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Original Research Paper

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ARE THE HAPTOGLOBİN LEVELS COMPARABLE TO THE ANTİOXİDANT STATUS LEVELS İN BİCYCLİNG?

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ABSTRACT

BACKGROUND AND AIMS: This study was designed to determine whether the haptoglobin levels are comparable to the antioxidant status levels. 40 athletes and 30 volunteers were enrolled in the study.

MATERIAL AND METHODS: Blood samples for serum malondialdehyde (MDA), total antioxidant status (TAS), haptoglobin, glucose, total cholesterol (TC), triglyceride (TG), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) and lipoprotein (a) (Lip a) determinations were obtained before and after cyclists completed 300 km bicycle ride.

RESULTS: Comparison of the results between the cyclists and control group yielded a significant difference in serum levels of TG, VLDL-C and LDL-C ($P<0.05$). The cycling induced a significant increase in HDL-C, VLDL-C, TG, MDA and TAS levels ($P<0.001$). Serum LDL-C and Lip a levels were greater before than after cycling ($P<0.05$). There was no significant difference among precycling and postcycling haptoglobin levels, whereas the haptoglobin level in control group was significantly higher than in the cyclists ($P<0.001$).

CONCLUSION: The change in serum antioxidant and lipid levels due to physical activity must be explained as a consequence of the duration of exercise. Further studies are needed to demonstrate haptoglobin's possible role in decreasing oxidative stress during exercise.

KEYWORDS : Bicycling, Antioxidant, Haptoglobin.

INTRODUCTION

There is a consensus view that physical activity decreases the incidence of hypertension, reduces the risk of coronary heart disease and improves blood lipid profile.[1] Studies have shown that active men and women have higher plasma concentrations of high density lipoprotein cholesterol (HDL-C), lower levels of very low density lipoprotein cholesterol (VLDL-C), total cholesterol (TC) and triglyceride (TG) and moderately lower levels of low density lipoprotein cholesterol (LDL-C) than sedentary ones.[2] Although consensus is growing on the association between the physical activity and the health; debate continues regarding the pro-oxidant nature of exercise.[3] An increase in the rate of oxygen consumption can result in an increased production of reactive oxygen species (ROS).[4] The ROS induce damage in all cellular macromolecules, such as lipids, proteins and DNA.[5] Exercise activates neutrophils which is accompanied by increased plasma levels of granular enzymes leading to the oxidation of LDL-C.[6]

Erythrocytes are susceptible to oxidative damage as a result of the high cellular concentrations of oxygen and hemoglobin.[7] When hemoglobin is released into the plasma, it would normally bind to haptoglobin and be safely carried to engage CD163 molecules where haptoglobin-hemoglobin complex is degraded within the macrophage.[8] Haptoglobin functions as an antioxidant because of its ability to bind free hemoglobin and thereby prevent iron-driven oxidative tissue damage.[9] LDL-C oxidation arises from the ability of iron to participate in the generation of powerful oxidant species such as hydroxyl radical, hydrogen peroxide and superoxide through the Haber-Weiss and Fenton reactions.[10] The aim of this study is to investigate whether rigorous bicycling, performed by professional athletes, results in increased serum levels of serum oxidants and antioxidant status. The estimation of haptoglobin levels can be useful for the accurate and rapid evaluation of antioxidant defenses. We tried to determine whether the haptoglobin levels are comparable to the antioxidant status levels. Since oxidative stress induces alterations in cholesterol handling, we also determined the effects of bicycling on triglyceride and cholesterol serum levels.

MATERIAL AND METHODS

Forty male athletes from the National Cycling Team (on a training camp) were included in this study. Their mean age was 22.4 years (range, 16-41 years) and body mass index (BMI) was 23.5 kg/m^2 (range, 19.75-27.15). They cycled 591 km/wk (range, 250-900 km) for an average of 8.7 years (range, 2-28 years). They had a cycling VO_{2max} of 60.6±7.0 ml.kg^{-1}.min^{-1}.

Thirty male students from university and medical staff who had not been involved in any intense sports training program for at least 1 year before the study were also included as control group. Their mean age and BMI were 24.4 years (range, 17-35 years), 24.2 kg/m^2 (range, 20.2-28.2), respectively.

Following an explanation of the experimental procedures, written informed consent was obtained from each subject. This study protocol adheres to principles outlined in the Declaration of Helsinki. All subjects underwent a physical examination, the previous week. Subjects with a history of any inflammatory, peripheral vascular, collagen, coronary heart, liver diseases or malignancies and a history of taking nitrates and antioxidant vitamins were excluded from the study. All subjects were nonsmokers. They were instructed to consume their usual diet during the study and not to take any medicine or dietary supplements.

Venous blood samples were obtained from all the subjects on the exercise test day and 1 hour after cycling course. The athletes rode a 300 km bicycle circuit in 6 hours. All blood samples were centrifuged at 3000 rpm for 10 minutes. Serum samples were stored at -80 °C until biochemical analysis.

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In this study, we evaluated the oxidant and antioxidant status of the subjects by quantifying serum concentrations of malondialdehyde (MDA), total antioxidant status (TAS) and haptoglobin. Serum MDA levels were measured according to the method of Yoshioka.[11] Serum TAS levels were measured with TAS kit (Randox Labs, Crumlin, UK). Serum haptoglobin, lipoprotein (a) (Lip a), glucose, TC, LDL-C, HDL-C and TG levels were measured using spectrophotometric method using Architect c 8000 autoanalyzer (Abbott, Abbott Park, IL, USA).

Hematological determinations (number of erythrocytes, hematocrit and hemoglobin concentration) were made using an automatic flow cytometer analyser Cell-Dyn Ruby (Abbott) system.

Statistical analyses were performed on SSPS (version 20; SPSS Inc, Chicago, Illinosis). The Kolmogorov-Smirnov normality test revealed that the data for HDL-C, LDL-C, TG, Lip a, TAS and haptoglobin was not normally distributed. Results are represented as median(minimum-maximum) for non-normally distributed data and as mean±SD for continuous normally distributed variables. Student's t test as a parametric test and Mann-Whitney U test as a nonparametric test were computed to examine the differences between the groups. For repeated measures, paired samples t test was used to compare normally distrubuted parameters and Wilcoxon test for non-normally distrubuted parameters, respectively. P value <0 .05 was considered statistically significant.

RESULTS

The basal characteristics of the athletes and control group are shown in Table 1. There were no statistically significant differences in age and BMI in the groups (P>0.05). A comparison of basal hematological parameters between cyclists and control group (Table 2) showed that the number of erythrocytes, hematocrit and hemoglobin concentrations were similiar.

Table 1. Comparison of age and BMI between athletes and control group.

Variable	Cycling Athlete	Control Group	P
Age (y)	22.4 (16-41)	24.4 (17-35)	0.08
BMI (kg/m2)	23.5 (19.75-27.15)	24.2 (20.2-28.2)	0.06

Table 2. Basal hematological parameters.

Variable	Cycling Athlete	Control Group	P
Erythrocytes (106/μL)	4.9 (0.008)	4.99 (0.007)	>0.05
Hematocrit (%)	45.5 (0.5)	45.6 (0.4)	>0.05
Hemoglobin (g/dl)	15.2 (0.2)	15.4 (0.2)	>0.05

The control group had only baseline measurements. They did not contribute to the postcycling part of the study. The main results of the investigation are summarized in Table 3. Glucose and TC levels did not change throughout the period studied. In the cyclists, there were no differences between pre- and postcycling HDL-C levels. Serum LDL-C and lipoprotein (a) levels were greater before than after cycling (P<0.05). The cycling induced a significant increase in VLDL-C, triglyceride, MDA and TAS levels (P<0.001). Comparison of the results between the cyclists and control group yielded a significant difference in serum levels of triglyceride, VLDL-C and LDL-C (P<0.05). Although there was no significant difference between cyclists and control group's lipoprotein (a) levels, cycling induced a decrease in precycling lipoprotein (a) levels (P=0.012). There was no significant difference among precycling and postcycling haptoglobin levels, whereas the haptoglobin level in control group was significantly higher than in the cyclists (P<0.001).

Table 3. Comparison of precycling, postcycling and control group values.

Variable	Precycling	Postcycling	Control Group	P1	P2
Glucose (mg/dl)	88.5 (15.9)	92.5 (21.0)	93.1 (10.7)	0.290	0.171
TC (mg/dl)	185.1 (37.2)	191.6 (35.3)	173.3(25.2)	0.210	0.134
HDL-C (mg/dl)	64.0(49-106)	64.5 (48-92)	32.5(24-53)	0.392	<0.001
LDL-C (mg/dl)	91.0(36-170)	87.0(24-167)	114.5 (48-143)	<0.002	0.021
VLDL-C (mg/dl)	21.0 (9-44)	36.0 (14-134)	24.0 (16-83)	<0.001	0.017
TG (mg/dl)	104.5 (46-221)	179.5 (70-671)	121.0 (82-415)	<0.001	0.018
Lip a (mg/dl)	10.4 (1.4-94.0)	10.2 (1.8-86.6)	16.5 (1.3-87.2)	0.012	0.132
MDA (μmol/ml)	9.6 (0.5)	11.2 (1.17)	8.0 (0.6)	<0.001	0.001
TAS (mmol/L)	1.82 (1.62-6.9)	1.93 (1.64-27.1)	1.47 (1.37-1.55)	0.001	<0.001
Haptoglobin (mg/dl)	37.5 (23-55)	37.0 (18-101)	138.5 (71-168)	0.115	<0.001

P1: precycling vs postcycling, P2: precycling vs control group.

DISCUSSION

Acute, severe exercise increases cholesterol mobilization from its reservoirs in trained people, whereas long term exercise does not mobilize cholesterol but eliminates LDL-C in well-trained people.[12] Agulio et al. demonstrated that acute exercise decreased LDL-C and increased HDL-C, whereas chronic exercise decreased LDL-C.[3] According to our study, serum LDL-C levels were greater before than after cycling (P<0.002). Moreover, TC levels did not change throughout the period studied. Thompson et al. observed an increase in HDL-C levels after acute exercise, but not after chronic exercise.[13] In our study, there were no differences between pre- and postcycling HDL-C levels in cyclists. However, there was a significant difference in HDL-C levels between cyclists and control group (P<0.001). Our results showed a significant difference in serum levels of triglyceride, VLDL-C and LDL-C between the cyclists and control group (P<0.05). And also, our result is in agreement with Rokitzki and associates who demonstrated a rise in plasma triglycerides and VLDL-C levels after the cycling stage.[14] Increased triglyceride observed after exercise may be related to the release of free fatty acids from the periphery as a fuel to maintain physical activity.

There is a strong association between serum lipoprotein (a) levels and cardiovascular risk factors. Lippi et al. recently emphasized that a moderate increase in plasma lipoprotein (a) is consistenly regarded as a conditional risk factor for cardiovascular disease.[15] Rigorous sporting activity might induce changes in the metabolism of lipoprotein (a).[2] Results of our investigations confirm that rigorous and acute aerobic physical activity induced a significant decrease in lipoprotein (a) levels (P=0.012). However, lipoprotein (a) levels did not differ between cyclists and control group in our study (P>0.05).

Most of the recent studies have shown that physical exercise is associated with oxidative stres.[16] Oxidative stress may result from either overproduction of free radicals or from insuffiency of antioxidant defense systems.[17] Hessel et al. demonstrated that exhaustive exercise induces an imbalance between ROS production and antioxidant defenses.[18] Increased lipid peroxidation (as represented by MDA and thiobarbituric acid reactive substances) in plasma after soccer games was suggested to ocur in male players.[19] While most of the recent

studies evaluated various antioxidants, we measured TAS levels which may be more informative about overall antioxidant defenses. Confirming these results, we found that the cycling induced a significant increase in MDA and also TAS levels ($P<0.001$) (Figure 1). Aguilo et al. demonstrated an increase in plasma α-tocopherol levels and also in VLDL-C levels.[3] In our study, the increase in TAS levels was coincident with the rise in triglyceride and VLDL-C levels. This increase may be due to antioxidants such as β-carotene, tocopherols and ascorbic acid mobilisation from tissue storages to the plasma circulation by synthesis of VLDL-C.

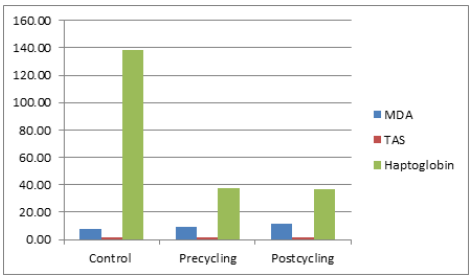

Figure 1. MDA, TAS and Haptoglobin levels.

On the other hand, through the generation of ROS, iron can damage lipids, proteins and nucleic acids.[20] In such conditions, upregulated haptoglobin expression might be a compensatory antioxidant protection. Bertaggia and associates pointed that haptoglobin is required to prevent oxidative stress in haptoglobin knockout .[21] Jelena et al. represented the antioxidative role of haptoglobin in the early phase of diabetes.[9] In our study, there was no significant difference among precycling and postcycling haptoglobin levels, whereas the haptoglobin level in control group was significantly higher than in the cyclists ($P<0.001$). This significant reduction of haptoglobin levels in cyclists could indicate a poor antioxidant protection attributable to haptoglobin. Reduced level of haptoglobin in exercise might be an indication of intravascular hemolysis during exercises. However, in our study, no differences were recorded for hematological values.

Taken together, our results indicate that, physical activity is very important in modifying serum antioxidant and lipid levels. However, the change in these parameters must be explained as a consequence of the duration of exercise. In view of its simplicity, measurement of serum haptoglobin levels could be useful for the accurate and rapid evaluation of antioxidant defenses. Our study showed that haptoglobin does not have a superior potential that of TAS. It is strongly recommended that larger-scale studies should be performed to demonstrate haptoglobin's possible role in decreasing oxidative stress during exercise.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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