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The effects of aerobic exercises on the serum oxidized LDL and total antioxidant capacity in non-active men

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Summary We investigated the effect of moderate and vigorous aerobic exercise (MAE and VAE) on serum oxidized low-density lipoprotein (ox-LDL) levels and total antioxidant capacity (TAC) in voluntary and untrained healthy subjects. All subjects were randomly divided into three groups, including VAE (80—85% of maximal reserve heart rate) ($n = 15$), MAE (60—65% of maximal reserve heart rate) ($n = 17$), and control ($n = 12$). Exercise groups exercised in three sessions per week for 8 weeks. MAE and VAE did not significantly alter serum ox-LDL, total cholesterol (TC) and TAC ($p > 0.05$), while serum high-density lipoprotein (HDL-C) and HDL-C/TC ratio were significantly increased ($p < 0.05$). In the exercise groups, maximal oxygen uptake (V_{O_2max}) was higher and body mass index (BMI) was lower than control group ($p < 0.05$). There was a significant positive correlation between baseline V_{O_2max} and TAC and HDL-C. TAC had a significant negative correlation with TC, LDL-C and BMI. We conclude that serum ox-LDL and TAC were not affected by exercise, however, a positive correlation between V_{O_2max} and TAC and a negative correlation between TAC and LDL-C as well as TC suggest a correlation between physical training and an improved antioxidant defense system.

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Introduction

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals are produced in metabolic and physiological processes, and harmful oxidative reactions may lead to oxidation of biological molecules such as lipids, proteins and DNA [1]. Antioxidant molecules prevent and/or inhibit these harmful reactions [2,3].

Antioxidants increase and plasma lipid peroxide levels decrease during exercise [4—6]. Thus the beneficial effects of chronic exercise are generated through an antioxidant defense mechanism [7]. In contrast, exercise-induced oxidative stress has also been reported [8—10]. However, the effect of different intensities of exercise on total antioxidant capacity (TAC) is not completely known.

Several studies indicate that oxidation of lipoproteins, especially low-density lipoprotein (LDL-C), has a role in the initiation and progression of atherosclerotic processes [11,12]. Therefore improving antioxidant status may be an important determinant in the control of cardiovascular disease via attenuation of LDL-C oxidation [13]. The aim of this study was to determine the effects of different intensities (vigorous versus moderate) of aerobic exercise on serum oxidized low-density lipoprotein (ox-LDL-C) levels and TAC.

Materials and methods

Subjects and study design

Participants had to be non-smokers, free of cardiovascular disease, metabolic disorders including diabetes and not taking cholesterol-lowering or blood pressure medications. Anyone consuming antioxidants or supplements such as Vitamin A, C or E was also excluded. Furthermore, they could not have been participating in regular physical activity. This information was collected using a health condition questionnaire and the Baecke questionnaire of habitual physical activity. Informed consent was obtained from all subjects after a full explanation of the study. Dietary intake of the subjects during the two months of our study was monitored using a 24-h dietary recall questionnaire. The study protocol was approved by the Ethics Committee of the Medical University of Tehran.

There were two exercise protocols, consisting of moderate aerobic exercise (MAE) and vigorous aerobic exercise (VAE). The two groups exercised for 8 weeks in three sessions per week, each session lasting 30—45 min. Sessions began with a 10—15 min warm-up followed by a 5—10 min cool-down. Core activity in each session included running at 80—85% of maximal reserve heart rate for the VAE group and walking at brisk and very brisk speeds and stepping up and down stairs at 60—65% of maximal reserve heart rate for the MAE group.

Physical and biochemical characteristics

Several variables were assessed at baseline (pre-test), after 4 weeks (mid-test), and after 8 weeks (post-test) of the training period. The body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Fasting blood samples were drawn into heparinized tubes from a cubital vein before and after the last session of exercise in the mid-test (4th week) and post-test (8th week). Plasma samples were stored at $-80\,^{\circ}C$ prior to undergoing analysis. Fasting serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL-C) concentrations were measured by an enzymatic colorimetric method (Pars Azmun, Tehran, Iran) [14]. LDL-C concentration was calculated with the Friedwald formula [15] for serum samples with triglyceride (TG) values less than 400 mg/dl. The assay sensitivity for all three tests was 1 mg/dl and the intra assay coefficients of variation for total cholesterol, triglycerides and HDL-C were 1.1%, 1.6% and 1.9%, respectively. Serum TAC was measured using a commercially available kit (Randox Laboratories, Crumlin, UK) as previously described [2,16]. In this method, the most potent radical, hydroxyl radical, is produced. First, a ferrous ion solution is mixed with hydrogen peroxide. The sequentially produced radicals such as the brown colored dianisidinyl radical cations, produced by the hydroxyl radical, are potent radicals. Then, the antioxidative effect of the sample against the potent free radical reactions is measured. The assay has excellent precision values, which are lower than 3%. The results are expressed in mmol/l. Serum ox-LDL (ml U/L) was measured using a commercially available sandwich enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). The assay sensitivity was 1 mU/L and its intra assay coefficient of variation was 8.5% [13,17]. We estimated the maximal oxygen uptake (V_{O_2max}) using the Fox protocol on the ergometer. This test is a simple method for predicting V_{O_2max} from the heart rate (HR) response to 5 min of cycle ergometry at a power output of 150 W and at a speed of 60 rpm [18].

Statistical analyses

SPSS 11 was used for data analyses. Values are expressed as mean ± SD. Paired *t*-test was used to compare pre- and post-test results. One-way ANOVA was used to evaluate the statistical significance for differences between groups. Least significant difference (LSD) test (post hoc analyses) was used for coupled comparisons between times, groups and time—group interactions. The Pearson correlation coefficient was utilized for correlation analyses. Statistical significance was considered if $p < 0.05$.

Results

Initially 20 males were recruited and randomly assigned to each of the two exercise groups, MAE and VAE, and 12 males to a control group. Subsequently, three dropped out of the MAE group and five from the VAE group because of injury or irregular participation. Thus, 45 males (25—45 years old) completed the study. The physical characteristics and demographic data for all subjects are presented in Table 1. Before the study (pre-test), the exercise and control groups had similar BMI, fitness levels (V_{O_2max}), serum TC, TG, LDL-C and HDL-C. At the end of the study, BMI in the VAE group was significantly lower than in the control group ($p < 0.05$), but serum TC and LDL-C did not alter in the exercise groups (VAE and MAE) compared to the control group. In addition, serum HDL-C and HDL-C/TC ratio were significantly higher in the VAE group compare to the control and the MAE groups ($p < 0.05$, Table 2). Our results also showed that neither vigorous nor moderate aerobic exercise had a significant effect on serum ox-LDL levels and TAC. We observed that V_{O_2max} was increased in the VAE group after 4 weeks compared to the control group ($p < 0.05$) (Table 2). There was a significant positive correlation between TAC and V_{O_2max} ($r = 0.36$, $p < 0.01$) (Fig. 1) and significant negative correlations were found between TAC and LDL-C ($r = -0.35$, $p < 0.05$), TAC and TG ($r = -0.42$, $p < 0.05$), TAC and TC ($r = -0.65$, $p < 0.05$) and TAC and BMI ($r = -0.34$, $p < 0.05$). Furthermore, a significant positive correlation was detected between HDL-C and V_{O_2max} ($r = 0.30$, $p < 0.04$) (see Fig. 2).

Discussion

The aim of this study was to evaluate the effect of moderate and vigorous intensities of aerobic exercise on TAC and serum ox-LDL levels. Our results demonstrated that neither vigorous nor moderate exercise changed TAC. There are several contradictory reports regarding the effect of exercise on oxidative stress. Although some studies have failed to observe exercise-induced oxidative stress [8,9,19], it is suggested that exercise may increase free radicals and ROS, which may interact with lipids, DNA and proteins [8—10]. In addition, other studies suggest that exercise training enhances antioxidant capacity [7,20—22], however no change in TAC after some exercise protocols has been also reported [23,24]. This variability may be due to different exercise protocols, training status, age and gender, as well as use of different markers of antioxidant status and methodology. It may also be related to the measurement of TAC in different tissues of the body. It is known that exercise stimulates antioxidant enzyme activity in skeletal muscles rather than liver, heart and lungs [25] and this may due to variation of oxygen uptake during exercise or basal metabolism in different tissues. Moreover, it should be considered that using TAC as a global indicator of oxidative stress in biological fluids is inadequate and this is one of the limitations of this study. It is better to use a range of measurements of individual antioxidants and markers of oxidative damage [26].

Table 1 Pre-test physical characteristics and demographic data

Variables	VAE[a] group ($N = 15$)	MAE[b] group ($N = 17$)	Control group ($N = 12$)
Age (years)	30.93 ± 6.38	34.94 ± 7.44	29.75 ± 5.31
Weight (kg)	69.50 ± 9.29	76.11 ± 9.39	75.54 ± 7.46
BMI (kg/m^2)	23.1 ± 3.35	25.90 ± 3.83	24.52 ± 1.88
V_{O_2max} (ml/kg/min)	42.78 ± 4.90	37.90 ± 5.32	41.21 ± 5.88
Systolic blood pressure (mm Hg)	119.06 ± 4.72	123.76 ± 9.86	122.16 ± 5.74
Diastolic blood pressure (mm Hg)	77.86 ± 7.03	83.05 ± 8.45	80.66 ± 6.55

No significant differences were observed using one-way ANOVA.
[a] Vigorous aerobic exercise.
[b] Moderate aerobic exercise.

Table 2 Effect of aerobic exercise on outcome variables at mid-test (4th week) and post-test (8th week) of the study (mean ± SD)

	VAE-group (n = 15)	MAE-group (n = 17)	Control group (n = 12)	Group × time effect (p-value)
Ox-LDL (ml U/l)				
Pre-test	56.00 ± 19.00	56.70 ± 17.82	48.25 ± 17.60	0.85
Mid-test	53.4 ± 14.74	58.94 ± 18.01	56.00 ± 18.23	
Post-test	51.13 ± 22.75	52.00 ± 19.00	63.91 ± 27.74	
TAC (mmol/l)				
Pre-test	1.56 ± 0.22	1.58 ± 0.29	1.55 ± 0.26	0.55
Mid-test	1.56 ± 0.22	1.68 ± 0.16	1.61 ± 0.25	
Post-test	1.66 ± 0.14	1.69 ± 0.16	1.64 ± 0.21	
HDL-C (mg/dl)				
Pre-test	40.93 ± 12.74	38.94 ± 6.94	35.58 ± 6.41	0.03
Mid-test	43.20 ± 9.54	39.41 ± 8.13	40.16 ± 7.29	
Post-test	49.73 ± 11.62[a]	44.35 ± 8.06	39.33 ± 7.32	
HDL-C/TC ratio				
Pre-test	0.23 ± 0.08	0.18 ± 0.05	0.17 ± 0.04	0.03
Mid-test	0.22 ± 0.05	0.20 ± 0.06	0.20 ± 0.04	
Post-test	0.28 ± 0.09[b]	0.23 ± 0.06	0.20 ± 0.04	
TC (mg/dl)				
Pre-test	199.06 ± 39.03	218.88 ± 45.78	202.16 ± 37.41	0.79
Mid-test	190.53 ± 30.45	204.11 ± 42.79	202.08 ± 22.88	
Post-test	177.60 ± 28.20	197.70 ± 33.70	193.08 ± 24.61	
TG (mg/dl)				
Pre-test	136.66 ± 71.60	157.52 ± 86.16	149.66 ± 73.68	0.15
Mid-test	123.73 ± 51.66	134.11 ± 65.70	170.50 ± 70.47	
Post-test	106.53 ± 43.74	122.23 ± 50.01	161.33 ± 90.98	
V_{O_2max} *(ml/kg/min)*				
Pre-test	42.78 ± 4.89	37.90 ± 5.32	41.21 ± 5.88	0.009
Mid-test	48.61 ± 5.42	42.33 ± 5.01	42.57 ± 5.57	
Post-test	50.80 ± 6.77[c]	43.88 ± 6.17	42.98 ± 5.87	
BMI (kg/m^2)				
Pre-test	23.01 ± 3.35	25.90 ± 3.83	24.52 ± 1.88	0.02
Mid-test	22.64 ± 3.28	25.43 ± 3.83	24.27 ± 1.97	
Post-test	21.34 ± 3.15[d]	24.88 ± 3.85	24.12 ± 2.11	

[a] $p < 0.03$, LSD test, group changes from pre-test to post-test, compared to control group.
[b] $p < 0.03$, LSD test, group changes from pre-test to post-test, compared to control and MAE group.
[c] $p < 0.009$, LSD test, group changes from pre-test to mid-test, compared to control group.
[d] $p < 0.02$, LSD test, group changes from pre-test to post-test, compared to control group.

Increased oxidative stress and lipoprotein oxidation have been linked to atherosclerotic diseases including coronary artery disease [27,28]. It has been shown that there is a positive relationship between plasma ox-LDL and cardiovascular mortality [29]. Recent studies have reported that regular moderate physical activities decrease oxidized lipoproteins and thereby influence the risk of atherogenesis in humans and animal models [30], though we did not find any significant change in serum ox-LDL in the exercise groups. It is possible that LDL susceptibility to oxidation is not associated with levels of circulating oxidized LDL [31]. It should be also pointed out that the effects of exercise training depend on the mode and type of exercise, e.g. regular exercise regimens versus acute exercise [32].

It has been found that LDL-C from professional athletes is less readily oxidized in vitro as compared to novices [28], suggesting that the level of physical fitness is one of the important factors which influences human capacity against oxidative stress. On the other hand, we found a significant positive correlation between V_{O_2max} and HDL-C or TAC, and a significant negative correlation between V_{O_2max} and LDL-C, which supported the pre-

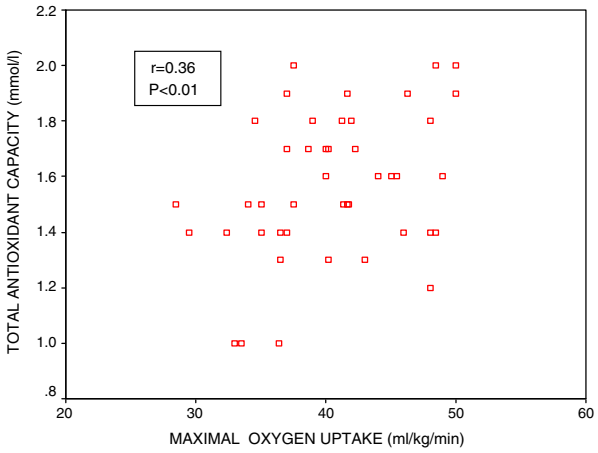

Figure 1 Correlation between mean total antioxidant capacity and maximal oxygen uptake based on the baseline values.

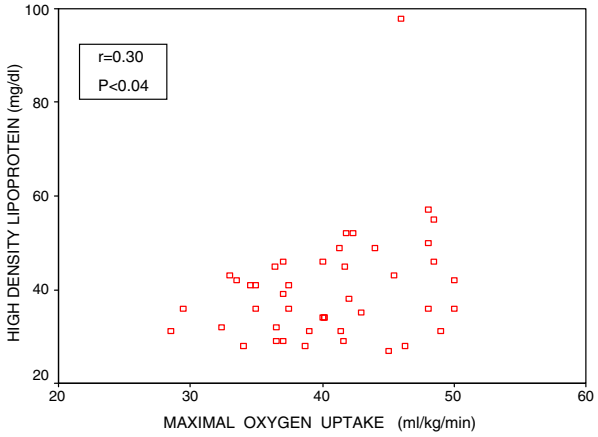

Figure 2 Correlation between mean high-density lipoprotein cholesterol and maximal oxygen uptake based on the baseline values.

vious studies [33,34]. The positive correlation between antioxidant status (TAC, HDL-C) and V_{O_2max}, and elevation of V_{O_2max} after VAE may be an indicator of the importance of regular aerobic exercise (up to 85% maximal reserve heart rate) towards improvement of antioxidant defense systems.

Kraus et al. [35] found that duration (volume) of weekly training is more efficient in modifying the lipid profile than intensity of training, but when exercise was carried out in long duration and high intensity, even more beneficial effects of training were observed. In our study, duration of exercise was similar (30—45 min/day) but VAE caused significant increases in HDL-C. Kokkinos et al. [36] noted a relationship between distance of weekly running and HDL-C modification, while our results suggest that there is a meaningful relation between intensity of aerobic exercise and HDL-C. It is reported that the effect of regular exercise training may be particularly helpful for people with low HDL cholesterol, elevated TG and abdominal obesity [37]. Our participants did not have a high HDL-C level (<40 mg/dl) at the beginning of the study and the fact that HDL-C is usually influenced more by exercise, even after intensive recreational training [38], suggests that a threshold of intensity of physical activity should be considered an important component of exercise prescriptions.

Conclusion

The results of the present study demonstrating an improvement of BMI, V_{O_2max} and HDL-C, and a lack of oxidative stress (TAC reduction and ox-LDL elevation) after vigorous aerobic exercise, up to 85% maximal reserve heart rate, suggest that recommendations to engage in low intensity exercise need to be re-examined.

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