lymphocyte ABCA1 Protein Expression , Plasma Apolipoprotein A-I and HDL-cin Overweight and Obese Boy Adolescents

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ABSTRACT

Early obesity and its transfer to the adulthood, increases likelihood incidence of coronary artery disease (CAD). ATP-binding cassette transporter (ABCA1) as a member of the ABC transporters family plays a crucial role in reverse cholesterol transport and CAD prevention. The purpose of this study was to investigate ABCA1 expression in lymphocytes, plasma apolipoprotein A-I and HDL-C in response to eight weeks interval aerobic endurance exercise (IERT) in overweight and obese boy adolescents(OAOBA). Thirty students (17.3±1.1yr, 85.73± 11.68 kg and 28.41±2.36 kg / m²) volunteered and randomly were assigned into training(n = 15) and control (n =15) groups . Exercise protocol was IERT(8 wk, 4 d/wk and 40 min/d).Cell hemolysis and sensitive Elisa method was used for Lymphocyte ABAC1 protein expression.T-student test was employed and data were analyzed by SPSS software (version 16). The independent-samples T-Test results were showed that after 8 weeks IERT, the levels of lymphocyte ABCA1 expression(p=0/000) and VO2max(p=0/000) were significantly increased and plasma levels of TG(p=0.017), TC(p=0.001), LDL-c/HDL-c(p=0.026),TC/HDL-c(p=0.002) and measures of BF%(p=0/015)and BMI(p=0.042) as anthropometric indicators were significantly decreased . Changes of other variables such increasing in Apo A-I, HDL-c, LDL-c and decreasing in body weight, were not significant. (p<0.05)
The findings of this study proved that 8-week IERT can be have positive effects on lymphocyte ABCA1 protein expression(as goalkeeper of reverse cholesterol process)and lipid profiles and ultimately in prevention of arteriosclerosis among OAOBA.

Keywords: ABCA1, ApoA-I, HDL-C, interval endurance training, overweight and obese boy adolescents
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INTRODUCTION

The prevalence of obesity is increasing at an alarming rate [1,3]. This phenomenon extends to children and adolescents in all countries of the industrialized world (3). Iran is also a growing trend in the prevalence of obesity and overweight [2].

Studies show that early obesity leads to increase likelihood of obesity in adulthood and will result to increasing prevalence of obesity-related diseases such as coronary artery disease (CAD), insulin resistance, diabetes, high blood pressure, arthritis, cancer, stroke and heart failure in future(4,6) . According to the study of Friedman et al [5],

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in 58% obese adolescents and children 5-17 years under study there are at least one of the cardiovascular risk factors in adulthood. Furthermore, the findings a long investigation that led to 57 years has shown, adults who are overweight in childhood and adolescence, 2 to 3 times more than others are at risk of dying from cardiovascular disease. [6]

Numerous population studies have shown an inverse correlation between plasma high density lipoprotein (HDL) levels and risk for CVD, implying that factors associated with HDL protect against atherosclerosis. Some of these factors appear to have antioxidant and anti-inflammatory effects [7], which may ablate processes that initiate atherogenesis. The most widely accepted view, however, is that HDL is atheroprotective because of its role in reverse cholesterol transport (RCT) [8].

Reverse cholesterol transport is a metabolic pathway where by excess cholesterol in peripheral tissues is transported to the liver for elimination from the body [9].ATP-binding cassette transporter A1(ABCA1)that widely expressed in macrophages, liver, small intestine, adrenal glands, endothelial cells and placental trophoblastic [10,5] and the liver producer lipid-poor apolipoprotein (apo)A-I that circulates to peripheral cells and picks up cholesterol and phospholipids, are plays a crucial role in plasma HDL-C metabolism and reverse cholesterol. ABC transporters are a superfamily of proteins that use ATP as a source of energy to transport substrates between different cellular compartments and from the cell [11, 12].

By a complex series of steps involving acquisition of more lipids and proteins and esterification of cholesterol, this partially lipidated apoA-I matures into spherical particles that represent the bulk of HDL. These particles are processed and remodeled by the combined actions of cholesteryl ester transfer protein, phospholipid transfer protein, scavenger receptor B1, and hepatic lipase, which transfer HDL cholesteryl esters to other lipoproteins and cells and regenerate lipid-poor apoA-I. The gatekeeper of this reverse cholesterol transport pathway is an ABCA1 [13–14].
It is believed that an increase in liver and small intestine apolipoprotein-A (Apo-A) release and higher ABCA1 expression in macrophage have strong impact on reverse cholesterol transport (RCT) process, plasma HDL-C formation and protect against atherosclerosis [15-16].

Also it has been suggested that ABCA1 is upregulated by several factors such as cholesterol influx, nutritional status, plasma glucose concentrations and physical activity [17-18].

Review the few existing studies on human samples such Butcher et al [19], the effect of a low-intensity exercise training (8 weeks of low-intensity exercise program consisting of walking 10,000 steps, three times a week) on lymphocyte ABCA1 and ABCG1, Ghanbari-Niaki et al, [20] the effect of a single circuit resistance exercise (9 exercises, 25 s per exercise, 3 sets of 3 nonstop circuits and 1 min. rest interval between the sets) at three given intensities (40%, 60%, 80% one-repetition maximum) and Rashidlamir et al[21],the effect of eight weeks of wrestling and wrestling technique based circuit training (two sessions/day, morning and evening) on lymphocyte ABCA1 gene expression and plasma apolipoprotein A-I, reflects the positive effect of exercise on ABCA1 expression and improving the RCT.

Due to lack of sufficient activity in among children and adolescents, and prevalence of early obesity increases likelihood of obesity-related diseases particularly CAD in adulthood, and since the ABCA1has an important role in RCT and subsequent prevention of CAD, in this study, researcher and colleagues intend to explore the effects of eight weeks IERT program on lymphocyte ABCA1 protein expression, plasma apolipoprotein A-I and lipid profilesin overweight and obese boy adolescents. Doing it for the first time in young human samples with the characteristics of obese and overweight, and using a unique exercise protocol that is implemented with advantages such as simplicity, low cost and being carried out interval are considered the importance of this research.

MATERIALS AND METHODS

Participants: Thirty obese and overweight (age 14-17 yr, 85.73 ± 11.68 kg , 28.41 ± 2.36 kg / m²) male adolescents were recruited from a high school in Takap City (West Azarbaijan Province, Iran). This study was approved by an institutional ethics review board at the department of sport physiology group of physical education faculty in Tehran University. All subjects gave their written informed consent to participate in the study. Subjects were asked to complete a medical examination and a medical questionnaire to ascertain that they did not take any medication, were free of cardiac, respiratory, renal, metabolic diseases. Subjects were not participating in regular physical activity

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except school physical education class. Body weight was measured with a digital scale (sensitivity of 0.1 kg) and height was measured to the nearest millimeter using JENIX (DS-102, Korea). BMI was calculated as weight in kilograms divided by the square of height in meters. Body fat percentage was calculated by Lipid Caliper with sensitivity of 0.2mm (YAGAMI, Japan) using Jackson and Pollock 3-site skinfold equations(22).Also VO_2 max was estimated by one mile Rockport Fitness Walking Test[39].

Study Design
Once subjects were recruited and baseline measurements were completed, randomly were assigned into exercise(n = 15) and control (n =15) groups. Subjects in exercise group participated in jump roping exercise in addition to regular physical education class, while the control group participated in only a regular physical education class. Anthropometric variables, lymphocyte ABAC1 protein expression and plasma Apo A-I, triglyceride(TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol(HDL-C)and fasting glucose were measured before and after 8 weeks of jump rope exercise.

Exercise Training
Subjects in the exercise group participated in supervised IERT four times per week, 40 min/d for 8 weeks. The detailed exercise training program is summarized in Table 1.

Blood Collection and Lymphocyte Preparation:
Participants attended the laboratory 48 hours before the first and 48 hours after the last session, at 8 a.m, after an overnight fasting and after having been abstained from exercise. A 10cc fasting venous blood from brachial vein was obtained. Blood samples were collected (3cc for lymphocyte isolation and 7cc for plasma variables measure) in test tubes and anticoagulated with EDTA. Plasma was separated by centrifuging for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection and divided into three aliquots. The aliquots were frozen and stored at −80 °C for subsequent analyses (within 2–3 weeks).

In order isolation of lymphocytes,3 cc of EDTA anticoagulated samples mixed with 5 ml lubricating buffer [preparing by: 50mmol Tris base (PH=7.6), 5mmol Carver magnesium (0.5mmolar), 109gr sucrose, 5mmolarTriton X (1%) and1 liter distilled water] and centrifuged at 3000 rpm for 15 minutes and after clear the supernatant again mixed with 5 ml phosphate buffered saline and centrifuged at 3000 rpm for 15 minutes. Finally, after clear the supernatant, white blood cells isolated and then were frozen and stored at −80 °C for further experiments.

Leukocyte ABCA1 protein expression
Total cell lysates were prepared by incubating the leukocyte cell pellets (n = 50) with 1 ml ice-cold lysis buffer (50 mM mannitol, 2 mM EDTA, 50 mM, pH 7.6) containing complete protease inhibitor (Goldbio.USA) and 0.1% w/v Triton X-100 for 60 minutes on ice. This was followed by five homogenization cycles (20 s each and 10 minutes resting time on ice after each homogenization step) in a fastprep homogenizer. The leukocyte homogenates were centrifuged at 15,000 g for 15 minutes at 4°C and the supernatants containing clear cell lysates were collected. Protein concentrations were determined by a bicinchoninic acid assay [24]. The clear leukocyte cell lysates were stored at −80°C until ABCA1 protein measurement was performed. Frozen leukocyte lysates were thawed and ABCA1 protein concentration was measured using an ELISA method(Human ABCA1 ELISA Kit, Cusabio, china). Briefly, either 100μl of standard sample were added to per well and incubated for 2 hours at 37°C. After removing the liquid of each well, 100μl of Biotin-antibody (1x) were added to each well and were incubated for 1 hour at 37°C.Then each well were washed three times with PBST. Next, 100μl of HRP-avidin (1x) were added to the wells and were incubated for one hour at 37 °. Then washing process was repeated 5 times again. Next, while protection from light, 90μl of TMB Substrate were added to each well and incubated for 15-30 minutes at 37°C. The enzymatic reaction was stopped by addition of 50 microliters sulphuric acid and absorbance measured at 450 nm on an ELISA plate reader (Sunrise, Tecan, Austria). ABCA1 protein concentrations of unknown samples were calculated from a calibration curve and results were expressed as ABCA1 protein pg/mg total protein.

Biochemical analyses
Apolipoprotein A-1 was determined by ELISA method(Human Apo A-I, Elisa, Assaypro Inc, USA). HDL-c was determined by direct Immuno method (HDL-C Immuno FS, Pars Azmoun,Tehran, Iran), the Intra-assay coefficient of variation and sensitivity of the method were 1.2% and 0.03 mmol/L. Plasma total Triglyceride (TG) was determined by enzymatic (GPO, Glycerol-3-Phosphate Oxidase) colorimetric method (Pars Azmoun, Tehran, Iran). The intraassay coefficient of variation and sensitivity of the method were 2.2% and 1 mg/dL respectively. Plasma

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total cholesterol (TC) was determined by enzymatic (CHOD-PAP, Cholesterol Oxidase-Amino Antipyrine) colorimetric method (Pars Azmoun, Tehran, Iran), the intrassay coefficient of variation and sensitivity of the method were 1.9% and 0.08mmol/L respectively. The procedure of Friedewald et al [23] was used to estimate low-density ipoprotein cholesterol (LDL-C). Fasting plasma glucose (FPG) was measured by an enzymatic colorimetric method using glucose oxidase(Glucose, Colorimetric Enzymatic, Parsazmun, Tehran, Iran).

Table 1. Interval endurance rope training(IERT) program

Week	Intensity (jumps/min)	Warm-up (5 mins)	Exercise duration (30 mins)	Cool down (5 mins)
1	60		1min of exercise,30 secs of rest	
2	60		1.5min of exercise,30 secs of rest	
3	60		2min of exercise,30 secs of rest	
4	70	Stretching	2.5min of exercise,30 secs of rest	Stretching
5	80		3min of exercise,30 secs of rest	
6	90		3.5min of exercise,30 secs of rest	
7	90		4min of exercise,30 secs of rest	
8	90		4min of exercise,30 secs of rest	

Statistics

The normality of distribution was checked for all variables with the Kolmogorov-Smirnov test. Pre-training and post-training differences were assessed with the Student's T-test (paired T-test for dependent samples and independent T-test for independent samples).A pvalue < 0.05 was used as the criterion of statistical significance. Statistical analysis was completed with the SPSSsoftware, version 16.0. Values are presented as mean ± SD.

RESULTS

Table 2: Pre and post exercise values between subjects of two groups

Variables	Pre			post		
	Exercise G	**Control G**	**p-value**	**Exercise G**	**Control G**	**p-value**
Age(yr)	17.35±1.07	16.9±1.15	0.91	--------------	--------------	------------
Height(cm)	175.8±7.3	171.2±10.6	0.175	-------------	--------------	-------------
Weight(kg)	87.26±11.05	90.02±10.5	0.49	83.9±10.14	89.8±9.78	0.116
BF(%)	29.37±1.85	29.17±2.29	0.79	27.43±1.3	29.11±2.13	0.015*
BMI (kg/m²)	28.24±2.56	28.31±2.49	0.93	26.96±2.37	28.85±2.48	0.042*
VO₂ max(ml/kg/min)	34.37±2.5	33.55±2.4	0.35	38.6±2.16	33.7±2.4	0.000*
ABCA1pg/mg/p	7.6±3.36	5.74±1.38	0.057	16.9±7.1	6.67±2.6	0.000*
APO-A1(mg/dl)	6.04±1.68	6.98±3/26	0.55	7.08±2.5	6.62±1.75	0.77
HDL-c(mg/dl)	41.06±4.8	46.1±7.05	0.03*	45.3±7.4	44/5±6.2	0.74
LDL-c(mg/dl)	124/1±27/7	141/5±27/6	0.097	120.6±22.03	150.2±28/02	0.37
TC(mg/dl)	200.3±38.03	219.7±38.6	0.177	183.8±25.5	226.8±34.9	0.001*
TG(mg/dl)	172.57±64.7	187.4±118.1	0.68	145.4±48.9	202.7±69.9	0.017*
LDL-c/HDL-c	3.05±0.76	3.12±0.65	0.79	2.81±0.63	3.29±0.7	0.026*
TC/HDL-c	4.91±0.99	4.82±0.86	0.78	4.13±0.8	5.12±0.77	0.002*
FG(mg/dl)	97.9±1.2	95.4±10.07	0.58	97.7±9.3	96.2±9.6	0.66

*Values expressed by mean and standard deviation (X±SD); * p < 0.05; %BF: percentage of body fat; BMI: body mass index; ABCA1: ATP-Binding cassette transporter A1; Apo A-I: Apolipoprotein A-I; TC: total cholesterol; TG- triglycerides;LDL-c: low density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; FG: fasting glucose.*

The age, body weight, height, BMI, percent body fat, VO₂ max, serum levels of fasting glucose, lipid profiles, plasma level of Apolipoprotein A-1, lymphocyte ABCA1 protein expression concentration of two groups at pre and post exercise are shown in Table 2. At baseline, there was no significant differences between two groups in all

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variables but in post exercise the levels of lymphocyte ABCA1 expression(p=0/000) and VO2max(p=0/000) were significantly increased and plasma levels of TG(p=0.017), TC(p=0.001), LDL-c/HDL-c(0.026),TC/HDL-c(p=0.002)and measures of BF%(p=0/015)and BMI(p=0.042) as anthropometric indicators were decreased. Changes of other variables such increasing inApo A-I, HDL-c, LDL-c and decreasing in body weight, were not significant.Significant level(p<0.05) was considered.(Table 2)

Also the paired T-Test results of subjects were showed that among training group there was asignificant increases in ABCA1 protein expression, Apo A-I ,HDL-C concentrations and VO₂ max(122.68%,P=0.000 ;17.12%,p= 0.029 ;10.32%,p=0.031 andP=0.000;6.48% respectively) and a significant reductions in weight (p=0.000; 4.18), percent body fat (p=0.000; 7.77), BMI (p=0.000; 4.29), TC (p=0.002; 8.23), TG (p=0.021; 20.49) and TC / HDL-c ratio (p=0.007; 15.87) in response to eight weeks interval rope training. Changes of other variables such as FG, LDL-c/HDL-c ratio, and LDL-c were not significant (Table3).

Table 3: The paired T-Test results of subjects in two groups

variables	Exercise G			Control G		
	pre	**post**	**%change**	**pre**	**post**	**%change**
Weight(kg)	87.26±11.05	83.9±10.14*	↓3.85	90.02±10.5	89.8±9.78	↑0.24
BF(%)	29.37±1.85	27.43±1.3*	↓7.08	29.17±2.29	29.11±2.13	↓0.02
BMI (kg/m²)	28.24±2.56	26.96±2.37*	↓4.53	28.31±2.49	28.85±2.48	↑1.9
VO₂ max(ml/kg/min)	34.37±2.5	38.6±2.16*	↑6.48	33.55±2.4	33.7±2.4	↑0.44
ABCA(pg/mg/p)	7.6±3.36	16.9±7.1*	↑122.68	5.74±1.38	6.67±2.6	↑16.37
APO-A1(mg/dl)	6.04±1.68	7.08±2.5*	↑17.12	6.98±3/26	6.62±1.75	↓5.15
HDL-c(mg/dl)	41.06±4.8	45.3±7.4*	↑10.32	46.1±7.05	44/5±6.2	↓3.47
LDL-c(mg/dl)	124/1±27/7	120.6±24.2	↓2.84	141/5±27/6	150.2±28	↑6.14
TC(mg/dl)	200.3±38.03	183.8±25.5*	↓8.23	219.7±38.6	226.8±34.9	↑3.23
TG(mg/dl)	172.57±64.7	145.4±48.9	↓15.74	187.4±118.1	202.7±69.9	↑8.16
LDL-c/HDL-c	3.05±0.76	2.81±0.63	↓15.87	3.12±0.65	3.29±0.7	↑5.44
TC/HDL-c	4.91±0.99	4.13±0.8*	↓15.88	4.82±0.86	5.12±0.77	↑6.22*
FG(mg/dl)	97.9±1.2	97.7±9.3	↓0.2	95.4±10.07	96.2±9.6	↑0.83

*Values expressed by mean and standard deviation (X±SD); * p < 0.05; %BF: percentage of body fat; BMI: body mass index; ABCA1: ATP-Binding cassette transporter A1; Apo A-I: Apolipoprotein A-I; TC: total cholesterol; TG:triglycerides; LDL-c: low density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; FG:fasting glucose*

DISCUSSION

The importance of regular exercise in preventing and treating chronic disease is generally accepted [26].The main findings of the present study were higher lymphocyte ABCA1 expression, plasma HDL-C, Apo-I and a reduction in plasma LDL-C concentrations following eight weeks IERT. Also there were significant changes in other lipid profiles and anthropometric indices.

In present study the increase of ABCA1 expression were in line with results of previously reported such as Butcher & et al [19],Hoang & et al [25],Ghanbari-Niaki et al [20] and Rashidlamir & et al[21].

The mechanism(s) by which the exercise trainingcan influence lymphocyte ABCA1 protein expression is(are) poorly understood. However, several possible mechanisms could be considered. It has been suggested that the modulating effect of fatty acids (FA) is mediatedby peroxisome proliferator-activated receptors (PPARs)and it is also well known that PPAR is a nuclear receptorsuch as 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, kidney and are also present in cells of the arterial wall including monocytes and macrophages[27,28].PPAR agonists' four fibrates (fenofibrate, bezafibrate, gemfibrozil, and LY518674) have been shown to regulate ABCA1 expression and HDL biogenesis [29, 30]. On the other hand, the effect of exercise training on PPAR mRNA expression has been taken into consideration by several researchers [31,32].Although it is likely that the modulating mechanism of ABCA1 expression in different tissues, such as muscle and leukocytes be different[26,54].On the other hand Butcher et al[19], have reported that 8 weeks of a low-intensity program Lead to significant changes in human leukocyte liver X-receptor

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(LXR) and proliferator-activated receptor-α (PPAR-α),also they found an increase in LXR and PPAR-α expression. They also suggested that importantly, ligand activation of PPAR-α also led to primary induction of LXR whose activation subsequently(after 4–8 wk exercise) triggered up-regulation of ABCA1and ABCG1 and therefore increased RCT [19].

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 [33,34,16]. Also is shown that cAMP can also be enhanced ABCA1 gene transcription [35].

In this study there were increasing in concentrations of ApoA-I and HDL-C and decreasing in LDL-c value flowing IERT program but these changes weren't significant , This results is consistent with the results of some studies [44,20,48,46,40,41,42,43] and is contrasted with some others [47, 26,42].It seems increases in ABCA1 expression and preβHDL (as the first product of apoA-I limitation), is the mechanism of increasing in Apo A-I [17, 36, 37].Also increasing of ABCA1 expression and Apo A-I and some other factors involved in the process of cholesterol reverse transport (such as LCAT, CETP, PLTP and Scavenger receptor BI (SR-BI) are the main mechanisms for increasing HDL-c [17, 38].

It seems the lack of significant changes in Apo AI and HDL-c and LDL-c is related to the volume and intensity of exercise. Studies has shown that effects of high volume and high intensity exercise on blood lipidprofiles and apolipoproteinsis more than the low or moderate exercise (41, 45, 42).Thus seems that the intensity and volume ofexerciseusedin this study has not sufficient to cause significant changes in these three variables.
Also the results of this study showed a significant decrease in plasma levels of TG (that might be due to an accelerated TG removal by skeletal muscle which plays a major part of TG removal by peripheral tissues (20)),TC,TC/HDL-c and LDL-c/HDL-c (as atherogenic indexes).These results are consistent with the other studies results (45, 42). The present study also clearly showed that IERT induced a significant changes in vo2max, BMI and BF(%) in OAOBA.

In summary, this is the first report and direct evidenced emonstrating that 8 weeks of IERT program enhances lymphocyte ABCA1 expression which is accompanied with a little elevated in plasma HDL-C, APO-A concentrations. The present results also indicated that IERT protocol could be taken into account as a modality of exercise for physical fitness improvement and weight control in all adolescents particularly OAOBA.

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