

Original article

Effects of aerobic training on serum paraoxonase activity and its relationship with PON1-192 phenotypes in women

Gulbin Rudarli Nalcakan ^{a,*}, S. Rana Varol ^a, Faruk Turgay ^a, Mesut Nalcakan ^b,
M. Zeki Ozkol ^a, S. Oguz Karamizrak ^c

^a Coaching Education Department, School of Physical Education and Sports, Ege University, Izmir 35100, Turkey

^b Esrefpasa Hospital, Izmir 35520, Turkey

^c Medical Faculty, Sports Medicine Department, Ege University, Izmir 35100, Turkey

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Abstract

Background: Paraoxonase 1 (PON1) is an antioxidant enzyme that protects high-density lipoprotein (HDL) and low-density lipoprotein against oxidation. Limited studies have addressed the influence of exercise on PON1 activity and its relationship with PON1 phenotypes. We investigated relationships between PON1-192 phenotypes, PON1 activity, aerobic exercise, and blood lipid and lipoprotein concentrations in middle-aged women.

Methods: An exercise group ($n = 50$) engaging in regular aerobic exercise and a control group ($n = 41$) were selected from a subset of 300 Caucasian women that met the inclusion criteria. Serum PON1, salt-stimulated PON1 (SSPON1), and arylesterase (ARE) activities; cholesterol levels and ARE activities of total HDL and HDL subgroups (HDLs) (supernatants obtained by polyethylene glycol); and blood lipid and lipoprotein concentrations were determined by standardized enzymatic methods. PON1-192 QQ (low activity), QR (moderate activity), and RR (high activity) phenotype groups were defined using serum SSPON1/ARE activity ratios. The R-carriers (RC) phenotype group consisted of the QR and RR groups combined.

Results: All lipid and lipoprotein concentrations were greater in the exercise group than in the control group. Regardless of phenotype, no significant differences were observed between the exercise and control groups in terms of serum PON1, SSPON1, or ARE activity associated with HDLs ($p > 0.05$), whereas PON1 activities in QQ-phenotyped women in the exercise group were significantly higher than those in the control group ($p < 0.01$), but not the RC group. A statistically significant interaction between PON1 phenotypes (QQ and RC groups) and exercise (exercise and control groups) on PON1 activity was found.

Conclusion: These results showed that a regular aerobic exercise program can improve PON1 activity depending on PON1-192 phenotype, but not on lipid and lipoprotein levels, in middle-aged Turkish women.

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Keywords: Aerobic exercise program; Arylesterase; Lipids; Lipoproteins; Paraoxonase; PON1-192 phenotype; Women

1. Introduction

Atherosclerosis is the main cause of death in developed countries. Regular aerobic exercise program reduces the risk of developing cardiovascular disease (CVD) by increasing serum high-density lipoprotein cholesterol (HDL-C) levels.¹ Paraoxonase 1 (PON1) prevents the oxidation of both HDL and low-density lipoprotein (LDL), impeding the development of atherosclerosis. The anti-oxidative property of HDL has been partly attributed to serum PON1 enzyme.²

PON1 enzyme hydrolyzes both paraoxon and phenylacetate substrates, which are referred to in the literature as paraoxonase and arylesterase (ARE) activities, respectively. PON1 can hydrolyze the oxidized lipids of LDL and HDL, which are known to promote atherosclerosis. PON1 enzyme is produced mainly in the liver and is released into the blood.^{2,3} HDL facilitates the secretion of PON1 from the liver into the blood and stabilizes the enzyme. PON1 is located on HDL and its subfractions (HDLs: HDL₂ and HDL₃) were found in the blood.⁴

PON1 activity can be affected by genetic factors, life style choices, disease status, and HDL-C levels;⁵ however, its activity is mainly determined by polymorphisms in the *PON1* gene. The *PON1* gene has several genetic polymorphisms, 1 set of which occurs in codon 192 (referred to as PON1-192 polymorphisms),

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* Corresponding author.

E-mail address: gulbinrn@gmail.com (G.R. Nalcakan).

whose Q and R alleles are associated with low and high PON1 activity, respectively.⁶

It was reported that PON1 activity, HDL₂-C, and HDL₃-C are related to CVD⁵⁻⁷ and that both serum PON1^{7,8} and ARE activities^{8,9} are lower in individuals with CVD than in controls. Similarly, it was found that blood HDL-ARE activities are lower in women with CVD than in controls and that these parameters are more predictive for CVD than HDL-C levels.¹⁰ In addition, ARE activity was shown to be polymorphism-independent.¹¹

Exercise is another major factor that affects PON1 activity.^{9,12,13} Therefore, to protect against CVD, it is important to monitor one's serum PON1 and HDLs-ARE activities as well as cholesterol levels, with the goal of improving these activities through an appropriate exercise program.

Tomás and coworkers¹³ reported that acute and 4-month exercise programs did not affect salt-stimulated PON1 (SSPON1) activity in a group of healthy Spanish men and women athletes, regardless of PON1-192 polymorphism. However, when PON1-192 polymorphism was considered, both types of exercise increased SSPON1 activity, and the effects of exercise on SSPON1 activity were modulated by PON1-192 polymorphism. Results from a separate study showed that PON1 activity increased significantly after maximal exercise independently of PON1-192 polymorphisms in rugby players, but ARE activity was not affected by exercise.¹² In a cross-sectional study, blood SSPON1 and ARE activities, as well as the distribution of PON1-192 genotypes, did not differ between endurance athletes and controls.¹⁴ Conflicting effects of genotype and exercise on PON1 activity and their relationships with PON1-192 polymorphisms were seen among athletes. Moreover, conflicting results were also observed with non-athlete women and men with CVD.⁹ The reasons underlying such discrepancies are unclear, as only a single study investigating the influence of PON1-192 phenotypes on PON1 activity is found in the literature.¹⁴

In addition, in both humans and mice, it was shown that PON1 activity is greater in women than in men,^{9,15} and it was reported that PON1 is regulated at the mRNA level in a gender-specific manner by some pro-inflammatory and anti-inflammatory substances.¹⁵

In parallel, it was also reported that estradiol may regulate the specific activity and/or stability of cell surface PON1.¹⁶ In most studies,^{8,9,14} blood ARE activities has been measured, which are known to be independent of PON1-192 polymorphisms. Thus, ARE and PON1 measurement methods and gender differences may influence the interpretation of results when examining the effects of PON1-192 phenotypes.

PON1 activity varies widely between individuals within the same genotype group and across ethnic variations.^{5,17} Therefore, similar studies may yield different results with healthy women and Turkish populations with a different ethnic group, although no such study has been published yet.

Furthermore, it is reported that the effect of physical activity on HDL-C concentration is related to PON1-192 polymorphisms.¹⁸ However, some studies have failed to demonstrate associations between PON1-192 phenotype and lipoprotein changes,^{19,20} while Hegele and coworkers²¹ found significant

associations between PON1-192 genetic variants and plasma HDL-C and triglyceride levels. Thus, the relationships between PON1 phenotypes and all blood lipid and lipoprotein concentrations are unclear, and these relationships have not been reported for Turkish subjects. Our hypothesis was that a regular aerobic exercise program can increase PON1 activity and lipid and lipoprotein concentrations depending on PON1-192 phenotype in middle-aged Turkish women. To test our hypothesis, we planned this cross-sectional study to investigate the relationships between PON1-192 phenotypes and regular aerobic exercise, PON1 and ARE activities, and all lipid and lipoprotein concentrations in middle-aged women.

2. Methods

2.1. Subjects

Three hundred women volunteers completed an anamnesis questionnaire providing information regarding their basic demographics, medical history, medication usage, frequency of smoking and alcohol consumption, and amount of physical activity. One hundred and sixty women met the inclusion criteria and were selected for medical examination. These criteria included (a) being ≥ 32 years of age and having regular menstrual cycles; (b) not being anemic or actively infected; (c) being free of illnesses predisposing to CVD; (d) not being a smoker and/or an alcohol user; (e) not taking medication affecting lipid, lipoprotein, or antioxidant metabolism; and (f) agreeing to participate in an exercise group (EG, $n = 50$) for 1 h for 3 days per week for at least 3 months, involving a supervised aerobic exercise program (step aerobics). The control group (CG, $n = 41$) included habitually active women (exercising less than 1 h/week) that were not engaged in a structured training program, nor had they been so engaged for at least 3 months prior to the study. Following medical history inquiries, physical examinations, and blood testing, 91 women met the inclusion criteria (Table 1).

Participants were informed about the details of the study and the probable risks and all provided written informed consent. The study was approved by the Ege University Medical Faculty's Ethics Committee.

2.2. Physical and physiological measurements

Physical examination consisted of anamnesis, ECG, anthropometry (height, weight, and body mass index (BMI)), and percentage of body fat (%BF). Body density and %BF values were calculated using widely recognized equations included in the book of Ratames and American College of Sports Medicine.²²

Physiological measurements included resting heart rate (HR), blood pressure (BP), and maximum oxygen consumption (VO_{2max}). VO_{2max} was determined by respiratory gas analysis (Quark b²; COSMED, Rome, Italy) on a cycle ergometer (Monark, Varberg, Sweden), using gradually increasing loads. VO_{2max} measurements were confirmed when 3 or more of the following criteria were met: (1) a plateau in VO_2 despite an increase in workload, (2) a respiratory exchange ratio higher than 1.2, (3) a peak HR at least equal to 90% of the age-predicted maximum, and/or (4) visible exhaustion.²³

Table 1
Physical and physiological features, serum lipid and lipoprotein levels, PON1, SSPON1, and ARE activities of exercise and control groups (mean \pm SD).

Parameters	EG (n = 50)	CG (n = 41)
Physical and physiological features		
Age (year)	41.0 \pm 4.3	39.5 \pm 4.4
BMI (kg/m ²)	25.3 \pm 2.5	24.5 \pm 3.6
%BF [†]	23.9 \pm 3.2	23.8 \pm 3.9
VO _{2max} (mL/min/kg) [†]	24.1 \pm 3.6 ^c	18.9 \pm 3.5
Resting HR (bpm)	70.8 \pm 8.7 ^b	75.0 \pm 5.9
SBP (mmHg) [†]	118.4 \pm 11.9	116.3 \pm 6.7
DBP (mmHg) [†]	75.4 \pm 9.1	73.3 \pm 6.1
Serum lipid and lipoprotein levels		
TC (mmol/L)	5.27 \pm 0.81 ^c	4.66 \pm 0.73
LDL-C (mmol/L)	3.49 \pm 0.74 ^b	2.93 \pm 0.93
HDL-C (mmol/L)	1.32 \pm 0.26 ^a	1.21 \pm 0.23
TG (mmol/L) [†]	1.06 \pm 0.57	0.91 \pm 0.39
HDL ₂ -C (mmol/L)	0.40 \pm 0.17	0.37 \pm 0.14
HDL ₃ -C (mmol/L)	0.92 \pm 0.16 ^a	0.83 \pm 0.20
TG/HDL-C [†]	0.80 \pm 0.51	0.75 \pm 0.38
PON1, SSPON1, and ARE activities		
PON1 (U/L) [†]	66.9 \pm 49.8	73.4 \pm 59.7
SSPON1 (U/L) [†]	307.2 \pm 210.9	373.5 \pm 247.1
ARE (KU/L)	128.6 \pm 36.6	121.5 \pm 29.0
HDL-ARE (KU/L)	115.7 \pm 25.2	109.1 \pm 23.9
HDL ₃ -ARE (KU/L)	78.8 \pm 22.3	69.5 \pm 25.6
HDL ₂ -ARE (KU/L)	36.9 \pm 15.0	39.7 \pm 16.8
SSPON1/ARE [†]	2.45 \pm 1.63	3.12 \pm 2.11

[†]Variables analyzed with Mann–Whitney *U* test.

^a *p* < 0.05, ^b *p* < 0.01, ^c *p* < 0.001, compared with CG.

Abbreviations: %BF = percentage of body fat; ARE = serum arylesterase activity; BMI = body mass index; CG = control group; DBP = diastolic blood pressure; EG = exercise group; HDL-ARE = HDL's arylesterase activity; HDL₂-ARE = HDL₂'s arylesterase activity; HDL₃-ARE = HDL₃'s arylesterase activity; HDL-C = high density lipoprotein cholesterol; HDL₂-C = HDL₂'s cholesterol level; HDL₃-C = HDL₃'s cholesterol level; HR = heart rate; LDL-C = low density lipoprotein cholesterol; PON1 = serum paraoxonase 1 activity; SBP = systolic blood pressure; SSPON1 = serum salt-stimulated paraoxonase 1 activity; TC = total cholesterol; TG = triglyceride; VO_{2max} = maximum oxygen consumption.

2.3. Blood sampling and analysis

Participants were instructed not to exercise, not to change their diet, and not to take any medication or supplements for at least 48 h prior to blood sampling. Overnight-fasting venous blood samples were drawn between 08:30 a.m. and 10:00 a.m. The sample-collection day was selected according to the women's menstrual cycles.

Blood samples were allowed to clot at 25°C for 30 min and centrifuged at 2000 *g* for 10 min. Hematological analyses were performed with a hematology analyzer (BC-3000 Plus; Mindray, Shenzhen, China). Serum samples were stored at -82°C until analysis. The analyses were performed within 1 month.

2.3.1. Assay of HDL, HDL₂, and HDL₃ supernatants (HDLs)

Supernatants of HDL and HDL₃ were separated from serum samples using polyethylene glycol (Ma: 20.000; Merck, Darmstadt, Germany) according to the differential precipitation method.²⁴ Serum baseline PON1, SSPON1, and ARE activities, as well as cholesterol and ARE activities of HDLs were analyzed on the same day by the following methods. The chole-

sterol and ARE activity levels of HDL₂ were estimated by calculating the difference between HDL and HDL₃.

2.3.2. Analysis of cholesterol and triglyceride levels

The cholesterol contents of HDLs (HDL-C, HDL₂-C, and HDL₃-C), serum total cholesterol (TC) and serum triglycerides (TG) levels were determined using standardized enzymatic methods with commercial kits (Dialab GmbH, Wien, Austria) with an autoanalyzer (Modular DP; Roche Diagnostics, Tokyo, Japan). LDL-C was calculated as described by Friedewald et al.²⁵

2.3.3. Analysis of PON1 activities

Serum PON1 and SSPON1 activities were determined using paraoxon (diethyl *p*-nitrophenylphosphate; Sigma Chemical Co., St. Louis, MO, USA) as the substrate with an autoanalyzer (Modular DP), as described previously.^{11,26} 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 μ L assay mixtures containing 1 mmol/L paraoxon, 1 mmol/L CaCl₂, and 5 μ L of serum in 50 mmol/L Tris–HCl buffer (pH 7.4), as described.¹¹ One unit of paraoxonase activity was defined as 1 μ mol *p*-nitrophenol formed per minute under the above assay conditions.

For SSPON1 assays,²⁶ the 800 μ L assay mixture consisted of 1 mmol/L paraoxon, 1 mmol/L CaCl₂, 5 μ L of serum, and 1 mol/L NaCl in 50 mmol/L glycine buffer (pH 10.5). Results are expressed as U/L for both PON1 and SSPON1. The within-run variation constants (CVs) were <1.5% for both enzyme assays.

2.3.4. Analysis of ARE

ARE activities associated with serum ARE and HDLs (HDL-ARE and HDL₃-ARE) were determined using phenylacetate substrate (Merck-Schuchardt, Darmstadt, Germany) by measuring phenol concentrations at 270 nm and 37°C with a UV–visible spectrophotometer (UV160A; Shimadzu, Kyoto, Japan), as described previously.¹¹ The 3 mL assay mixture contained 1 mmol/L phenylacetate, 0.9 mmol/L CaCl₂, and 5 μ L of serum in 9 mmol/L Tris–HCl buffer (pH 8). One unit of ARE hydrolyzes 1 mmol of substrate per minute is represented as (KU/L) of ARE. All ARE activity measurements were performed within the same assay. Within-run variation constants (CVs) were <5.5%.

2.3.5. Distribution of PON1-192 phenotypes

The paraoxonase phenotype distribution among the subjects was determined using a dual-substrate (paraoxon and phenylacetate) method.¹¹ SSPON1/ARE activity ratios were used to classify the phenotypes of each participant as EG, CG, QQ (low paraoxonase activity), QR (moderate paraoxonase activity), or RR (high paraoxonase activity) phenotypes. The RR and QR groups together were defined as R-carriers (RC).

2.4. Statistical analyses

Data were analyzed using the SPSS program Version 15.0 (SPSS Inc., Chicago, IL, USA), following normality

(Kolmogorov–Smirnov Test) and homogeneity (Levene Test) testing. Natural logarithmic transformation was performed on dependent variables to normalize the non-normal data and meet the homogeneity of variance assumption for parametric tests. The interactions between PON1-192 phenotype and exercise for dependent variables were assessed using a 2 × 2 (main group × phenotype subgroups) 2-way analysis of variance (ANOVA). One-way ANOVA with the *post hoc* least significance difference (LSD) test was used to compare dependent variables between the EG phenotype subgroups (EQQ, EQR, ERR, and ERC) and CG phenotype subgroups (CQQ, CQR, CRR, and CRC). Differences in the dependent variables investigated between the EG and CG were assessed by performing an unpaired Student's *t* test. In the case of non-normal distributions, nonparametric versions of the Kruskal–Wallis *H* test and Mann–Whitney *U* test were used for statistical analysis. Dependent variables analyzed with non-parametric tests were identified using the “†” symbol in the tables. Relationship levels between the variables investigated were determined either by calculating Pearson Product-Moment Correlation Coefficients (raw data were used for variables having normal distributions and log transformed data were used for variables that met the normality assumption after log-transformation) or Spearman's Rank Correlation Coefficient (raw data were used for non-normal variables).

3. Results

Participants with SSPON1/ARE ratios ≤1.93, between 2.45 and 4.89, or ≥5.48 were defined as individuals having QQ (low activity), QR (moderate activity), or RR (high activity) phenotypes, respectively. Thus, 46 participants (50.5% of all participants) had the QQ (low activity) phenotype, 10 (11.0%) had the RR (high activity) phenotype, and 35 (38.5%) belonged to the QR (moderate activity) phenotype group. The RC phenotype group consisted of QR and RR groups combined, to obtain statistically reliable results. The trimodal frequency distribution of participants in this study was similar to that observed in other Caucasian groups.^{1,2,27}

3.1. Physical and physiological characteristics of participants

EG and CG subjects displayed similar anthropometric characteristics. The mean training experience for the EG was 3.12 ± 3.29 years. VO_{2max} (*p* < 0.001) and resting HR (*p* < 0.01) values were statistically different between EG and CG (Table 1). Considering phenotyping, VO_{2max} was significantly higher in EQQ (*p* < 0.001) and ERC (*p* < 0.001) than those in CQQ and CRC, respectively; resting HR was significantly lower in EQQ (*p* < 0.05) and ERC (*p* < 0.05) than those in CQQ and CRC, respectively (Table 2).

Table 2

Serum PON1, SSPON1, ARE activities, and lipid and lipoprotein levels in exercise and control groups according to PON1-192 phenotyping (mean ± SD).

Parameters	EG				CG			
	EQQ (<i>n</i> = 29)	EQR (<i>n</i> = 18)	ERR (<i>n</i> = 3)	ERC (<i>n</i> = 21)	CQQ (<i>n</i> = 17)	CQR (<i>n</i> = 17)	CRR (<i>n</i> = 7)	CRC (<i>n</i> = 24)
Physical and physiological features								
Age (year)	41.8 ± 4.05	39.3 ± 4.54	43.0 ± 2.65 ^b	39.8 ± 4.47	41.1 ± 4.30	39.2 ± 4.61	36.6 ± 2.51 ^b	38.4 ± 4.23
VO _{2max} (mL/min/kg)	24.3 ± 3.91 ^c	23.8 ± 3.58 ^b	23.4 ± 1.31 ^a	23.8 ± 3.33 ^c	18.3 ± 3.70	19.4 ± 3.70	19.5 ± 2.31	19.4 ± 3.30
Resting HR (bpm)	70.5 ± 7.91 ^a	71.9 ± 9.84	67.3 ± 11.6	71.3 ± 9.92 ^a	75.7 ± 5.98	74.5 ± 6.36	74.9 ± 4.81	74.6 ± 5.85
Lipid and lipoprotein levels								
TC (mmol/L)	5.37 ± 0.84 ^b	5.09 ± 0.71	5.36 ± 1.13	5.13 ± 0.76 ^a	4.68 ± 0.62	4.77 ± 0.77	4.32 ± 0.90	4.64 ± 0.81
LDL-C (mmol/L)	3.58 ± 0.75 ^a	3.29 ± 0.64	3.51 ± 1.32	3.32 ± 0.72	2.80 ± 1.17	3.08 ± 0.73	2.86 ± 0.78	3.01 ± 0.73
HDL-C (mmol/L)	1.30 ± 0.26	1.35 ± 0.27	1.33 ± 0.08 ^a	1.35 ± 0.25 ^a	1.21 ± 0.26	1.24 ± 0.23	1.09 ± 0.12	1.20 ± 0.22
TG (mmol/L)	1.08 ± 0.68	0.99 ± 0.36	1.13 ± 0.61	1.01 ± 0.39	0.86 ± 0.36	0.98 ± 0.42	0.81 ± 0.42	0.93 ± 0.42
HDL ₂ -C (mmol/L)	0.37 ± 0.16	0.42 ± 0.18	0.53 ± 0.06 ^a	0.44 ± 0.18	0.38 ± 0.17	0.38 ± 0.14	0.33 ± 0.09	0.36 ± 0.13
HDL ₃ -C (mmol/L)	0.93 ± 0.16	0.93 ± 0.17	0.78 ± 0.13	0.91 ± 0.17	0.82 ± 0.23	0.86 ± 0.20	0.76 ± 0.10	0.83 ± 0.18
TG/HDL-C	0.83 ± 0.58	0.73 ± 0.33	0.85 ± 0.51	0.75 ± 0.38	0.71 ± 0.36	0.79 ± 0.42	0.74 ± 0.45	0.78 ± 0.42
PON1, SSPON1, ARE activities								
PON1 (U/L)	34.7 ± 10.2 ^b	99.6 ± 40.1 ^d	182.0 ± 30.9 ^{de}	111.4 ± 48.3 ^d	23.2 ± 11.9	102.3 ± 51.2 ^d	124.9 ± 61.5 ^d	108.9 ± 54.1 ^d
SSPON1 (U/L)	156.9 ± 39.6	471.8 ± 141.8 ^d	772.0 ± 43.6 ^{de}	514.7 ± 169.7 ^d	133.4 ± 58.9	476.0 ± 151.4 ^d	707.5 ± 116.2 ^{df}	543.5 ± 176.1 ^d
ARE (KU/L)	128.7 ± 39.3	130.0 ± 35.5	119.0 ± 15.4	128.4 ± 33.3	120.7 ± 28.3	124.9 ± 30.8	115.2 ± 29.4	122.0 ± 30.1
HDL-ARE (KU/L)	114.8 ± 27.9	119.9 ± 20.7	98.3 ± 20.1	116.8 ± 21.6	108.1 ± 26.2	108.7 ± 23.5	112.7 ± 22.5	109.9 ± 22.8
HDL ₃ -ARE (KU/L)	79.7 ± 23.9	80.0 ± 19.6	62.4 ± 20.8	77.5 ± 20.3	64.9 ± 25.1	70.4 ± 29.1	78.5 ± 16.0	72.7 ± 25.9
HDL ₂ -ARE (KU/L)	35.1 ± 14.3	39.9 ± 17.0	35.9 ± 7.5	39.4 ± 15.9	43.1 ± 15.8	38.4 ± 16.6	34.3 ± 20.5	37.2 ± 17.4
SSPON1/ARE	1.3 ± 0.2	3.7 ± 0.7 ^d	6.6 ± 0.8 ^{df}	4.1 ± 1.2 ^d	1.1 ± 0.3	3.8 ± 0.8 ^d	6.4 ± 1.5 ^{d,g}	4.6 ± 1.6 ^d

Note: Mann–Whitney *U* test was used to compare variables including non-normal data between independent groups: TG, TG/HDL-C, HR for EQR, VO_{2max} for EQQ, HR for ERC, all variables for ERR and CRR, HDL₂-C for CQQ, HDL₂-C and TG/HDL-C for CQR and CRC.

^a *p* < 0.05, ^b *p* < 0.01, ^c *p* < 0.001, compared with same phenotypes in CG; ^d *p* < 0.001, compared with QQ within group; ^e *p* < 0.05, ^f *p* < 0.01, ^g *p* < 0.001, compared with QR within group; ^h *p* < 0.01, compared with EQQ and CQQ.

Abbreviations: ARE = serum arylesterase activity; CG = control group; CQQ = QQ-phenotype in control group; CQR = QR-phenotype in control group; CRC = R-carriers phenotype (QR + RR) in control group; CRR = RR-phenotype in control group; EG = exercise group; EQQ = QQ-phenotype in exercise group; EQR = QR-phenotype in exercise group; ERC = R-carriers phenotype (QR + RR) in exercise group; ERR = RR-phenotype in exercise group; HDL-ARE = HDL's arylesterase activity; HDL₂-ARE = HDL₂'s arylesterase activity; HDL₃-ARE = HDL₃'s arylesterase activity; HDL-C = high density lipoprotein cholesterol; HDL₂-C = HDL₂'s cholesterol level; HDL₃-C = HDL₃'s cholesterol level; HR = heart rate; LDL-C = low density lipoprotein cholesterol; PON1 = serum paraoxonase I activity; SSPON1 = serum salt-stimulated paraoxonase activity; TC = total cholesterol; TG = triglyceride; VO_{2max} = maximum oxygen consumption.

3.2. Measurement of all lipid and lipoprotein concentrations

HDL-C and HDL₃-C ($p < 0.05$), TC ($p < 0.001$), and LDL-C ($p < 0.01$) levels were significantly greater in the EG than in the CG (Table 1). TC ($p < 0.01$) and LDL-C ($p < 0.05$) levels were higher in EQQ phenotype group than in those of the CQQ phenotype group. The ERC phenotype group had greater TC ($p < 0.05$) and HDL-C ($p < 0.05$) levels, as compared to the CRC group (Table 2). However, no differences were found in all lipid and lipoprotein concentrations between the CQQ and CRC phenotype groups (Table 2).

3.3. Serum PON1, SSPON1, and ARE activities

No significant difference was found between the EG and CG in terms of serum PON1 and SSPON1 activities, ARE, and HDLs-ARE activities (Table 1). Evaluation of the effects of exercise according to PON1-192 phenotypes revealed that serum PON1 activity of the EQQ group was greater than that in the CQQ group ($p < 0.01$). Although HDL₃-ARE activities in the EQQ group were 23% higher than those in the CQQ group, this difference was not statistically significant. ARE activities were also not significantly different among the phenotype groups (Table 2). As expected, serum PON1 and SSPON1 levels, as well as SSPON1/ARE ratios were significantly greater among QR, RR, and RC phenotyped individuals than those with the QQ phenotype ($p < 0.001$), with or without taking into account of exercise status.

Two-way ANOVA showed a significant interaction between PON1 phenotypes (QQ and RC groups) and exercise (exercise and control groups) on PON1 enzymatic activity ($F(1, 87) = 4.01$; $\eta^2 = 0.044$; $p = 0.048$). Significant positive correlations were found between serum PON1 and SSPON1, SSPON1/ARE ratio, HDL₃-ARE activities ($p < 0.01$), and HDL-ARE activities ($p < 0.05$), and between HDL-C and HDL-ARE, HDL₂-C, HDL₃-C ($p < 0.01$), HDL₂-ARE, HDL₃-ARE, and ARE ($p < 0.05$).

4. Discussion

The main finding of the present study was that serum PON1 activity in the EQQ phenotype group was greater than that in the CQQ group ($p < 0.01$), but not the ERC group. Moreover, a significant interaction was found between regular aerobic exercise and PON1-192 phenotype on PON1, whereas no similar interaction was found for all lipid and lipoprotein concentrations. The results show that there are the beneficial effects of regular aerobic exercise training on PON1 activity and that this effect is related to PON1-192 phenotype, but not to all lipid and lipoprotein concentrations. Although HDL₃-ARE activity was 23% greater in the EQQ group compared to the CQQ group, this was not a statistically significant difference. This finding supports also the beneficial effects of regular exercise on PON1 activity. No apparent PON1 phenotype effects on ARE activities or HDLs-ARE activities were observed in the present study, suggesting that ARE activity is not related to phenotype.

4.1. The effects of regular exercise on all lipid and lipoprotein concentrations

Regardless of phenotyping, HDL-C ($p < 0.05$), HDL₃-C ($p < 0.05$), TC ($p < 0.001$) and LDL-C ($p < 0.01$) levels were greater significantly in the EG than CG. Considering phenotyping, while EQQ subjects had greater serum lipid and lipoprotein concentrations than their controls, ERC subjects had higher TC and higher HDL-C levels. In fact, exercise intensity, frequency,⁶ duration of the exercise program, weekly exercise energy expenditures surpassing 2000 kcal,²⁷ and the need for more vigorous physical activity compared with men²⁸ were found to be important factors in attaining improved lipid profiles in women. Therefore, exercise-linked energy expenditure can be thought of as insufficient for improving all lipid and lipoprotein concentrations in the present study.

It has been shown that PON1-192 QQ genotype group members have elevated antiatherosclerotic lipid and lipoprotein concentration profiles compared to RC in the highest tertile of physical activity and that the effect on HDL concentration levels is modified by PON1-192 polymorphisms.¹⁸ Similar to previous studies,^{19,20} we did not observe a significant difference in all lipid and lipoprotein concentrations between the CQQ and CRC phenotype groups. Differences among these studies may be mainly due to differences in the ethnic groups studied and the frequencies of various PON1-192 polymorphisms in the groups. The present study shows that the PON1 phenotype is not related to any lipid and lipoprotein concentrations notwithstanding the effects of regular exercise.

4.2. The effects of regular exercise on PON1 and ARE activity

The beneficial effects of exercise on PON1 enzyme activities observed in this study did not parallel the observations of Brites et al.¹⁴ with highly trained male endurance athletes in a cross-sectional study (the only published study similar to our own measurement methods). Brites et al.¹⁴ did not detect any effects of PON1 phenotype or exercise on serum PON1 and ARE activities in men. Tomás et al.¹³ determined that SSPON1 activities were decreased in RC, increased in QQ homozygous youths of both genders who trained aerobically, and that these effects were dependent on PON1-192 polymorphisms. Another study in athletes showed that the increase in PON1 activity following maximal exercise does not depend on the PON1-192 polymorphism,¹² in contrast to results presented by Tomás et al.¹³ In addition, in a population study, Ferré et al.²⁹ also found that the lifestyle factors as physical activity do not play significant role on PON1 activity except PON1-192 polymorphism. Although the studies by Otocka-Kmiecik et al.,¹² Tomás et al.,¹³ and Ferré et al.²⁹ were polymorphism studies unlike the present study, these results showed that the PON1-192 genotype groups may give difference answers on PON1 activity to exercise. The differences between these studies may due to differences in ethnicity, age, gender, and methods for measuring PON1 activity in these studies.

The most significant differences found in the study were the increased PON1 ($p < 0.01$) and HDL₃-ARE (23%; $p > 0.05$)

activities in the EQQ group, compared with their controls. Because serum ARE activity is also used as an indicator of PON1 enzyme protein levels,³⁰ PON1 induction may have played a role in the observed increases. A previous study demonstrated that small HDL₃ particles have greater PON1 activity than large HDL₂ particles.³¹ In addition, ARE activity is independent of PON1-192 polymorphisms. Therefore, elevated HDL₃-ARE activity in the EQQ subgroup shows an improvement owing to the aerobic exercise program that was independent of the PON1 phenotype.

It has been reported that PON1 activity is related to blood HDL-C levels.⁵ However, PON1 or ARE activities did not correlate with HDL-C levels in the present study, and the difference in HDL-C levels between the EQQ and CQQ groups was not significant. Therefore, our study stands in contrast to previous work and suggests that PON1 activity in the EQQ group is unrelated to HDL-C levels. Oxidative stress can inhibit PON1 activity, whereas improvements in the antioxidant system by regular aerobic exercise can improve PON1 activity.^{2,5,13,14} The presence of greater PON1 and HDL₃-ARE activity in exercise group can also exhibit the increased antioxidant capacity by exercise training.

A limitation of this cross-sectional study was that it only enabled comparisons with a single environmental effect, which was the participation in an exercise-training program of at least 3 months in duration. Thus, caution should be used in interpreting results, as the starting lipid profile of the subjects might have been biased. Nevertheless, the exclusion and inclusion criteria imposed were designed to reduce the selection bias to an acceptable level.

The attempt to correlate PON1 activity levels with potential PON1-192 phenotypes through an indirect method^{11,26} does not replace the need for genotyping allele frequencies, which themselves account for approximately half of the variation in PON1 activities. Although genotyping was beyond the scope of the current study, analyzing the trimodal frequency distribution of PON1 activities may be promising for future larger scale genotype-environmental effect interaction studies.

5. Conclusion

Participation in the aerobic exercise program significantly improved PON1 activities. The PON1-192 phenotype is related to the beneficial effects of regular aerobic exercise on PON1 activity, but not lipid and lipoprotein levels, in middle-aged women.

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Authors' contributions

GRN carried out all phases of the study; FT executed all the biochemical analysis and drafted the manuscript; SRV con-

ceived of the study and participated in its design and coordination; MZO organised the determination of participants and carried out the physical and physiological measurements of the study; MN carried out medical examinations of the participants and assisted all the physical, physiological and biochemical analysis; SOK participated in biochemical analysis and drafted the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

None of the authors declare competing financial interests.

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