Effects of Long-Term Training, Periods of Detraining and Retraining on Paraoxonase 1 Activity in Child Swimmers

Yüzücü Çocuklarda Uzun Süreli Antrenman, Antrenmansız ve Yeniden Antrenman Dönemlerinin Paroksonaz 1 Aktivitesi Üzerine Etkileri

ABSTRACT Objective: Antioxidant paraoxonase 1 enzyme (PON1) is a preventive enzyme against atherosclerosis. Aerobic exercise increases, oxidative stress decreases PON1. Effects of intensive and high volume swimming training which may create oxidative stress and decrease PON1 activity in children are unknown. Therefore, aim of the study was to investigate the effects of long-term swimming training (TP), detraining (DTP) and retraining periods (RTP) on blood PON1 activity in children. Material and Methods: 10 trained healthy (11.1±0.6) years old male swimmers joined the study. Critical speed (CS) as endurance level indicator; basal PON1 (PON1), salt-stimulated PON1 (SPON1), arylesterase (AE) activities; high density lipoprotein cholesterol (HDLC) total oxidant status (TOS); total antioxidant status (TAS) were determined at the end of TP, DTP and RTP every two months consecutively. Oxidative stress index (OSI: TAS/TOS) calculated. Results: AE activity at TP was greater than DTP (p<0.01) despite the high OSI (p<0.01). AE activity increased at RTP (p<0.05) despite the increased TOS (p<0.05). No change in PON1 activities and HDL-C levels during periods. Changes in AE activities were independent from HDL-C, CS. Conclusion: Results suggest that TP and RTP trainings improved AE activity independently from HDL-C and aerobic fitness levels despite the oxidative stress in trained children.

Keywords: Paraoxonase 1 enzyme; swimming; training; detraining; child; lipid

ÖZET Amaç: Antioksidan paraoksonaz 1 (PON1) atheroskleroza karşı koruyucu bir enzimdir. Aerobik egzersiz PON1 1 Bu çalışmada antrenman (AP), deantrenman (DAP) ve reantrenman periyodlarının (RAP) PON1 aktiviteleri üzerine etkileri araştırıldı. Gereç ve Yöntemler: 10 sağlıklı (11,1±0,6 yıl) yaşta antrene erkek yüzücü katıldı. AP sonunda, DAP ve RAP'dan ikiser ay sonra açık venöz kanları alındı. Dayanıklılık seviyesi, kritik hız (KH), serum örneklerinden bazal PON1, tuzla uyarılmış PON1 (TPON1), arilesteraz (AE) aktiviteleri, yüksek dansiteli lipoprotein kolesterol (HDLC), total oksidan statü (TOS), total antioksidan statü (TAS) düzeyleri ölçüldü. Oksidatif stres indeksi (OSI: TAS/TOS) hesaplandı. Bulgular: AP'deki AE aktivitesi, yüksek OSI'ye rağmen DAP'dakinin daha büyüktü (p<0,01). AE aktivitesi, DAP'taki artıştan sonra RAP'da orta düzeyde bir artış gösterdi (p<0,05). Diğer paraoksonaz aktiviteleri ve HDL-C seviyeleri çalışma boyunca anlamlı bir değişiklik göstermedi. Sonuç: Hem AP hem de RAP'ın, oksidatif stres reçine çocuklarda AE aktivitesini iyileştirdi, fakat DAP, bu kanamalı yok etti. Bu sonuçlar, çocuklarda antrenmanın atheroskleroz için önemli bir risk faktörü olduğunu teyit etti.

Anahtar Kelimeler: Paraoksonaz 1 enzimi; yüzme; antrenman; de antrenman; çocuk; lipid

Leading cause of death in developed and developing countries are cardiovascular diseases (CVD). Although the clinical manifestations of CVD start in the middle age, it is reported that the atherosclerotic process starts to develop in childhood. Therefore, it is required to minimize
or reduce the known risk factors for CVD starting from childhood and adolescence. Besides traditional risk factors such as blood lipids and lipoproteins (BLLP), serum paraoxonase 1 (PON1; EC.3.1.8.1, aryl dialkylphosphatase) is also important risk factor for CVD which has an antioxidant and antiatherosclerotic enzyme located on high density lipoprotein (HDL). It has the capacity to hydrolyze both the toxic organophosphorous compound paraoxone and phenyl acetate. Therefore, this enzyme was called as both the paraoxonase (PON1) and the arylesterase (AE). PON1 metabolizes oxidized lipids of low density lipoprotein (LDL) and HDL which begins atherosclerotic process. Studies in humans have also shown that serum PON1 activity is very low at birth and increases in time, reaching a plateau at 15 months of age. It is quite constant over time, once it reaches adult values. PON1 activity can vary depending on genetic state (As PON1 polymorphisms), ethnicity, pathological and environmental conditions. For example, it was found that blood PON1 activity was lower in the persons with diabetes mellitus type 2, atherosclerosis and smoking individuals, whereas plasma oxidant levels was higher according to controls. In addition, it was reported that the negative relationships were found between coronary heart disease (CHD) and PON1 and AE activities. Furthermore, it was reported that PON1 phenotype (as its activity) is a better predictor of vascular disease than is PON1-192 or PON155 genotype. Therefore, to able to know PON1 activity levels as an atherosclerotic risk factor and to follow up it is important for both first and second protective measures. Physical activity (PA) is associated with a reduced risk of coronary heart disease and PON1 activity. It was found that PON1 activity significantly greater in trained adolescence athletes when compared with controls; moderate exercise increased serum PON1 activity in smokers who have lower serum PON1 activity when compared with sedentary. There are also studies which were been showed that the aerobic training didn’t change PON1 activity significantly or that no association between physical exercise and serum PON1 activity was found. Intensity, duration and type of exercise might be played role on the differences between the studies. Swimming is generally performed in aerobic intensity as physical activity. However, swimming trainings with high intensity and volume are applied in most countries including also Turkey in children. During aerobic exercise, oxygen consumption increases too much in skeleton muscle when compared with resting status and it can cause oxidant stress. It is known that PON1 activity is inhibited by oxidative stress. Oxygen consumption was found higher in children than adults during exercise. Accordingly, it was found that, the child swimmers exhibited increased oxidative stress and that children is more susceptible to oxidative stress induced by chronic exercise. Therefore, long duration swimming training may decrease paraoxonase enzyme activity by increasing oxidative stress in children. However, the effects of annual swimming season on PON1 activity are unknown. Instead of sectional effects, effects of one year-duration swimming training period can be a more dependable and beneficial observation tool on indicated risk factors, to prepare optimum training plans. Therefore, we hypothesized that (8-month) long-duration swimming training (TP) and retraining periods (RTP) would reduces PON1 activity, whereas detraining (DTP) following TP increase it by reducing oxidative stress in child swimmers. To discuss more numerous studies, we performed three different activities of same PON1 enzyme in the present study.

MATERIAL AND METHODS

STUDY GROUPS

10 trained healthy (11.1± 0.6) years old male swimmers joined the study including Ege University swimming club. Three measurement sessions were performed. Initial measurement session (TP) was realized 4 days after Turkish National Swimming Championship (Contains 4 session of 2-day race program) which was been held at the end of swimming season. Second measurement was conducted after two months of detraining period (DTP). The last measurement was done 2-month after retraining period (RTP) subsequently. TP shows the 8-
month training period from RTP to TP. 10 swimmers to TP measurement, 14 swimmers to DTP measurement and 12 swimmers participated to the last RTP measurement. Total 6 athletes who were not participated in all three measurements were excluded. 10 common swimmers that participated to all 3 measurements were taken under consideration. All swimmers were not realized any swimming activity, made diet change and took any medicine, vitamin supply or similar matters that will effect on oxidative defense system in DTP. Swimmers were warned not to modify their diets last one week and not to train hard last three days prior to the tests. In addition, participants were warned not to carry out regular exercise. Physical activities of the objects were standardized as short-time aerobic walking and swimming activities during DTP. These warnings, the rules and any occurrence of any sick were accepted as the excluding criteria. Swimmers who are determined as healthy after anamneses form and biochemical tests examination were accepted to study. In TP, training frequency was six days per week; total volume was between 25 and 30K. In RTP, swimmers trained one session a day and six days a week. In the first week, they swam 18K in total; in the second week total volume was increased to 21K; and in the third week it was reached to a volume of 24 to 27K. Continuous, interval loading methods were used in END 1, END 2, SPR 3 intensity zones in training sessions.

INFORMED CONSENT STATEMENT
The aim, benefits, test applications, possible risks of the study were explained verbally and written to participant children and to their parents and a written consent was taken from the parents.

ETHICAL APPROVAL
The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee. Ege University Medical Faculty Ethics Committee was approved the study on 27.04.2009 with 08-12/7 decision number. The study was conducted according to these committee considerations.

PHYSICAL MEASUREMENT METHODS
Body compositions (height, body weight) measurements were measured using the body Composition analyzer (Tanita SC-330 S, Tokyo, Japan).

BODY MASS INDEX (BMI)
BMI was calculated via height and body weight values according to following formula; BMI= Weight (Kg)/height (m).2

SWIMMING PERFORMANCE TEST (CRITICAL SPEED)
Critical speed (CS) is used as aerobic endurance capacity criteria. Maximum 50m freestyle and 400m freestyle swimming times were measured with 1-day break and full rest. Critical speed was calculated according to following formula “400m-50m/ 400m time in seconds -50m time in seconds= m/s” according to reference.15 Whole measurements including physical and exercise tests were realized in the morning between 09:00/11:00 o clock subsequent 12 hours of fasting.

SAMPLING AND ANALYSIS OF BLOOD SAMPLES
Overnight fasting blood venous samples were obtained at 09.00 am at the end of TP, DTP and RTP. The measurement of TP was performed 4 days after Turkish National Swimming Championship. Bloods samples were centrifuged at 2000g for 10 min after waited 30 min in room temperature. Then serum samples were separated from whole blood. The samples were kept at-70℃ until the biochemical assays were performed in a single batch. Biochemical parameters were measured from serum samples within a month.

PARAOXONASE ACTIVITIES
Basal PON1 (PON1), salt (NaCl)-stimulated PON1 (SPON1) and arylesterase (AE) activities were determined;

MEASUREMENT OF SERUM BASAL PON1 ACTIVITY
Serum PON1 and SPON1 activities were determined by an autoanalyzer (Beckman, CX7, USA)
using paraoxon (Sigma Chemical Co, St. Louis, USA) as substrate. PON1 and SPON1 activities were measured after the reaction of paraoxon hydrolysis into p-nitrophenol and diethylphosphate catalysed by the enzyme. Both PON1 activity was determined from initial velocity of p-nitrophenol production (Subtracting the spontaneous paraoxon hydrolysis) at 412 nm, 37°C. Basal PON1 assay mixture included 1.0 mM paraoxon, 1.0 mM CaCl2 and serum in 50 mM Tris-HCl buffer, pH= 7.4. One unit of paraoxonase activities is defined as 1.0 µmol of p-nitrophenol formed per min. The intra and inter variation coefficients (CV) of both PON1 and SPON1 was lower 1.09%.

MEASUREMENT OF SPON1 ACTIVITY

Serum SPON1 activity was determined. In this method; 1.0 M NaCl was added to the same mixture in a 50 mM glycine buffer, pH 10.5. It was made in same conditions and with method of basal PON1.

MEASUREMENT OF AE ACTIVITY

Serum AE activity was measured using phenylacetate (Merck-Schuchardt, Munich, Germany) as a substrate by means of a spectrophotometer (Shimadzu UV160A, Japan), measuring phenol at 270 nm and 37°C. The assay mixture (3 ml) contained 1 mM phenylacetate, 0.9 mM CaCl2 and 5 µl of serum in 9 mM Tris-HCl buffer, pH 8.0. One unit of arylesterase hydrolyses 1.0 µmole of phenylacetate per minute. Serum dilution rates of 1:3-1:4 were used accordingly. Nonenzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. The intra and inter coefficient of variation (CV) was under 5.4 %.

OTHER BIOCHEMICAL PARAMETERS

The levels of triglycerides (TG), total cholesterol (TC), high density (HDL-C) and low density lipoprotein cholesterol (LDL-C) by auto analyzer (Beckman, CX7, USA) with standard enzymatic-colorimetric methods using commercial kits (Di-alab GmbH, Austria). LDL-C levels were calculated by the formula (LDL-K=TC-TRG/5-HDL-C).17

STATISTICAL METHODS

Data were analyzed using SPSS for Windows (Release 22 Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to determine whether the data were normally distributed and it showed a normal distribution. Therefore, parametric analysis methods were used. Means and SDs were used for descriptives. Binary comparisons were done by paired t test. It was used “Pearson test” for correlation analysis. It was used 0.05 values for significance.

RESULTS

PHYSICAL AND PHYSIOLOGICAL PROFILE

The results were given in tables below. Body weight increased significantly between TP and DTP, and then decreased after RTP, while BMI was only decreased at RTP. Height and CS increased significantly throughout the study (Table 1).

BLOOD LIPIDS AND LIPOPROTEINS (BLLP)

TC significantly increased between DTP and RTP (p<0.01) and TC value at RTP was higher than TP. TG at TP was greater than DTP (p<0.05) and RTP (p<0.01), but didn’t change between DTP and RTP. HDL-C didn’t change significantly at any period. LDL-C significantly increased in each period (p<0.01) (Table 2). But BLLP levels remained within normal ranges throughout season.

THREE DIFFERENT ACTIVITIES OF PON1 ENZYME (TDPON1)

AE activity of TP decreased after DTP (p<0.01) and increased after RTP (p<0.05) but, no significant difference was found between TP and RTP. Serum basal PON1 and SPON1 activities did not changed after each period significantly. Although the decrease in SPON1 activity was insignificant between TP and DTP, it was at an important level (23.9%) (Figure 2). (Table 3, Figure 1)

CORRELATIONS

At TP; between basal PON1 and SPON1 (r=0.64, p=0.048), between AE and basal PON1: (r=0.64, p=0.048), between AE and SPON1 (R=0.99, p=0.001). At DTP; between AE and PON1: (r=0.67, p=0.036), between AE and SPON1(r=0.76, p=0.011), between PON1 and SPON1 (r=0.90,
p=0.001). At RTP; between basal PON1 and SPON1 (r=0.99, p=0.001). Because of same enzyme, these relationships are expected correlations. No significant relationship was found between basal PON1, SPON, AE activity and including HDL-C and other BLLP parameters following periods.

![Table 1: Physical and physiological measurement data (Mean ± SD) and comparisons.](image)

![Table 2: Blood lipids and lipoproteins and comparisons.](image)

![Figure 1: AE activity during periods.](image)

![Figure 2: PON1 and SPON1 activity during periods.](image)
DISCUSSION

The main findings of the present study are; TP and RTP were increased AE activity independently from oxidative stress contrast to our hypothesis, but were not increased the other PON1 activities whereas DTP removed the beneficial training effects. (Table 3, Figure 1).

THE EFFECTS OF THREE SWIMMING PERIODS ON TDPON1 ACTIVITIES

Serum both AE and SPON1 activities in TP decreased in DTP. Thus, serum TDPON1 activities in DTP can be accepted as their baseline values (Figure 1 and Figure 2). As we know, there is no similar study which were been investigated the effects of a whole swimming season on PON1 activities. The present study may be the initial on human in literature. Therefore, we can not compare it with a similar study. However, the studies were showed that aerobic exercises were not affect SPON1 activity similarly to the present study. In a crosssectional study, unlike the present study, serum PON1 activity were significantly greater in trained adolescence athletes than their controls which is only the study in healthy children. Unlike above studies, both anaerobic wrestling exercise and a maximal exercise (ME) were increased PON1 activity in rugby players, but not AE activity. Unlike the present study (As activity measurement method), moderate aerobic exercise was increased serum PON1 activity in smokers; another aerobic cardiac therapy exercise was increased AE activity in the patients with CHD. Two-week-long supervised diet and aerobic exercise program was increased significantly PON1 activity, but not arylerastase activity in obese children. Similarly to the above study, no change in blood AE activity was observed significantly after aerobic exercise training in both diabetes mellitus and in healthy subjects. In a population study, no relation was seen in PON1 activity with physical activity. As seen, the variations in different PON1 activities due to exercise were usually similar in patients and healthy athletes. The differences between the studies may mainly due to intensity, duration, frequency and type of exercise used as well as PON1 polymorphism (As PON1-Q192R) and other factors in study design. Basal PON1 or SPON1 activity were used in the present study. The present study is the first study emphasizing the importance of activity measurement methods in literature that is important for both patients and healthy persons’ therapy by exercise and their follow up. The results would be clearly very different as in the above studies.

THE RESPONSES OF TDPON1 ACTIVITIES TO THREE SWIMMING PERIODS AND POSSIBLE MECHANISMS

It is well known that oxidative stress can inhibit or decrease PON1 activity. In our another study carried out with the same swimmers and the same periods of the present study; blood oxidant stress index (OSI) (Calculated as blood total oxidant status (TOS) / total anti oxidant status (TAS) ratio) and TOS levels at TP were greater than DTP and RTP (p<0.01), whereas TAS lowered significantly at TP

### TABLE 3: PON1, SPON1 and AE activities and TAS levels and comparisons.

<table>
<thead>
<tr>
<th>Activity</th>
<th>TP&amp;DTP</th>
<th>DTP&amp;RTP</th>
<th>TP&amp;RTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE (KU/L)</td>
<td>20.00</td>
<td>3.95</td>
<td>3.50**</td>
</tr>
<tr>
<td>SPON1 (U/L)</td>
<td>383.79</td>
<td>75.86</td>
<td>1.25</td>
</tr>
<tr>
<td>PON1 (U/L)</td>
<td>67.92</td>
<td>55.10</td>
<td>-0.35</td>
</tr>
</tbody>
</table>

Values were shown as means ± SD; TP: training period; DTP: detraining period, RTP: retraining period; AE: Arylesterase activity; PON1: basal paraoxonase activity; SPON1: salt-stimulated Paraoxonase activity; KU: Kilo unite; TAS: Total antioxidant status. *p<0.05, ** p<0.01.
according to DTP (p<0.05). TOS increased at RTP again (P<0.05), but not OSI. The TAS increase at RTP was insignificant, but the difference was moderately strong (d=0.77, pw=0.592) according to the statistical power analysis. It was assumed that the extreme oxidative stress at TP may be appeared due to national competition at the end of TP. Aerobic exercise can generate reactive oxygen species (ROS) that will occur oxidative stress. Therefore, the oxidative stress observed at TP can be the results of activation of the mitochondrial respiratory chain system triggered by intensive exercises. Serum basal TDPON1 activities were not related with TAS, TOS and OSI parameters throughout periods. These results show that the variations in AE activity were not affected from oxidative stress contrary to our hypothesis. However, although trained child swimmers exhibited increased oxidative stress, it was also shown that they have increased antioxidants and reduced risk of oxidative stress. This situation may be the result of increased antioxidant capacity which is enough to compensate the existing oxidative stress in the present study. HDL provides optimum physiological acceptor complex, in terms of both stimulating PON1 secretion and stabilizing secreted peptide. Therefore, both the lipid and the protein components of HDL are important for achieving optimum activity of PON1. Therefore, it is expected a relationship between PON1 and HDL-C as in literature. However, HDL-C levels were not related to TDPON1 activities and it didn’t also change significantly throughout periods. It was demonstrated that PON1 is not a fixed component of HDL and it has protective functions outside the lipoprotein environment. This property of PON1 may be the reason of finding no significant relationship between PON1 and HDL-C in our study. A primary determinant of serum PON1 level is the availability of the enzyme released by the liver, the principal site of PON1 production. Because of polymorphism, there is a wide range of serum concentrations and activities of PON1 in humans. AE activity is related to PON1 enzyme protein level and it can be also used as an indicator of PON1 enzyme protein. But AE activity was not affected from polymorphism like basal PON1 and SPON1 in the present study. Therefore, the increase in AE activity might be the result of an increase of PON1 protein levels due to the training. The difference in TDPON1 activities response in swimming periods may be mainly the rate-limiting step for phenylacetate since the rate of hydrolysis of phenylacetate was much more (about 1000 times) than paraoxon. Another reason may be different training thresholds for TDPON1 activities. Insignificant decrease found in SPON1 activity between TP and DTP (Figure 2) may be explained by such a threshold. Because TP’s training duration time was longer (8 months) than of RTP (two months). Although RTP increased TC and LDL-C levels, these parameters were lower at TP than RTP. Furthermore, BLLP levels remained within normal ranges throughout periods. Improvements in BLLP levels are generally related with physical composition and physiological improvements in these studies. However, in the present study HDL-C didn’t change significantly and the changes in BLLP levels were not related with the changes in physical composition and physiological improvements following the periods. Therefore, the main reason of absence of changes in body fat in swimmers and staying within normal ranges in the present study is similar to literature. As known, the atherosclerotic process precedes the clinical manifestations of CVD in years, or decades, whereas physical activity is an important protective factor for the prevention and control of CVD. Therefore, these variations in BLLP and AE activity as risk factors should be carefully monitored throughout a whole swimming season in child swimmers.

STUDY LIMITATIONS

Lack of swimmer participant situation was limited our investigation in PON1-Q192R polymorphism and PON1 protein level issues. Thus, a study with enough population of objects including PON1-
Q192R polymorphism also is recommended. At least in part, the results of this study can fill this deficiency in literature.

CONCLUSION

Both TP and RTP increased AE activity despite oxidative stress whereas DTP removed the beneficial effects. These results show that sedentary status is an important risk factor for atherosclerosis. The different measurement methods of PON1 activity may also affect the study results.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

REFERENCES


