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EFFECT OF ANAEROBIC TRAINING ON SERUM PARAOXONASE 1 (PON1) ACTIVITY AND ROLE OF PON1-L55M POLYMORPHISM

UČINEK ANAEROBNEGA TRENINGA NA AKTIVNOST SERUMSKE PARAOKSONAZE 1 (PON1) IN VLOGA POLIMORFIZMA PON1-L55M

ABSTRACT

Paraoxonase (PON) enzyme family (paraoxonase 1, 2 and 3) has antiatherosclerotic properties. The decreased PON1 enzyme activity (EA), PON1 level and PON1-L55M polymorphism (PON1P) are risk factors for atherosclerosis. Effects of anaerobic training on PON1 levels and the role of PON1P are unclear. In present study, the effects of anaerobic training on serum PON1 level, PON1EA, high density lipoprotein (HDL) and its subgroups' paraoxonase activities (HDLPON1EA, HDL2PON1EA, HDL3PON1EA) as well as the role of PON1P were investigated. The trained male athletes group (handball, basketball, volleyball) (AG: n=36, age=20.56±2.42 years) and the control group (CG: n=39, age=22.26±3.44 years) participated in this study. The PON1 and HDL's PON1 enzyme activities, the protein levels of PON1 enzyme, oxidized low-density lipoprotein (oxLDL) levels and the PON1P (from genomic DNA samples) were determined. Serum PON1EA, HDLPON1EA, HDL2PON1EA and HDL3PON1EA enzyme activities of the athletic homozygous LL and M carrier (Mc) groups were not significantly different from sedentary, however the indicated enzyme activities of the athletic LL homozygous group were significantly higher than athletic Mc group ($p < 0.05$). While the control genotype groups were compared, the control LL (CLL) genotype group had higher serum PON1EA (38.7%), HDLPON1EA (37.2%), HDL2PON1EA (41.9%) and HDL3PON1EA (33.1%) values than control Mc (CMc) genotype. These findings indicate that the genetically higher PON1EA and HDL and its subgroups' PON1EA in LL genotype group may have an important role in the beneficial effects of anaerobic training. However, the Mc genotype group was genetically negatively affected from anaerobic training. Therefore, it was concluded that high intensity training may be a risk factor for atherosclerosis in athletes with Mc genotype.

Keywords: Paraoxonase 1, Paraoxonase 1-L55M polymorphism, Anaerobic training, Atherosclerosis.

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IZVLEČEK

Encimska družina paraoksonaz (PON) – paraoksonaza 1, 2 in 3 – ima antiaterosklerotične lastnosti. Znižana encimska aktivnost PON1 (EA), raven PON1 in polimorfizem PON1-L55M (PON1P) predstavljajo dejavnike tveganja za aterosklerozo. Vplivi anaerobnega treninga na raven PON1 in vloga PON1P še niso povsem pojasnjeni. V tej študiji so raziskovali vplive anaerobnega treninga na serumsko raven PON1, encimsko aktivnost PON1 (PON1EA), lipoprotein visoke gostote (HDL) in paraoksonazno aktivnost njegovih podskupin (HDLPON1EA, HDL2PON1EA, HDL3PON1EA), kot tudi vlogo PON1P. V raziskavi so sodelovali moški športniki (rokomet, košarka, odbojka) – športna skupina (AG: n = 36, starost = 20,56 ± 2,42 let) in kontrolna skupina (CG: n = 39, starost = 22,26 ± 3,44 let). Določili so PON1 in HDL-odvisno encimsko aktivnost PON1, ravni beljakovin encima PON1, ravni oksidiranega lipoproteina nizke gostote (oxLDL) ter polimorfizem PON1P (iz genomskih DNA vzorcev). Serumske vrednosti PON1EA, HDLPON1EA, HDL2PON1EA in HDL3PON1EA pri športnikih z homozigotnim LL genotipom in nosilcih M alela (Mc skupina) niso bile statistično značilno različne od neaktivnih posameznikov. Vendar pa so bile omenjene encimske aktivnosti v športni skupini z LL genotipom statistično značilno višje kot pri športnikih Mc skupine ($p < 0,05$). Pri primerjavi genotipskih skupin v kontrolni skupini je imel LL genotip (CLL) višje vrednosti serumske PON1EA (za 38,7 %), HDLPON1EA (za 37,2 %), HDL2PON1EA (za 41,9 %) in HDL3PON1EA (za 33,1 %) v primerjavi z Mc genotipom (CMc). Ti izsledki kažejo, da ima genetsko višja PON1EA ter višja PON1 aktivnost HDL in njegovih podskupin pri osebah z LL genotipom lahko pomembno vlogo pri koristnih učinkih anaerobnega treninga. Nasprotno pa je bil Mc genotip genetsko neugodno prizadet zaradi anaerobnega treninga. Zato so raziskovalci zaključili, da je lahko visoko intenziven trening dejavnik tveganja za aterosklerozo pri športnikih z Mc genotipom.

Ključne besede: Paroksonaza 1, Paroksonaza 1-L55M polimorfizem, anaerobni trening, aterosklerozo

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INTRODUCTION

Coronary heart diseases (CHD) are among the leading main causes of death in the world. Low protein levels and activities of the paraoxonase enzyme family members (PON1, PON2 and PON3) are considered as a risk factor for CHD (Mackness et al., 1998). This multigene family is localized between q21.3 and q22.1 on the long arm of chromosome 7 in humans (Hegele, 1999). PON1 is the first identified and most studied member of this enzyme family (Deakin & James, 2004) and it has much more clarity compared to PON2 and PON3 (Li et al., 2003). It is known that PON1 has antioxidant, anti-inflammatory and antiatherosclerotic properties by protecting high-density lipoproteins (HDL) and low-density lipoproteins (LDL) from oxidation (Rajkovic et al., 2011; Priyanka et al., 2019). Oxidized low-density lipoproteins (oxLDL) causes endothelial dysfunction and poses a risk for coronary artery diseases (Levitan et al., 2010). Therefore, knowing PON1 and oxLDL levels is extremely important in predicting coronary artery disease.

PON1 has the ability to hydrolyze paraoxon (PON1 enzyme activity, PON1EA, PON1 activity) and phenylacetate (arylesterase activity, ARE) in addition to being able to hydrolyze the oxidized lipids of HDL and LDL (Gan et al., 1991). This enzyme mainly synthesised from the liver is located on HDL and its subgroups (HDLs: HDL2 and HDL3) found in the blood (Aviram et al., 1998; Deakin et al., 2002). Additionally, PON1 can dissociate from the HDL in physiological conditions and increased free PON1 is associated with diseases with high oxidative stress (OS) (Kontush et al., 2010). Studies showed that PON1 activity, HDL2 and HDL3 levels were associated with CHD (Mackness et al., 2003; Deakin & James, 2004). Therefore, knowing the PON1 activities of HDL and its subgroups may be important role to understand risk factors for CHD.

Factors such as age, diet, smoking, environmental conditions, disease status affect PON1 activity (James et al., 2000; Senti et al., 2001) however, its activity is mainly determined by polymorphisms. It has been shown that there are more than 160 polymorphisms in the coding region of the PON1 enzyme, introns that do not participate in protein coding and in the promoter region (Costa et al., 2005). Studies indicate that polymorphisms in the promoter region of PON1 T(-107)C, G(-824)A, G(-907)C have a strong effect on gene expression and enzyme levels in serum (Li et al., 2003). However, the most common polymorphisms due to amino acid changes in the coding region of PON1 occur in the 55th and 192nd codons. While the polymorphism in the 55th codon arises from the Leucine/Methionine (L/M) change, the polymorphism in the

192nd codon arises from the Glutamine/Arginine (Glu/Arg) change. A study on PON1-Q192R polymorphism revealed differences in PON1 activity according to Q and R alleles (Superko et al., 2012). Another study on the PON1-L55M polymorphism (PON1P) reported differences in PON1 activity and concentration according to the L and M alleles. Garin et al. (1997) showed that the amino acids sequence in leucine/methionine polymorphism at position 55 (L55M) has been associated with changes in PON1 serum concentrations and has also been reported to have an association with cardiovascular disease. In addition, it is known that PON1 polymorphisms are associated with CHD (Zhang et al., 2021).

Another important factor affecting PON1 activity is exercise. In studies investigating the relationship between exercise and PON, aerobic exercises (acute/chronic) were mostly examined. Most of these studies showed that aerobic training increased PON1 activities and protein levels (Mahdirejei et al., 2015; Taylor et al., 2015; Benedetti et al., 2018; Russo et al., 2018). In the study by Nalcakan et al. (2016) it was reported that aerobic exercises increased PON1 activity and especially in the QQ phenotype, PON1 activity was significantly higher than control group. There are also studies showed that regular aerobic training had not significantly effect on PON1 activities and protein levels (Silva et al., 2011; Kotani et al., 2012).

In the literature review, it is seen that the number of studies examining the relationship between anaerobic training and PON levels is quite limited. It is not clear to what extent PON enzymes are affected depending on the amount of OS created by high-intensity anaerobic training. In present study, the effects of anaerobic training on serum PON1 levels, PON1EA, HDL and its subgroups' paraoxonase activities (HDLPON1EA, HDL2PON1EA, HDL3PON1EA) as well as the role of PON1P were investigated.

METHODS

Participants

An athlete group (AG: n=36, age=20.56±2.42 years) consisting of healthy male aged between 18-35 years who regularly engage in anaerobic training (basketball, volleyball, handball), not being a smoker, alcohol, drugs or antioxidants users and a control group (CG: n=39, age=22.26±3.44 years) who have not exercised for at least 3-4 months, non-obese (Body mass index, BMI <30) joined in this study. The experimental procedures including possible risks were verbally explained to the participants after which they signed informed consent. The

present study was approved by the local university Ethics Committee and all data was collected in accordance with the Declaration of Helsinki.

Training details

AG trained for a total of 13 h, 5 days a week, 2,5 h/day. The training sessions included high-intensity running, technical-tactical exercises, vertical-horizontal jumpings, plyometrics, strength and power exercises (2 days/week) with high intensity loading. Additionally, aerobic and anaerobic endurance and speed training were executed.

Physical measurements

An electronic medical scale (Seca 769, Germany) was used for measuring the height and body weight of the participants and all measurements were done with shorts and without socks. BMI was calculated based on height and body weight by using $BMI = m \text{ (kg)} / h^2 \text{ (m}^2\text{)}$ formula (McArdle et al., 2000).

Blood Sampling and Analysis

Following the physical measurements venous blood samples were obtained in 9-mL serum vacuum tubes, one with EDTA and heparinized blood at rest 3-4 hours after lunch. Serum blood with EDTA samples and heparinized blood tubes were kept at room temperature for 20 minutes. Serum blood tubes for 10 minutes centrifuged at 2000g and serum samples were separated. The serum samples were stored in the freezer (-82°C) for the measurements of the following parameters. DNA samples required for polymorphisms were isolated from blood with EDTA. All biochemical variables were determined within 1 month after obtaining the serum samples.

Analysis of PON1 and oxLDL protein concentrations

Concentrations of PON1 and oxLDL were determined using enzyme-linked immunosorbent assays by measuring absorbance at 450 nm on a microplate reader (Diareader ELX800G; Dialab GmbH, Vienna, Austria). $\frac{1}{2}$ dilution was applied to serum samples for the analysis of PON3 and oxLDL levels. The commercial kits (Elabscience Biotechnology, Wuhan, China) were used for analysing oxLDL levels. Serum concentrations of PON1 and oxLDL were determined by means of standard curves constructed with purified PON and oxLDL proteins in a single batch. The coefficients of variation of PON1 and oxLDL were less than 10%.

Analysis methods of PON1 activity

Spectrophotometer device (Shimadzu UV 1700, Japan) was used to measure serum PON1 activity. Measurements of serum PON1 activity were conducted manually based on the method used by Eckerson et al. (1983). According to this method, serum PON1 activity was determined using paraoxon (diethyl p-nitrophenylphosphate, Sigma Chemical Co, St. Louis, MO, USA) as the substrate with an autoanalyzer (Modular DP, Japan). Paraoxon hydrolysis rates were determined by recording the absorbance at 412 nm and 37°C, which provided a measurement of p-nitrophenol release. PON1 activities were measured in 800-mL assay mixtures containing 1 mmol/L paraoxon, 1 mmol/L CaCl₂, and 5 mL of serum in 50 mmol/L TrisHCl buffer (pH 7.4), as described. One unit of paraoxonase activity was defined as 1 mmol p-nitrophenol formed per min under the above assay conditions.

Analysis HDL and it's subgroups' PON1 activity

HDL-containing (HDL and HDL3) supernatants were isolated from serum according to method of Kostner et al. (1985) depending on a differential precipitation process. The method is based on the selective precipitation of lipoproteins by varying the pH and the polyethylene glycol (PEG 20000) polymer (Ma: 20.000, Merck, Darmstadt, Germany) concentrations of the reagent. In this method, the cholesterol and PON1 activity levels of HDL2 were estimated by calculating the difference between HDL and HDL3.

Analysis of lipids and lipoprotein levels

Serum total cholesterol (TC) and serum tri-glycerides (TG) levels were measured according to standardized enzymatic methods by using commercial kits (Dialab GmbH Wien, Austria) and autoanalyzer (Modular DP, Roche Diagnostics, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was determined as described by Friedewald et al. (1972).

Determination of total antioxidant status (TAS) and total oxidant status (TOS)

Total antioxidant status (TAS) and total oxidant status (TOS) were measured from serum samples by using a microplate reader (Biotek Epoch2, USA) and commercial kits (Rel Assay Diagnostics, Turkey). In this method, by adding the dark blue-green ABTS radical to the sample, the substance combined with the antioxidants in the sample creates a new color. The change in absorbance seen at 660 nm is associated with the participant's total antioxidant level. The oxidants present in the sample oxidize the ferrous ion-chelate complex to ferric ion. Ferric ion forms a colored complex with chromogen in acidic medium. The color intensity which can

be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. It was given as mmol Trolox Eq/L for TAS and mmol H₂O₂ Eq/L for TOS. The ratio of serum TOS to TAS was accepted as oxidative stress index (OSI).

Distribution of PON1-L55M phenotypes

DNA isolation was carried out from blood samples with EDTA using a commercial kit (DNeasy Blood Tissue Kit, QIAGEN®-Hilden, Germany) and following the procedure specified in the kit. Genotyping: In order to investigate the SNP regions, the relevant region of the gene was amplified by PCR (SimpliAmp, Thermal Cycler, Singapore) and DNA sequence analysis was performed (MiSeq System, Illumina) and genotypes were determined.

PON1-Revers Primer: 5'- ACACTCACAGAGCTAATGAAAGCC -3'

PON1-Forward Primer: 5'- GAAGAGTGATGTATAGCCCCAG -3'

PON1 activity ratios were used to classify the phenotypes of each participant as LL, LM, MM. Due to the small number of MM and LM homozygote genotype groups, the M carrier (Mc = MM + LM) were combined to form carrier group in order to make the statistical analysis safer.

YOYO IR-1 test

Participants performed the YOYO IR-1 test which is an incremental increasing interval shuttle run test at track & field (Figure 1).

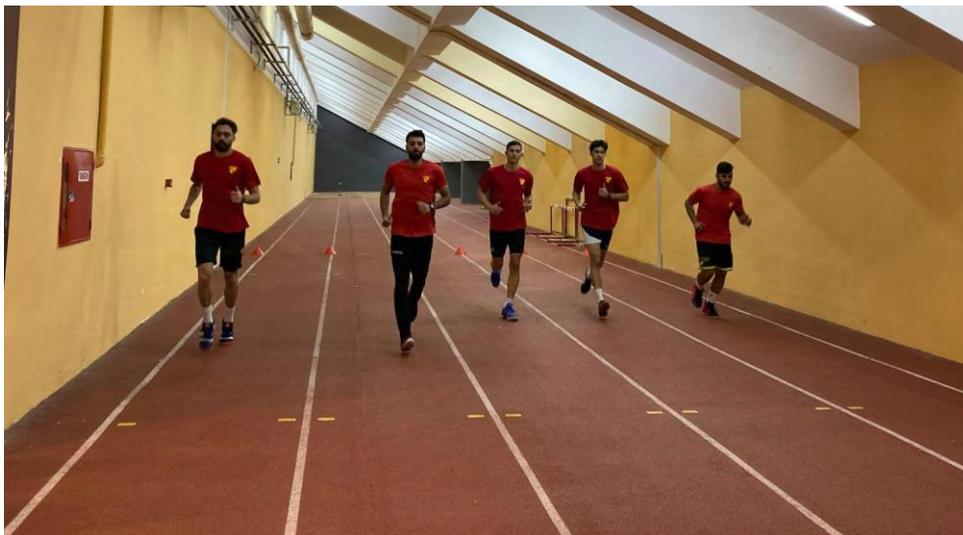


Figure 1. Image of participants during YOYO IR-1 test

In this test, shuttle runs of 2x20 m were run and active rest was carried out at a distance of 10 m (2x5m) for 10 seconds between each shuttle run (after the completed distance of 40m).

Individuals in the test were asked to adjust their speed according to the auditory signals. The test was terminated when the 40m distance was not completed even though the signal was received and the next 40m was not completed either (Bangsbo et al., 2008). This test was completed in 10-20 minutes depending on the condition level of the participants.

Statistical analyses

SPSS Windows version 24.0 package program was used in the statistical analysis. The Shapiro Wilk test was used to examine whether the obtained data showed a normal distribution. The Student t test was used to compare the normal data features in two independent groups and the Mann Whitney U test was used to compare the non-normal data features in two independent groups. Relationships between numerical variables were analysed with the Spearman correlation coefficient. In the comparison of numerical data in more than two independent groups, Kruskal Wallis test and Dunn multiple comparison test were used for non-normal data. As descriptive statistics, mean \pm standard deviation, median (min-max) were given for numerical variables, and number and % values were given for categorical variables. In addition, the effect of exercise and polymorphism interaction on all parameters were examined by two-way analysis of variance (ANOVA). The significance level was accepted as $p < 0.05$ in all statistical analyses.

RESULTS

VO₂max and YOYO values of athletic group (AG) were found higher than control group (CG) ($p < 0.05$). The oxLDL values of AG were higher compared to CG ($p < 0.05$). While no significant difference was found between AG and CG groups in TOS values ($p > 0.05$), TAS values of AG were lower than CG ($p < 0.05$). Moreover, OSI values of AG were higher than CG ($p < 0.05$). However, no difference was found between AG and CG in terms of PON1 protein levels and activities ($p > 0.05$) (Table 1).

Table 1. Physical, physiological and biochemical parameters of athletic and control groups (means±standard deviation).

	AG (N=36)	CG (N=39)	T	P
AGE (years)	20.56 ± 2.42	22.26 ± 3.44	2.45	0.016
HEIGHT (cm)	190.01 ± 7.61	178.81 ± 7.44	-6.44	0.001
WEIGHT (kg)	87.08 ± 14.25	77.2 ± 11.85	-3.27	0.002
BMI (kg/m ²)	24.03 ± 3.05	24.08 ± 2.77	0.06	0.949
YOYO (m)	1127.78 ± 504.5	857.95 ± 272.02	-2.91	0.005
VO ₂ max (ml)	45.87 ± 4.24	43.61 ± 2.29	-2.90	0.005
HDL2PON1EA (U/L)	40.59 ± 32.66	43.9 ± 44.27	0.36	0.716
HDL3PON1EA (U/L)	54.83 ± 38.72	50.8 ± 33.26	-0.48	0.629
HDLPON1EA (U/L)	95.42 ± 70.27	94.7 ± 72.77	-0.04	0.965
SERUMPON1EA (U/L)	107.09 ± 80.08	104.06 ± 81.98	-0.16	0.872
PON1 (ng/mL)	292.73 ± 315.12	355.75 ± 31.68	0.87	0.387
oxLDL (pg/mL)	279.94 ± 54.72	255.48 ± 39.42	-2.23	0.029
TAS (mmol/L)	0.87 ± 0.3	1.02 ± 0.25	2.35	0.021
TOS (μmol/L)	18.39 ± 3.7	17.59 ± 7.39	-0.58	0.560
OSI (TOS/TAS)	26.12 ± 19.9	18.29 ± 10.38	-2.16	0.034
TC (mg/dL)	156.36 ± 44.94	157.03 ± 26.32	0.08	0.937
LDL-C (mg/dL)	78.75 ± 25.06	85.38 ± 25.97	1.13	0.263
TG (mg/dL)	101.83 ± 52.18	118.79 ± 58.97	1.32	0.193
HDL-C (MG/DL)	50.83 ± 12.27	47.79 ± 8.81	-1.24	0.219

Notes: p<0.05, p<0.01; **AG** = Athletic group; **CG** = Control group; **BMI** = Body mass index; **HDL2PON1EA** = HDL2's paraoxonase 1 activity; **HDL3PON1EA** = HDL3's paraoxonase 1 activity; **HDLPON1EA** = HDL's paraoxonase 1 activity; **SERUMPON1EA** = Serum paraoxonase 1 activity; **PON1** = Paraoxonase 1; **oxLDL** = Oxidized low-density lipoprotein; **TOS** = Total oxidant status; **TAS** = Total antioxidant status; **OSI** = Oxidative stress index (TOS/TAS ratio); **TC** = Total cholesterol; **LDL-C** = Low-density lipoprotein cholesterol; **TG** = Total tri-glycerides; **HDL-C** = High-density lipoprotein cholesterol.

Paraoxonase findings of genotype groups

VO₂max and YOYO values of LL phenotype in athletes (ALL) were found higher compared to the control group (CLL) (p<0.01). No significant difference was found between the athletic and control groups in terms of PON1 protein levels and activities in the LL phenotype and M carrier group (p>0.05). Moreover, there was no significant difference between the athletic and control groups in terms of biochemical parameters in the LL phenotype and Mc carrier group (p>0.05) (Table 2).

Table 2. Comparison of biochemical parameters of ALL/CLL groups and AMc/CMc groups in PON1-L55M genotype (means±standard deviation).

	ALL (n=16)	CLL (n=16)	P	AMc (n=20)	CMc (n=23)	P
AGE (years)	20.41±3.03	21.15±2.01	0.273	20.64±2.64	23.65±4.14	0.037
HEIGHT (cm)	191.5±5.8	177.8±6.1	0.001	188.8±8.7	179.5±8.3	0.001
WEIGHT (kg)	86.43±8.95	75.89±9.22	0.003	87.6±17.61	78.11±13.51	0.053
BMI (kg/m ²)	23.58±2.51	24.02±2.67	0.634	24.4±3.44	24.12±2.9	0.774
YOYO (m)	1245±518.43	837.5±222.7	0.009	1034±485.63	872.17±305.75	0.192
VO ₂ max (ml)	46.86±4.36	43.43±1.87	0.007	45.09±4.08	43.73±2.57	0.208
HDL2PON1EA (U/L)	55.49±38.4	58.3±56.85	0.871	28.68±21.56	33.88±30.42	0.527
HDL3PON1EA (U/L)	69.65±41.45	63.16±30.29	0.617	42.98±32.76	42.2±33.11	0.938
HDLPON1EA (U/L)	125.13±79	121.46±78.83	0.896	71.66±53.2	76.08±63.45	0.807
SERUMPON1EA (U/L)	142.29±91.8	134.82±94.88	0.823	78.93±57.3	82.66±65.59	0.845
PON1 (ng/mL)	341.65±432.27	298.91±225.85	0.728	253.59±17.26	395.28±359.22	0.117
oxLDL (pg/mL)	270.8±56.28	248.69±38.18	0.203	287.25±53.75	260.21±40.42	0.067
TAS (mmol/L)	0.82±0.3	1.08±0.26	0.015	0.92±0.3	0.99±0.24	0.406
TOS (µmol/L)	17.82±3.48	18.39±7.3	0.781	18.85±3.89	17.04±7.57	0.341
OSI (TOS/TAS)	29.5±27.49	17.82±7.07	0.110	23.42±10.77	18.62±12.31	0.184
TC (mg/dL)	162.13±60.44	153.63±23.69	0.604	151.75±28.03	159.39±28.27	0.380
LDL-C (mg/dL)	75.31±24.01	79.69±23.24	0.604	81.45±26.17	89.35±27.5	0.342
TG (mg/dL)	100.44±59.69	129.13±70.81	0.225	102.95±46.9	111.61±49.56	0.561
HDL-C (mg/dL)	52.19±9.1	48.06±9.56	0.221	49.75±14.47	47.61±8.47	0.551

Notes: p<0.05, p<0.01; **ALL** = LL-phenotype in athlete group; **CLL** = LL-phenotype in control group; **AMc** = M-carriers phenotype (LM + MM) in athlete group; **CMc** = M-carriers phenotype (LM + MM) in control group; **BMI** = Body mass index; **HDL2PON1EA** = HDL2's paraoxonase 1 activity; **HDL3PON1EA** = HDL3's paraoxonase 1 activity; **HDLPON1EA** = HDL's paraoxonase 1 activity; **SERUMPON1EA** = Serum paraoxonase 1 activity; **PON1** = Paraoxonase 1; **oxLDL** = Oxidized low-density lipoprotein; **TOS** = Total oxidant status; **TAS** = Total antioxidant status; **OSI** = Oxidative stress index (TOS/TAS ratio); **TC** = Total cholesterol; **LDL-C** = Low-density lipoprotein cholesterol; **TG** = Total tri-glycerides **HDL-C** = High-density lipoprotein cholesterol

While LL phenotype and the M carrier group were compared in the athletic group, serum PON1 activity was lower in the M carrier group (p<0.05). Additionally, HDLPON1 activity of athletic group was found lower in the M carrier group than LL phenotype (p<0.05). Moreover, the PON1 activity of the HDL subgroups (HDL2PON1EA, HDL3PON1EA) of the M carrier group in the athletic group was lower than LL phenotype (p<0.05). While LL phenotype and the M carrier group were compared in the control group, no difference was found in terms of biochemical parameters (p>0.05) (Table 3). However, the control LL (CLL) genotype group had higher serum PON1EA (38.7%), HDLPON1EA (37.2%), HDL2PON1EA (41.9%) and HDL3PON1EA (33.1%) values than control Mc (CMc) genotype.

Table 3. Comparison of biochemical parameters of ALL/AMc groups and CLL/CMc groups in PON1-L55M genotype (means±standard deviation).

	ALL (n=16)	AMc (n=20)		CLL (n=16)	CMc (n=23)	
			P			P
AGE (years)	20.41±3.03	20.64±2.64	0.798	21.15±2.01	23.65±4.14	0.155
HEIGHT (cm)	191.5±5.8	188.8±8.7	0.282	177.8±6.1	179.5±8.3	0.472
WEIGHT (kg)	86.43±8.95	87.6±17.61	0.799	75.89±9.22	78.11±13.51	0.572
BMI (kg/m ²)	23.58±2.51	24.4±3.44	0.432	24.02±2.67	24.12±2.9	0.916
YOYO (m)	1245±518.43	1034±485.63	0.217	837.5±222.7	872.17±305.75	0.701
VO ₂ max (ml)	46.86±4.36	45.09±4.08	0.222	43.43±1.87	43.73±2.57	0.684
HDL2PON1EA (U/L)	55.49±38.4	28.68±21.56	0.012	58.3±56.85	33.88±30.42	0.090
HDL3PON1EA (U/L)	69.65±41.45	42.98±32.76	0.045	63.16±30.29	42.2±33.11	0.051
HDLPON1EA (U/L)	125.13±79	71.66±53.2	0.021	121.46±78.83	76.08±63.45	0.054
SERUMPON1EA (U/L)	142.29±91.8	78.93±57.3	0.016	134.82±94.88	82.66±65.59	0.068
PON1 (ng/mL)	341.65±432.27	253.59±178.26	0.446	298.91±225.85	395.28±359.22	0.667
oxLDL (pg/mL)	270.8±56.28	287.25±53.75	0.378	248.69±38.18	260.21±40.42	0.372
TAS (mmol/L)	0.82±0.3	0.92±0.3	0.327	1.08±0.26	0.99±0.24	0.285
TOS (μmol/L)	17.82±3.48	18.85±3.89	0.412	18.39±7.3	17.04±7.57	0.584
OSI (TOS/TAS)	29.5±27.49	23.42±10.77	0.370	17.82±7.07	18.62±12.31	0.815
TC (mg/dL)	162.13±60.44	151.75±28.03	0.499	153.63±23.69	159.39±28.27	0.508
LDL-C (mg/dL)	75.31±24.01	81.45±26.17	0.473	79.69±23.24	89.35±27.5	0.259
TG (mg/dL)	100.44±59.69	102.95±46.9	0.888	129.13±70.81	111.61±49.56	0.368
HDL-C (mg/dL)	52.19±9.1	49.75±14.47	0.561	48.06±9.56	47.61±8.47	0.877

Notes: p<0.05, p<0.01; **ALL** = LL-phenotype in athlete group; **AMc** = M-carriers phenotype (LM + MM) in athlete group; **CLL** = LL-phenotype in control group; **CMc** = M-carriers phenotype (LM + MM) in control group; **BMI** = Body mass index; **HDL2PON1EA** = HDL2's paraoxonase 1 activity; **HDL3PON1EA** = HDL3's paraoxonase 1 activity; **HDLPON1EA** = HDL's paraoxonase 1 activity; **SERUMPON1EA** = Serum paraoxonase 1 activity; **PON1** = Paraoxonase 1; **oxLDL** = Oxidized low-density lipoprotein; **TOS** = Total oxidant status; **TAS** = Total antioxidant status; **OSI** = Oxidative stress index (TOS/TAS ratio); **TC** = Total cholesterol; **LDL-C** = Low-density lipoprotein cholesterol; **TG** = Total tri-glycerides; **HDL-C** = High-density lipoprotein cholesterol.

No statistically significant difference was found between the 2 genotype groups in terms of L55M genotype distribution or their alleles frequencies (Table 4).

Table 4. Genotypes and alleles frequency of PON1-L55M polymorphism in athletic and control groups.

Polymorphism	Genotype /Allele	Group	AG (n = 36)	CG (n = 39)	Total (n = 75)	χ^2 /p
PON1-L55M	Genotype	LL	16 (44.4)	16 (41.0)	32 (42.7)	0.311/0.856
		LM	16 (44.4)	17 (43.6)	33 (44.0)	
		MM	4 (11.1)	6 (15.4)	10 (13.3)	
	Allele	L	48 (66.7)	49 (62.8)	97 (64.7)	
		M	24 (33.3)	29 (37.2)	53 (35.3)	

Notes: p<0.05; Note: AG, CG, and Total are presented as number (%). Abbreviations: AG = athletic group; CG = control group; L = leucine; M = methionine; PON = paraoxonase.

Important correlations

In athletic group; A significant correlation was found between YOYO and oxLDL ($r=-0.391$; $p<0.05$). In the ALL group; a significant correlation was found between serum PON1EA and HDLPON1EA ($r=0.991$; $p<0.001$). Additionally, PON1 activities of HDL subgroups were also

correlated with each other. Significant correlations were found between HDLPON1EA and HDL2PON1EA ($r=0.988$; $p<0.001$) and HDL3PON1EA ($r=0.990$; $p<0.001$). Also, a significant correlation was found between HDL2PON1EA and HDL3PON1EA ($r=0.958$; $p<0.001$). In AMc group; a significant correlation was found between serum PON1EA and HDLPON1EA ($r=0.995$; $p<0.001$) and TBARS ($r=-0.499$; $p<0.05$). Significant correlations were found between HDLPON1EA and HDL2PON1EA ($r=0.969$; $p<0.001$) and HDL3PON1EA ($r=0.987$; $p<0.001$). Also, a significant correlation was found between HDL2PON1EA and HDL3PON1EA ($r=0.915$; $p<0.001$).

In control group; No significant correlation was found between parameters in the control group. In the CLL group; a significant correlation was found between serum PON1EA and HDLPON1EA ($r=0.991$; $p<0.001$). Moreover, PON1 activities of HDL subgroups were also correlated with each other as athletic group. Significant correlations were found between HDLPON1EA and HDL2PON1EA ($r=0.952$; $p<0.001$) and HDL3PON1EA ($r=0.817$; $p<0.001$). A significant correlation was found between HDL2PON1EA and HDL3PON1EA ($r=0.599$; $p<0.05$). In CMc group; a significant correlation was found between serum PON1EA and HDLPON1EA ($r=0.997$; $p<0.001$). Significant correlation was detected between HDLPON1EA and HDL2PON1EA ($r=0.999$; $p<0.001$). Moreover, a significant correlation was found between HDLPON1EA and HDL3PON1EA ($r=0.999$; $p<0.001$). Also, a significant correlation was found between HDL2PON1EA and HDL3PON1EA ($r=0.995$; $p<0.001$).

DISCUSSION

The main finding of the present study was that no significant difference was found between AG and CG in terms of PON1 protein level and activity. However, serum PON1EA, HDL2PON1EA, HDL3PON1EA and HDLPON1EA values were found significantly higher in athletic LL phenotype (ALL) compared to the M carrier group (AMc) ($p<0.05$). No difference was found between the LL phenotype in the control group (CLL) and the M carrier groups (CMc). In addition, there were no significant differences between the AG and CG groups in terms of TG, TC, HDL-C and LDL-C levels ($p>0.05$) whether polymorphisms were taken into account or not.

The effects of anaerobic training on PON1 protein levels and activities

While genotypes are not taken into consideration there were no significant differences in the PON1 protein levels and activities between athletes and control groups ($p > 0.05$) although OSI (including oxLDL) levels were higher ($p < 0.05$). These findings can be interpreted as anaerobic training have no effect on athletes' PON1 protein level and serum PON1 activity but increases OSI (including oxLDL). There are many studies in the literature stating that exercise increases PON1 protein levels and activities. However, those studies showed the effect of aerobic not anaerobic exercises/training on PON1. Sang et al. (2015) showed that walking and low-intensity running training performed 5 times a week for 10 weeks increased PON1 activity. Another study showed that PON1 protein level and activity increased significantly after a single session of aerobic exercise on the treadmill (Taylor et al., 2015). There is a study in the literature reporting that PON1 protein levels increased after ultramarathon running (Benedetti et al., 2018). Russo et al. (2018) stated that aerobic training for 3 months increased PON1 activity. There are other studies reporting that endurance training and walking exercises significantly increased PON1 protein levels and activity (Kotani et al., 2012; Mahdirejei et al., 2015). A study showed that PON1 concentration and activity increased after an endurance test performed on a treadmill (Otaska-Kmiecik et al., 2021). In addition, another study stated that repeated endurance tests (3 endurance tests) performed on the treadmill increased PON1 activity (Otaska-Kmiecik et al., 2023). However, in the studies mentioned above the effects of aerobic processes have been revealed and it has been shown that low-medium intensity exercises (acute/chronic) have a positive effect on PON1. In our study, the effect of long-term training (chronic anaerobic training) in handball, basketball and volleyball branches where anaerobic processes are at the forefront, on PON1 was investigated. There are also studies in the literature reporting the positive effect of high-intensity exercises on PON1. Zibad et al. (2015) reported that strength training performed with the intensity in 80% of 1 repetition maximum increased the activity of PON1. Turgay et al. (2015) showed that long-term anaerobic exercise trainings increase PON1 activity. However, TaheriChadorneshin et al. (2017) stated that the effect of maximum effort training on PON1 activity and lipid profile have not yet clear. In our study, we examined anaerobic training which includes significantly intense high-intensity exercises and found that these trainings had no effect on PON1 protein level and activity. The main reason for this may be that the OSI values of the athletic group were higher than the control group. The high OSI value may have suppressed the PON1 increase.

The genotype groups' serum PON1 levels and activities profile (the role of PON1 polymorphism-PON1P)

In this present study, serum PON1EA, HDL2PON1EA, HDL3PON1EA and HDLPON1EA values were significantly higher in athletic LL phenotype (ALL) than M carrier group (AMc) ($p < 0.05$). However, in control group, there was no significant difference between the LL phenotype (CLL) and the M carrier groups (CMc) ($p > 0.05$). These findings show that the M carrier group was affected negatively by anaerobic training for the specified parameters whereas the LL group affected positively in terms of both serum PON1EA and PON1 activities of HDL subgroups. In this respect, our study findings are similar to the study results of Yigittürk et al. (2020). In the study by Yigittürk et al. (2020) examining the effect of hypoxic training on PON1 and the role of PON1-Q192R and PON1-L55M polymorphisms, it was stated that training under hypoxic conditions the PON1 activity was increased, especially in the QQ and LL groups compared to carrier groups. However, unlike our study they examined the effect of training under hypoxic conditions.

The mentioned differences between the genotype groups for serum PON1 protein levels and serum or HDL subgroups' PON1 activities may mainly be due to the PON1P. It is well known that low PON1 protein levels and enzyme activity are related to the CHD. In the light of this information, it can be concluded that athletes in the LL genotype group may have an advantage in terms of prevention of CHD when they perform high intensity exercises such as jumping, interval running, strength, speed and plyometric training which are used in basketball, volleyball and handball. On the contrary it can be said that M carrier group is negatively affected by anaerobic training and thus has risk for CHD due to the decrease in PON1 protein level and activity with these training. Therefore, future studies are needed to investigate training protocols for the prevention of CHD in the M carrier group.

CONCLUSION

These findings indicate that LL genotype had higher serum PON1EA. LL genotype group also had higher HDL and its subgroups' PON1EA. Therefore, it can be said that the LL genotype group performing anaerobic training is more advantageous in preventing CHD. However, the M carrier genotype group was genetically negatively affected from anaerobic training and this may be considered as a risk factor for CHD. It can be concluded that carry out studies on special training protocols to protect the M carrier genotype group which is negatively affected by

anaerobic training from CHD by reducing the potential risks related to OS. In addition, findings of present study show that anaerobic training has no significant effect on all lipids and lipoprotein levels. Moreover, this study indicates that the PON1P is not related to all lipid and lipoprotein concentrations. In this study, the sample group was limited to athletes in anaerobic sports branches such as basketball, volleyball and handball. It is recommended to conduct further studies with a large number of participants in different branches, with different gender, age, diet, environmental conditions.

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Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Data availability statement

The datasets generated for this study are available on request to the corresponding author.

Ethics approval

This project was approved by the Ege University Medical Research Ethics Committee, İzmir, Turkey with 20-10T/48 reference number. All data was collected in accordance with the Declaration of Helsinki. Participants were informed about the details of the study and all provided written informed consent.

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