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The Effect of Eight Weeks of Wrestling and Wrestling Technique Based Circuit Training on Lymphocyte ABCA1 Gene Expression and Plasma Apolipoprotein A-I

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Abstract: The aim of the present study was to investigate the associated effect of wrestling and wrestling technique based circuit training on ABCA1 gene expression in lymphocytes. For this purpose, Sixteen well-trained wrestlers (17-22 Yr, 55-75 Kg, 19-25 kg/m^2) volunteered and randomly were assigned into training (n = 8) and control (n =8) groups. Experimental group was asked to complete a wrestling technique based circuit training (WTBCT) protocol combined with wrestling training (two sessions/day, morning and evening) for 8 weeks and control group remained sedentary. Blood samples were collected 48 hours before the first session and 48 hours after the last session to measure biochemical variables and to collect lymphocyte for ABCA1 gene expression. Subjects had an overnight fasting. A semi-quantitative technique (RT-PCR) was used for lymphocyte ABCA1 expression. T-student test was employed and data were analyzed by SPSS software (version 16). The results showed no significant differences in pretest ABCA1 gene expression levels, but given training protocol resulted in a higher and significant ($P<0.031$) ABCA1 gene expression level in training group when compared with control group. Significant increase ($P<0.02$) and decrease ($P<0.004$) were observed in plasma HDL-C and LDL-C concentrations respectively and Apo A-I concentration remained unchanged in training group when compared with control group. The present results indicated that WTBCT protocol combined with wrestling training was able to increase lymphocyte ABCA1 expression which was accompanied by higher plasma HDL-C and lower LDL-C concentrations. It seems that in this experimental condition, an anaerobic-based sport combined with WTBCT might be considered as a protocol to stimulate RCT process and cardiovascular improvement and to prevent cardiovascular disease.

Key words: ABCA1 % Apo A-I % Circuit training % Reverse cholesterol transport

INTRODUCTION

The beneficial effects of physical exercise and training particularly aerobic-based exercise and sports on human and animal organs and body function at different intensity and modality levels have been well established [1-6]. Physical exercise (acute /chronic) and sports also have been shown to have impact on blood cell compositions, particularly on leukocyte counts, metabolism, metabolites, peptide and gene expression in different species [7-15]. The formation of HDL and its remodeling by plasma factors is a complex process and requires several factors such as ATP-binding cassette transporter (ABC), lipoprotein lipase (LPL) and lecithin cholesterol acyltransferase (LCAT). ATP-binding cassette transporter type A1 (ABCA1) from large family of ABC is a multispan molecule with high expression in the liver, small intestine, testis, heart, adrenal gland, lung, leukocyte and macrophage and other tissues [16-17].

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 [18-20].

It has been suggested that ABCA1 plays a role in inflammation and it is upregulated by several factors such as cholesterol influx, nutritional status, plasma glucose concentrations and physical activity [21-23].

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To our knowledge, only three studies have been focused on the effect of physical activity on ABCA1 expression [7, 24-25]. In the first study, ABCA1 expression was measured in human skeletal muscle biopsies and leukocytes and physical activity (habitual exercise) were assessed using International Physical Activity Questionnaire (IPAQ) [25].

In the second study by Butcher *et al*., (2008), 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 were employed [24].

Ghanbari-Niaki *et al*., (2011) reported that 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) increased leukocytes counts and ABCA1 expression in peripheral blood lymphocyte [7].

To our knowledge, no study has been focused on the effects of anaerobic-based sports such as wrestling and judo as a weight-related sport and somewhat similar to power-based exercise on leukocyte, particularly on lymphocyte ABCA1 expression. Thus, this study was conducted to investigate lymphocyte ABCA1 expression in response to an 8 weeks of wrestling training combined with a wrestling based technique circuit training (WBTCT) protocol in young male freestyle wrestlers. The second aim of this study was to see if any possible ABCA1 expression is accompanied by plasma HDL-C and Apo-A concentrations.

MATERIALS AND METHODS

Participants: Sixteen well-trained wrestlers were randomly assigned into experimental (n=8) and control (n=8) groups. 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. Experimental group performed 8 weeks and six sessions (morning and afternoon)/ week of wrestling technique based circuit training (WTBCT) and control group remained sedentary in this period. All subjects were accustomed to the training protocol.

Training Protocol: Each session was divided into two part; part one: wrestling training including 3 sets of two-min. wrestling training with 30 seconds of passive rest between times (after the last set, 5 minutes of passive rest) and part two: wrestling technique based circuit training including 8 stations of common wrestling skills, 5 meters apart and each subject was asked to perform each skill powerfully and then run to the next station and perform another skill (according to the protocol). Subjects performed the protocol (8 stations, one set in first session and gradually to 3 sets the last week of training). Subjects exercised with an average of 85%-95% HR max for all active tasks. Training protocol lasted eight weeks [26].

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 10^{cc} fasting venous blood from brachial vein was obtained. Blood samples were collected in test tubes and anticoagulated with EDTA. Peripheral blood mononuclear cells were isolated by lymphocyte (Cedarlane Laboratories Limited, Burlington, ON, Canada) density gradient centrifugation at 900 g according to the manufacturer's instructions and the pellet containing lymphocytes were used for further analysis.

Study Design: Subjects' weights were measured with a (sensitivity of 0.1 kg) digital scale before the initial sampling and at the end of the research program. Heartbeat was constantly monitored by the polar device (F1tm model). Body fat percentage was measured by Lipid Caliper Lafayette using three fold thickness method [27].

ABCA1 Expression and Abundance:

ABCA1 Expression: Freshly prepared blood lymphocytes were powdered with cold mortar and pestle and used for the isolation of RNA. Total RNA was extracted by the guanidine thiocyanate method. mRNA was purified using mRNA Isolation Kit (Roche, Germany) according to the manufacturer's instructions. Two-hundred nanogram of mRNA was used to synthesize first strand cDNA in a 20/ ll volume using oligo (dT) primer in the first-strand synthesis kit (Fermentase, Germany). Relative expression levels of ABCA1 mRNA in the lymphocyte were determined using a semi-quantitative PCR method. The following primers were used to amplify rat ABCA1 and b-actin (as an internal control) cDNA: ABCA1-Forward: 50-CGT CCT CCT TGT CAT CTC TG-30. ABCA1-Reverse: 50-TAA CTT TCT TTC ACT TTC TCG TC-30. b-actin-

Forward: 50-TCC TGT GGC ATC CAT GAA ACT-30. b-actin-Reverse: 50-ATC GTG CAC CGC AAA TGC TTC-30. ABCA1 cDNA was amplified giving a 237-bp product. PCR (Polymerase Chain Reaction) was formed for 35 cycle of denaturation of 94°C for 30 sec, annealing of 55.5°C for 30 sec and extension at 72°C for 50 sec. Reactions were set up using a twofold serial dilution of template cDNA to assess the best dilution of template in PCR. Template cDNA was standardized by amplification of a 315-bp internal control of b-actin, a housekeeping gene. All the reactions were repeated a minimum of three times to ensure repeatability. All PCR products were electrophoresed on an agarose gel and bands visualized by ethidium bromide staining and quantitated by computer integrated densitometry (Kodak, CT). Levels of mRNA were expressed as the ratio of signal intensity for the b-actin gene.

Lipoproteins and Apolipoprotein A-I: Plasma high density lipoprotein cholesterol (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 respectively. The procedure of Friedewald *et al*., was used to estimate low-density lipoprotein cholesterol (LDL-C). Apolipoprotein A1 was determined by ELISA method (Wuham USCN Sciences Co. LTD, Wuhan, China).

Statistics: All results are expressed as means ± SD. All variables were compared by unpaired t-test. Correlation was calculated using the Pearson Product Moment correlation. All statistical analysis was performed by SPSS (Version 16).

RESULTS

Participants' Characteristics: The mean age of the participants was 17±1 years for both groups, BMI was 21.06±3.11kg/m^2 for control group and 22.10±2.22 for experimental group, body fat percentage was 14.28±4.15 for control group and 15.97±3.02 for experimental group. Weight and other lipid-related parameters are shown in Table 1.

ABCA1 and Reverse Cholesterol Transport: Data analysis revealed that the expression of ABCA1 in lymphocyte remained unchanged in control group, but there was a significant difference between post and pre ABCA1 gene expression levels in the training group. In addition, a significant difference was observed between groups (P= 0.031, t=2.80) (Fig. 1).

Plasma HDL-C concentration was higher in the experimental group following the 8 weeks (P=0.02, t=2.54). Also, there was a significant decrease in plasma LDL-C concentration in experimental group compared to the control group (P=0.004, t=3.29) and Apo A-I increased in experimental group but it was not significant (p=0.156, t=1.47) (Fig. 2).

CONCLUSION

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 WBTCT program for 8 weeks. The increase of ABCA1 expression was on a per cell basis and therefore was independent of the increase of WBC counts.

Table 1: Participants' characteristics

	Control		Experimental	
Variable	Pre-test	Post-test	Pre-test	Post-test
BMI (kg/m^2)	21.06±3.110	21.17±3.550	22.10±2.220	19.88±3.42**
Weight(kg)	62.51±11.90	62.14±12.99	64.11±14.02	60.32±12.86**
Body fat percentage	14.28±4.150	14.48±4.340	15.97±3.020	13.54±6.55**
HDL-C (mg/dL)	38.10±2.510	38.23±2.690	37.70±2.900	39.10±2.72*
LDL-C (mg/dL)	74.70±12.15	74.75±10.13	94.30±8.640	90.40±7.79**
Apo A-I (mg/dL)	158.23±8.530	157.45±5.770	164.38±3.370	164.79±10.24
Lymphocyte (n×1000 /μS)	2.70±0.840	2.68±0.690	2.35±0.450	2.15±1.23*
ABCA1mRNA-lymphocyte (RU)	76.16±6.340	76.15±4.450	77.80±3.530	90.18±4.77*

*Significance of the changes at the level of 0.05 and

**significance of the changes at the level of 0.001, as compared to the control group

Means±SD are shown. RU: relative units

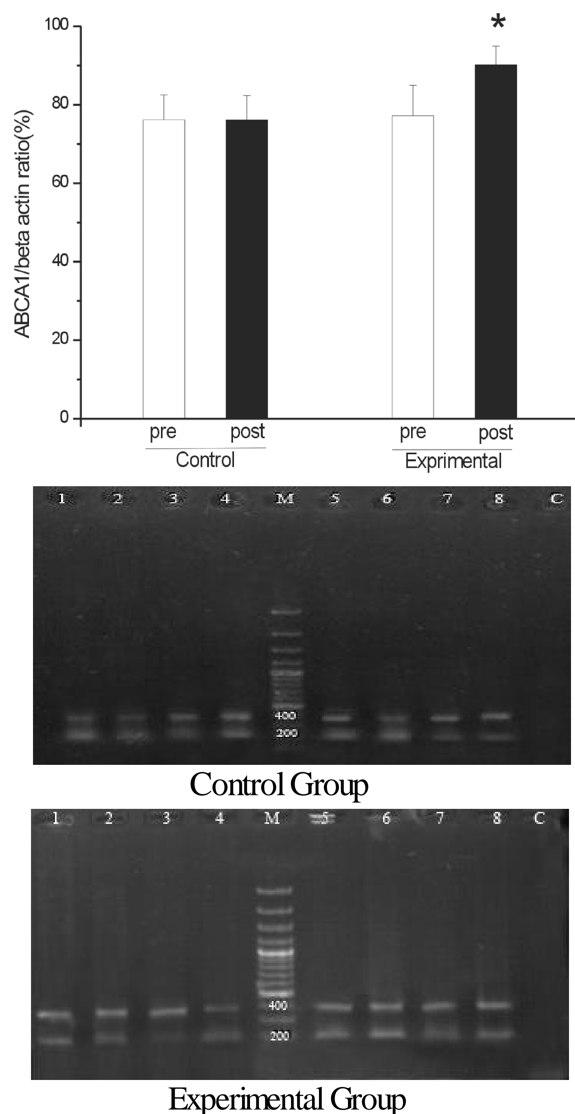

Fig. 1: Semi-quantitative RT-PCT of lymphocyte ABCA1 mRNA expression in two groups before and after the training protocol. Data expressed as mean±SD

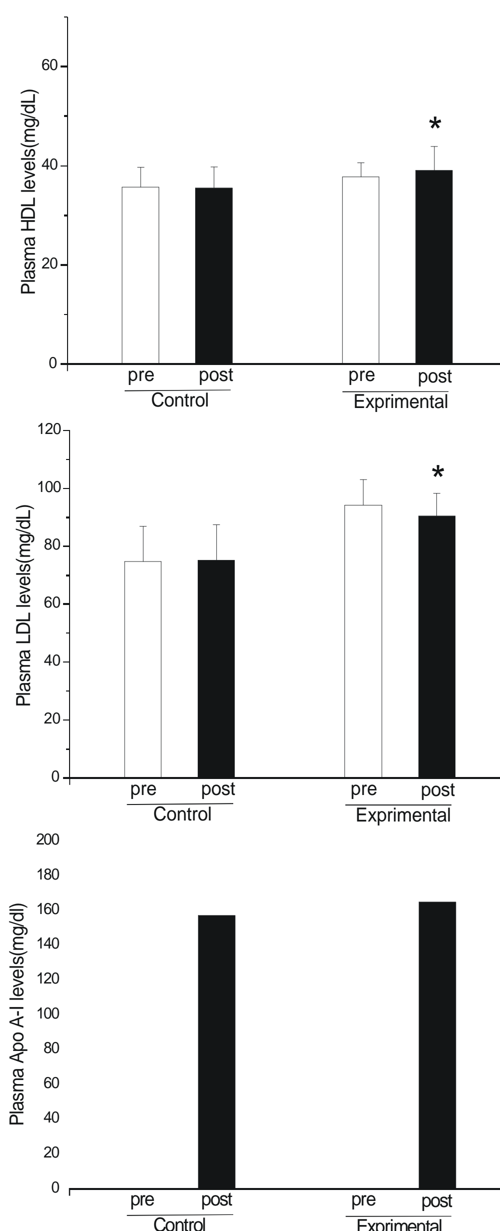

Fig. 2: Plasma HDL-C, LDL-C and apolipoprotein A-I concentrations after 8 weeks of research program in control and experimental group. Data expressed as mean±SD

Ghanbari-Niaki *et al*. (2011) reported that ABCA1 was expressed in lymphocyte at any given intensities (40%, 60%, 80% 1RM) [7].

ABCA1 is a cell membrane transporter that facilitates delivery of phospholipids from cell membranes to lipid-poor apoA-1 with the formation of discoidal apoA-I-containing HDL and it plays a pivotal role in forming plasma HDL.

Hoang *et al*., (2008) reported that participants with high physical activity had higher levels of ABCA1 expression in leukocytes [25].

According to Butcher *et al*., (2008), a low-intensity training program (8 weeks of low-intensity exercise program consisting of walking 10,000 steps, three times a week) resulted in a 3.46 and 3.06 fold increase in lymphocyte ABCA1 and ABCA1 expression but they did not find a significant change in mRNA expression of ABCA1 or ABCG1 in the control group [24].

Data of plasma HDL-C and LDL-C concentrations were also in line with several results previously reported by investigators [28-33].

The mechanism(s) by which the WBTCT protocol can influence lymphocyte ABCA1 mRNA 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 mediated by peroxisome proliferator-activated receptors (PPARs) and it is also well known that PPAR is a nuclear receptor such as liver X receptor (LXR) and retinoid X receptor (RXR) that regulates the expression of genes controlling lipid and glucose metabolism.

Three PPAR isoforms (", $/*, () 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 [34-36].

Fatone *et al*., (2010) reported that two sessions of combined aerobic per week (at 55%-70% of maximal oxygen uptake) and resistance circuit training (at 60%-80% of 1 repetition maximum) resulted in a significant increase in PPAR-" after 6 and 12 months whereas PPAR-(increased only after 6 months [37]. Spangenburg *et al*., (2009) suggested that acute exercise and training for 12 weeks resulted in a higher extended PPAR mRNAs expression in soleus muscle and plantaris muscle, but PPAR-(mRNA levels were lowest in skeletal muscle [38].

Butcher *et al*., (2008) have investigated the effect of 8 weeks of a low-intensity program on human leukocyte liver X-receptor (LXR) and proliferator-activated receptor ((PPAR-() and they found an increase in LXR and PPAR-(expression. They also suggested that importantly, ligand activation of PPAR-(also led to primary induction of LXR whose activation subsequently (after 4–8 wk exercise) triggered up-regulation of ABCA1 and ABCG1 and therefore increased RCT [24].

In summary, this is the first report and direct evidence demonstrating that 8 weeks of WBTCT program enhances lymphocyte ABCA1mRNA expression which is accompanied with an elevated plasma HDL-C, APO-A and a reduction in plasma LDL-C concentrations. The present results also indicated that WBTCT protocol could be taken into account as a modality of exercise for cardiovascular improvement in wrestlers who are practicing in an anaerobic-based sport. Further investigation is suggested to see the effect of this WBTCT protocol on other related factors such as LXR and PPAR types.

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