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Research Article

Serum Paraoxonase 1 Activity Is Associated with Fatty Acid Composition of High Density Lipoprotein

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Introduction. Cardioprotective effect of high density lipoprotein (HDL) is, in part, dependent on its related enzyme, paraoxonase 1 (PON1). Fatty acid composition of HDL could affect its size and structure. On the other hand, PON1 activity is directly related to the structure of HDL. This study was designed to investigate the association between serum PON1 activity and fatty acid composition of HDL in healthy men. *Methods*. One hundred and forty healthy men participated in this research. HDL was separated by sequential ultracentrifugation, and its fatty acid composition was analyzed by gas chromatography. PON1 activity was measured spectrophotometrically using paraxon as substrate. *Results*. Serum PON1 activity was directly correlated with the amount of stearic acid and dihomo-gamma-linolenic acid (DGLA). PON1/HDL-C was directly correlated with the amount of miristic acid, stearic acid, and DGLA and was inversely correlated with total amount of ω6 fatty acids of HDL. *Conclusion*. The fatty acid composition of HDL could affect the activity of its associated enzyme, PON1. As dietary fats are the major determinants of serum lipids and lipoprotein composition, consuming some special dietary fatty acids may improve the activity of PON1 and thereby have beneficial effects on health.

1. Introduction

Serum paraoxonase 1 (PON1) is a 45 kDa glycoprotein which can catalyze the hydrolysis of various organophosphates and nerve agents [1, 2] and also metabolize some drugs and prodrugs by its lactonase activity [3]. This enzyme which is located on high density lipoprotein (HDL) particles protects low density lipoprotein (LDL) phospholipids against oxidation [4]. Decreased PON1 activity has been addressed in several diseases such as coronary artery diseases (CAD) [5], type I diabetes [6], obesity [7], and renal failure [8].

It is evident that PON1 activity is influenced by a variety of agents like environmental, pharmacological, and lifestyle factors as well as age and sex [2, 5, 9–11]. Dietary fats have been suggested as an important relevant factor [12, 13]. Studies have presented that dietary fatty acids may

affect PON1 activity [14]. Polyenoic fatty acids have shown considerable inhibitory effect on PON1 activity [15], while monoenoic acids (especially oleic acid) protect PON1 from oxidative inactivation [16]. It has been also indicated that replacement of dietary saturated fats with trans fats in healthy men and women leads to a small reduction in the serum PON1 activity [17].

Serum PON1 is almost exclusively found in association with HDL particles. The lipid composition of HDL can influence its size [18] and structure and is determined by a combination of various factors including dietary intake, metabolism, and storage [19]. So, dietary fats may affect PON1 activity via changing the phospholipid composition of HDL. On the other hand, it seems that any variation in HDL fatty acid composition could alter the function of HDL and the activity of its related enzyme, PON1.

The association between serum PON1 activity and the lipid content of HDL has not yet been investigated. Therefore, in this study we investigated the association between serum PON1 activity and fatty acid composition of HDL in healthy male subjects.

2. Subjects and Methods

2.1. Subjects. 140 healthy men aged between 20 and 50 with a mean value of 32.5 ± 8.2 years were selected from a representative sample of the Iran center population. All participants were nonsmoker and nonobese (mean body mass index (BMI) 23.6 ± 3.3 kg/m^2) volunteers, without diabetes (fasting blood sugar (FBS) ≤ 110 mg/dL), cardiovascular disease, familiar hypercholesterolemia, and dislipidemia or any other recognized lipid-related disorders. A questionnaire containing demographic characteristics, medical history, and lifestyle information was completed for each subject by interview. All participants signed an informed consent document approved by the Isfahan University of Medical Sciences Ethics Committee.

2.2. Biochemical Analysis. A 12–14 h fasting blood sample (20 mL) was taken from each subject by venipuncture. Serum samples were separated by centrifugation at 3000 RPM for 15 min, and then 100 μL aliquots of each sample were stored at −80°C until the measurement of the serum PON1 activity. Serum HDL cholesterol (HDL-C) was determined at the day of sampling using enzymatic methods.

2.3. Serum PON1 Activity. Serum PON1 activity was assayed using 2 mM paraoxon (diethyl p-nitro-phenyl phosphate, Sigma Chemical Co., St. Louis, Mo, USA) as the substrate in 100 mM tris buffer (pH 8.0) containing 2 mM of $CaCl_2$ [20]. A 40 μL aliquot of serum was added to 500 μL of substrate medium, and the initial rate of hydrolysis (generation of p-nitrophenol) was monitored at 412 nm, using a continuously recording spectrophotometer (UV 3100, Shimadzu, Kyoto, Japan) over a period of 2 min. A PON1 activity of 1 Unit/L was defined as 1 μmole of p-nitrophenol formed per minute. The molar extinction coefficient of p-nitrophenol was taken at 17,000 M/cm [21]. All assays were performed in duplicate at 25°C. The coefficient of variation (CV%) for 10 measurements was 2.9%.

2.4. HDL Separation. Serum HDL was separated (1.063 g/cm^3 < ρ < 1.21 g/cm^3) by a three-stage method [18]. Briefly, 5.9 mL of serum added to 3 mL of solution A (containing 0.195 mmol NaCl, 0.62 mmol NaOH, 0.01% (w/v) EDTA-Na_2, d = 1.006 g/mL) in polyallomer ultracentrifuge tube (Optiseal, Beckman/Coulter, Fullerton, California, USA) in a discontinuous density gradient fashion, and centrifuged at 60000 rpm for 12 h at 16°C, with the acceleration of "5" and deceleration of "7." The very low density lipoproteine (VLDL) fraction accompanied with 3 mL of the upper layer solution was removed, and then 3 mL of solution B (containing 24.8 g NaBr in 100 mL of solution A) was added to the residual content of tubes for the second stage and centrifuged at 60000 rpm for 12 h at 16°C, acceleration: "9" and deceleration: "7." Then the LDL fraction accompanied by 3 mL of the supernatant was removed. In the last stage the residual content of tubes was mixed with 3 mL of solution C (containing 78.32 g of NaBr in 100 mL of solution A) and centrifuged for another 12 h at 16°C, acceleration: "9" and deceleration: "7". After centrifugation, the HDL fraction appearing as a yellow band at the supernatant was separated and stored at −80°C for further analysis. The centrifugations were done in a Beckman Coulter Optima L-100 XP ultracentrifuge equipped with a 90Ti fixed angle rotor. Protein concentration of the collected HDL fraction was measured using the Lowry method [22].

2.5. Fatty Acids Measurement of HDL

2.5.1. Calibration Standards and Chemical Compounds. A commercially available standard mixture of 37 fatty acid methyl ester (FAME) and a series of individual standards of fatty acid methyl esters including myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), myristoleic acid (C14:1), palmitoleic acid (C16:1), oleic acid (C18:1n9), linoleic acid (C18:2n6), α-linolenic acid (ALA, C18:3n3), di-homo gamalinolenic acid (DGLA, C20:3n6), Cis-11,14,17-eicosatrienoic acid (ETE, C20:3n3), arachidonic acid (AA, C20:4n6), cis-5,8,11,14,17-eicosapentaenoic acid (EPA, C20:5n3), Cis-4,7,10,13,16,19-docosahexaenoic acid (DHA, C22:6n3), and nonmethylated heptadecanoic acid (HDA, C17:0) (as internal standard) (all obtained from Sigma Chemical Co., St. Louis, Mo, USA) were used for calibration. Boron trifluoro methanol (BF_3-M) and butylated hydroxyl toluene (BHT) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA), and all other reagents, chemical compounds, and solvents were obtained from Merck (Merck Co., Darmstadt, Germany).

2.5.2. Lipid Extraction. Lipids of HDL fraction were extracted according to the Folch method [23]. 100 μL of HDL samples were added to a 3 mL of chloroform-methanol (2 : 1 v/v) in a test tube. 100 μL BHT (5 mg/mL in chloroform) as antioxidant and 100 μL heptadecanoic acid (1000 μg/mL in chloroform) as internal standard (to monitor the recovery rate) were added to the mixture, and the tubes were vortexed

vigorously for 1 min. 600 μL of 0.9% NaCl solution was mixed again prior to a brief centrifugation (100 RPM, 3 min) to separate the phases. The chloroform phase containing lipids (lower layer) was separated by a pasture pipette and transferred to another test tube. Anhydrous sodium sulfate was added to remove any remnant water, and the lipid extract was transferred to a 15 mL Teflon liner screw cap tube and dried under nitrogen gas at room temperature before methylation [18].

2.5.3. Fatty Acid Methyl Ester Preparation. The fatty acid methyl esters were prepared according to the method of Morison and Smith, using BF_3 [24]. 1 mL hexane and 1 mL BF_3/methanol (13%) were added to the dried lipid extract in 15 mL Teflon liner screw cap tube and mixed well. The mixture was heated at 100°C in an oven for 45 min. After cooling, 0.5 mL hexane and 0.5 mL HPLC grade water were added to the mixture, shacked and centrifuged. Then, the methyl esters fraction in the upper hexane layer was separated by a Pasteur pipette and dried under nitrogen gas. Fatty acid methyl esters were dissolved in 200 μL hexane prior to GC analysis.

2.5.4. Instrumentation, Chromatography and Detection Method. Fatty acid composition of HDL was measured by a gas chromatography method. GC analyses were done by a Younglin GC-FID system model, Acme 6000 M (Young Lin Co., Hogye, Anyang, Korea), equipped with an FID detector. The injector and detector temperature was held at 260°C, and the flame was maintained with 40 mL/min H_2 and 300 mL/min air. Helium was used as the detector auxiliary gas at a flow of 28 mL/min. Chromatography was carried out using an SP-2560 fused silica capillary column 100 m × 0.25 mm × 0.2 μm film thickness (Supelco Co., Bellefonte, PA). Helium was used as the carrier gas at a flow rate of 18 cm/s with constant flow compensation. Sample injections of 1 μL were performed with a split ratio of 17 : 1 for the analysis. The oven temperature was programmed from 140 to 245°C at a rate of 4°C/min after an initial time hold of 5 min and the final time hold of 15 min (the total analysis time was 45.00 min).

2.6. Statistical Analyses. Statistical analyses were performed using SPSS version 16.0 (SPSS, IL, USA). Quantitative parameters were compared between groups by one way ANOVA using Tukey correction for post hoc multiple comparisons except for nonnormal distributed variables that were analyzed by a nonparametric Kruskal-Wallis test. Correlations between variables with normal distribution were calculated by Pearson correlation coefficient analysis. Spearman's rho correlation coefficient analysis was used to calculate the correlation between variables that were not normally distributed (i.e., serum PON1 activity, PON1/HDL-C ratio, PON1/Apo A-I ratio, palmitoleic acid, linolenic acid, arachidic acid, ETA, EPA, DHA, EPA: DHA, and ω3 index). All data were expressed as mean ± standard error or median and interquartile range. A P value < 0.05 was considered statistically significant.

3. Results

Subjects were divided into three experimental groups in accordance with their serum HDL-C levels to analyze the association between serum PON1 activity and HDL fatty acid composition (group 1: HDL ≤ 39 mg/dL; 49 subjects, group 2: 39 mg/dL < HDL ≤ 49 mg/dL; 46 subjects and group 3: 49 mg/dL < HDL; 45 subjects). All subjects in the three groups were age matched, and mean values for HDL-C were 32.3 ± 4.1, 43.5 ± 2.9, and 61.9 ± 7.2 mg/dL in groups 1, 2, and 3, respectively.

Table 1 shows the fatty acid composition of HDL as percent of total fatty acids in three experimental groups. The major fatty acid of HDL in all groups was palmitic acid and the minor one was ETE. The proportion of saturated fatty acids (SFA) in group 1 of experiment was significantly higher compared to that in group 2 ($P < 0.001$) and group 3 ($P = 0.005$). Among the SFAs, palmitic acid and stearic acid were significantly higher in group 1 when compared to that in groups 2 and 3 ($P < 0.01$). There was no significant difference in monounsaturated fatty acids (MUFA) of HDL particles among three groups. Polyunsaturated fatty acids (PUFA) were significantly lower in group 1 in comparison with group 2 ($P < 0.01$) and group 3 ($P < 0.05$). Among the PUFAs, ETE was significantly higher in group 1 compared to that in groups 2 and 3 ($P < 0.05$). Inversely, AA was significantly lower in group 1 compared to that in group 2 ($P < 0.05$) and group 3 ($P < 0.001$). The proportion of ω3 fatty acids to total fatty acids was significantly higher ($P < 0.05$), and the proportion of ω6 fatty acids to total fatty acids was significantly lower ($P < 0.01$) in group 3 of the study in comparison with the other groups. A significant trend was seen for higher SFA to unsaturated fatty acids (UFA), SFA : MUFA, and SFA : PUFA ratios in group 1 of the study. The ratio of AA : EPA was significantly higher in group 3 compared to that in group 1 ($P < 0.01$) and group 2 ($P < 0.05$).

Associations of serum PON1 activity, PON1/HDL-C ratio, and PON1/apo A-I ratio with HDL fatty acid composition in all participants are indicated in Table 2. Serum PON1 activity was directly correlated with stearic acid ($r = 0.190$, $P < 0.05$) and DGLA ($r = 0.260$, $P < 0.01$). Serum PON1 activity adjusted for serum apo A-I level (PON1/HDL-C ratio) was directly correlated with miristic acid ($r = 0.190$, $P < 0.05$), stearic acid ($r = 0.237$, $P < 0.01$), and DGLA ($r = 0.257$, $P < 0.01$). There was a negative correlation between the PON1/HDL-C ratio and total ω6 fatty acids of HDL particles ($r = -0.198$, $P < 0.05$). Serum PON1 activity adjusted for serum apo A-I level (PON1/apo A-I ratio) was directly correlated with stearic acid ($r = 0.179$, $P < 0.05$) and DGLA ($r = 0.278$, $P < 0.01$) but not with miristic acid. It also was directly correlated with palmitoleic acid ($r = 0.197$, $P < 0.05$). A negative correlation was seen between PON1/apoA-I ratio with total ω6 fatty acids of HDL particles ($r = -0.184$, $P < 0.05$).

The correlations between PON1 activity and fatty acids of HDL in each individual group of the study are shown in Table 3. As indicated in group 1 of the study with the serum HDL-C levels lower than 40 mg/dL, serum PON1 activity strongly correlated with MUFAs ($r = 0.487$, $P < 0.01$)

Table 1: Comparison of HDL fatty acid composition between groups.

Fatty acids	Group 1 HDL ≤ 39	Group 2 39 < HDL ≤ 49	Group 3 49 < HDL	*P* value (G1 & G2)	*P* value (G1 & G3)	*P* value (G2 & G3)
SFA	42.52 ± 0.42	40.48 ± 0.24	40.97 ± 0.23	0.000	0.005	0.581
C14:0 (myristic acid)	0.49 ± 0.02	0.43 ± 0.02	0.40 ± 0.01	0.088	0.002	0.416
C16:0 (palmitic acid)	25.94 ± 0.26	25.21 ± 0.26	25.37 ± 0.30	0.154	0.300	0.924
C18:0 (stearic acid)	15.78 ± 0.23	14.53 ± 0.17	14.90 ± 0.16	0.000	0.004	0.354
C20:0 (arachidic acid)	0.32 ± 0.01	0.31 ± 0.02	0.30 ± 0.02	0.921	0.895	0.998
UFA	57.48 ± 2.8	59.52 ± 1.6	59.03 ± 2.2	0.000	0.005	0.581
MUFA	10.34 ± 0.19	10.58 ± 0.20	10.37 ± 0.22	0.689	0.995	0.749
C14:1 (myristoleic acid)	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.185	0.209	0.997
C16:1 (palmitoleic acid)	0.65 ± 0.03	0.73 ± 0.05	0.64 ± 0.04	0.357	0.994	0.307
C18:1n9c (oleic acid)	9.6 ± 0.16	9.64 ± 0.17	9.51 ± 0.18	0.748	0.975	0.867
PUFA	47.14 ± 0.48	48.93 ± 0.29	48.66 ± 0.47	0.009	0.031	0.889
C18:3n3 (ALA)	1.21 ± 0.08	1.43 ± 0.09	1.29 ± 0.07	0.136	0.775	0.431
C20:3n3 (ETE)	0.16 ± 0.02	0.14 ± 0.02	0.11 ± 0.01	0.050	0.036	0.313
C20:5n3 (EPA)	0.68 ± 0.04	0.99 ± 0.17	0.59 ± 0.04	0.070	0.811	0.015
C22:6n3 (DHA)	6.61 ± 0.33	6.96 ± 0.33	6.15 ± 0.20	0.664	0.513	0.129
C18:2n6c (LA)	23.85 ± 0.43	23.80 ± 0.44	24.23 ± 0.54	0.997	0.834	0.800
C20:3n6 (DGLA)	3.83 ± 0.13	3.79 ± 0.19	3.66 ± 0.17	0.984	0.727	0.830
C20:4n6 (AA)	11.60 ± 0.31	12.82 ± 0.25	13.53 ± 0.39	0.026	0.000	0.217
SFA : UFA	0.74 ± 0.01	0.68 ± 0.01	0.70 ± 0.01	0.000	0.004	0.562
SFA : MUFA	4.17 ± 0.08	3.88 ± 0.07	4.02 ± 0.08	0.030	0.365	0.446
SFA : PUFA	0.91 ± 0.02	0.83 ± 0.01	0.85 ± 0.01	0.000	0.009	0.630
Total $\omega 3$	7.86 ± 0.35	8.53 ± 0.38	7.25 ± 0.19	0.298	0.367	0.015
Total $\omega 6$	39.28 ± 0.55	40.41 ± 0.44	41.41 ± 0.44	0.208	0.004	0.293
$\omega 6 : \omega 3$ ratio	5.37 ± 0.19	5.13 ± 0.22	5.91 ± 0.18	0.671	0.134	0.018
AA : EPA	19.62 ± 1.31	20.31 ± 1.65	26.64 ± 1.79	0.950	0.006	0.016
EPA : DHA	0.11 ± 0.01	0.16 ± 0.03	0.10 ± 0.01	0.125	0.955	0.068

ALA: α-linolenic acid, ETE: eicosatrienoic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, SFA: saturated fatty acids, UFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids. Data are expressed as mean ± SE and indicated the percent of fatty acid to total fatty acids.

especially palmitoleic acid ($r = 0.457$, $P < 0.01$) and oleic acid ($r = 0.474$, $P < 0.01$). In this group, PON1 activity positively correlated with EPA ($r = 0.352$, $P < 0.05$) and DGLA ($r = 0.402$, $P < 0.01$) and negatively correlated with LA ($r = -0.332$, $P < 0.05$). The ratio of SFA : MUFA was inversely ($r = -0.414$, $P < 0.01$) and the ratio of EPA : DHA was directly ($r = 0.339$, $P < 0.05$) correlated with serum PON1 activity in the first group of the study. In the second group with serum HDL-C levels between 40 and 50 mg/dL, correlations between PON1 activity and fatty acids of HDL were completely different. PON1 activity negatively correlated with myristic acid ($r = -0.296$, $P < 0.05$), arachidic acid ($r = -0.315$, $P < 0.05$), myristoleic acid ($r = -0.342$, $P < 0.05$), ALA ($r = -0.301$, $P < 0.01$), ETE ($r = -0.297$, $P < 0.01$), EPA ($r = -0.295$, $P < 0.05$), and the ratio of EPA : DHA ($r = -0.310$, $P < 0.05$) and positively correlated with AA ($r = 0.425$, $P < 0.01$). There were no significant associations between PON1 activity and HDL fatty acids in the third group of participants with the serum HDL-C levels higher than 50 mg/dL.

4. Discussion

The associations between fatty acid composition of HDL and the activity of its related enzyme, PON1, in healthy male subjects with different ranges of serum HDL-C levels were investigated in this study. Results indicated that serum PON1 activity was directly correlated with stearic acid and DGLA levels. Serum PON1 activity adjusted for serum HDL-C level (PON1/HDL-C ratio) also was directly correlated with miristic acid, stearic acid, and DGLA levels.

It is evident that PON1 activity is influenced by a variety of agents including environmental, pharmacological and lifestyle factors as well as age and sex [2, 5, 9–11]. Dietary fats have been proposed as an important relevance factor [12–14].

Table 2: Bivariate correlations between serum PON1 activity, PON1/HDL-C ratio, and PON1/apo A-I ratio with fatty acid composition of HDL particles in all participants.

Fatty acids (percent of total FA)	Serum PON1 activity	*P* value	PON1/HDL-C ratio	*P* value	PON1/apo A-I ratio	*P* value
SFA	0.097	NS	0.136	NS	0.109	NS
C14:0 (myristic acid)	0.070	NS	0.190	0.028	0.123	NS
C16:0 (palmitic acid)	0.029	NS	0.039	NS	0.037	NS
C18:0 (stearic acid)	0.190	0.027	0.237	0.006	0.179	0.038
C20:0 (arachidic acid)	−0.064	NS	0.026	NS	−0.004	NS
UFA	−0.097	NS	−0.136	NS	−0.109	NS
MUFA	0.112	NS	0.111	NS	0.178	0.039
C14:1 (myristoleic acid)	−0.024	NS	0.034	NS	0.044	NS
C16:1 (palmitoleic acid)	0.141	NS	0.157	NS	0.197	0.022
C18:1 (oleic acid)	0.102	NS	0.095	NS	0.164	NS
PUFA	0.057	NS	−0.115	NS	−0.107	NS
C18:3n3 (ALA)	−0.108	NS	−0.070	NS	−0.069	NS
C20:3n3 (ETE)	−0.052	NS	0.047	NS	−0.059	NS
C20:5n3 (EPA)	−0.043	NS	0.124	NS	0.104	NS
C22:6n3 (DHA)	−0.037	NS	0.007	NS	−0.020	NS
C18:2n6c (LA)	−0.100	NS	−0.106	NS	−0.133	NS
C20:3n6 (DGLA)	0.260	0.002	0.257	0.003	0.278	0.001
C20:4n6 (AA)	0.038	NS	−0.075	NS	−0.010	NS
SFA : UFA ratio	0.097	NS	0.136	NS	0.109	NS
SFA : MUFA ratio	−0.074	NS	−0.054	NS	−0.134	NS
SFA : PUFA ratio	0.077	NS	0.133	NS	0.111	NS
Total $\omega 3$ fatty acids	−0.047	NS	0.022	NS	−0.010	NS
Total $\omega 6$ fatty acids	−0.111	NS	−0.198	0.022	−0.184	0.033
$\omega 6 : \omega 3$ ratio	−0.005	NS	−0.088	NS	−0.062	NS
AA : EPA ratio	−0.036	NS	−0.148	NS	−0.107	NS
EPA : DHA ratio	0.054	NS	0.067	NS	0.084	NS

ALA: α-linolenic acid, ETE: eicosatrienoic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, SFA: saturated fatty acids, UFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, NS: not significant.

Studies have exhibited that dietary fatty acids may influence PON1 activity [15]. Polyenoic fatty acids have demonstrated a considerable inhibitory effect on PON1 activity [16], while monoenoic acids (especially oleic acid) protect PON1 against oxidative damages [25]. Furthermore, replacement of dietary saturated fats with trans fats in healthy men and women moderately decreases the activity of PON1 [26].

HDL is a phospholipid and protein containing particle to which PON1 is also attached. The lipid composition of HDL which can influence its structure is determined by a combination of various factors including dietary intake, metabolism, and storage [19]. So, dietary fats may affect PON1 activity via changing the phospholipid composition of HDL. On the other hand, it seems that any variation in HDL fatty acid composition may affect its three-dimensional structure and physical characteristics including size and zeta potential of HDL particles and consequently alter the function of HDL and the activity of its related enzyme, PON1.

In this research we investigated the individual correlations between PON1 activity and fatty acid composition of HDL in each group of the study, separately (Table 3). The association between PON1 activity and HDL fatty acid composition was completely different in each group of the study. In subjects with serum HDL-C levels of 39–49 mg/dL (group 2 of the study) SFAs had negative correlations with serum PON1 activity. This correlation was not found in other groups. MUFAs (especially palmitoleic acid and oleic acid) were positively correlated with serum PON1 activity in subjects with serum HDL-C levels lower than 40 mg/dL (group 1 of the study), while in group 2 this correlation was different and myristoleic acid was inversely associated with PON1 activity. Among PUFAs in group 1, EPA and DGLA were positively and LA was negatively correlated with PON1 activity. In group 2 this correlation was different again in that ALA, ETE, and EPA were negatively and AA was positively correlated with serum PON1 activity. These findings are in

Table 3: Bivariate correlations between serum PON1 activity and fatty acid composition of HDL particles between groups.

Fatty acids (percent of total FA)	Serum PON1 activity		
	Group 1 (HDL ≤ 39)	Group 2 (39 < HDL ≤ 49)	Group 3 (49 < HDL)
SFA	0.021	−0.147	0.038
C14:0 (myristic acid)	0.155	−0.296*	−0.074
C16:0 (palmitic acid)	−0.044	−0.192	0.078
C18:0 (stearic acid)	−0.060	0.133	0.078
C20:0 (arachidic acid)	0.006	−0.315*	−0.106
UFA	−0.021	0.147	−0.038
MUFA	0.487**	−0.184	−0.126
C14:1 (myristoleic acid)	0.106	−0.342*	−0.168
C16:1 (palmitoleic acid)	0.457**	−0.058	−0.203
C18:1 (oleic acid)	0.474**	−0.189	−0.099
PUFA	−0.211	0.251	0.033
C18:3n3 (ALA)	−0.235	−0.301*	0.112
C20:3n3 (ETE)	−0.141	−0.297*	0.130
C20:5n3 (EPA)	0.352*	−0.295*	−0.106
C22:6n3 (DHA)	−0.260	0.191	−0.077
C18:2n6c (LA)	−0.332*	−0.145	0.201
C20:3n6 (DGLA)	0.402**	0.144	0.136
C20:4n6 (AA)	0.222	0.425**	−0.250
SFA : UFA ratio	−0.006	−0.144	0.040
SFA : MUFA ratio	−0.414**	0.082	0.165
SFA : PUFA ratio	0.091	−0.215	0.008
Total ω3 fatty acids	−0.231	0.002	−0.086
Total ω6 fatty acids	−0.037	0.177	0.074
ω6 : ω3 ratio	0.190	−0.054	0.127
AA : EPA ratio	−0.227	0.205	0.047
EPA : DHA ratio	0.339	−0.310*	−0.007

ALA: α-linolenic acid, ETE: eicosatrienoic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, SFA: saturated fatty acids, UFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
(* Significant at the level of 0.05).
(** Significant at the level of 0.01).

accordance with those of Nguyen and Sok investigation [16]. There was no correlation between serum PON1 activity and fatty acid composition of HDL in subjects with serum HDL-C levels higher than 49 mg/dL (the third group of our study).

To the best of our knowledge there is no investigation evaluating the relationship between HDL fatty acid composition and PON1 activity. The association between serum PON1 activity and dietary factors has been reported in some studies. For example, Tomas and coworkers suggested that high intake of oleic acid from olive oil has a positive effect on serum PON1 activity in men especially in subjects with the PON1-192 RR polymorphism [27]. Findings of another study demonstrated that meals rich in olive oil can increase postprandial serum PON1 activity in diabetic women [28]. In an in vitro study, beneficial protecting and stabilizing effect of oleic acid on PON1 against oxidative inactivation was observed [25].

We found a negative correlation between the PON1/HDL-C ratio and total ω6 fatty acids of HDL particles in all subjects, but no correlation was found between serum PON1 activity and total ω3 fatty acids or the ratio of ω6 : ω3 in all subjects. Calabresi et al. have reported that a ω-3 polyunsaturated fatty acid concentrate can enhance plasma PON1 concentration and HDL_2 subfraction in familial combined hyperlipidemia patients [12]. Also, in another study the inhibitory effect of polyenoic fatty acids, especially linoleic acid, on PON1 activity was observed [9]. In addition, opposite to the triolein-rich diet that caused a significant increase in PON1 activity, fish oil showed significant decrease in an animal study [14]. One point that deserved further attention was the relatively strong association between MUFAs (myristoleic acid, palmitoleic acid, and oleic acid) and PON1 activity only in group 1 (HDL-C ≤ 39). The reason of the relations is not clear, but it may be a protective compensation mechanism by which the fatty acids play in the subjects with low levels of HDL-C.

Contrary to the presented studies, some researchers have not found any relationship between PON1 activity and daily intake of total lipids and SFAs [11]. It is suggested that dietary fats may change the serum PON1 activity via affecting the synthesis and secretion of phospholipids and apo-A-I within very high density lipoprotein (VHDL), the main transporter of PON1 and LCAT molecules in blood [14].

Although there is a relative agreement between the results obtained in the present study with the others, some controversy is observed even between other researches, which can

be due to many factors beside the genetic variations. HDL lipid composition's determinants including dietary intake, metabolism, storage, and exchange among compartments are one of them [19]. In the mentioned studies only the effect of dietary fatty acids has been defined; however, there is a strong interaction between all the determinants of HDL lipid composition.

5. Conclusion

The protective role of HDL against oxidative stress-related disease seems to be a complicated and multifactorial procedure that depends on the HDL particle size, number, composition, distribution, and its related enzyme PON1. The major determinants of HDL fatty acid composition are serum and dietary fats. Fatty acid composition of HDL is the major factor which can influence the metabolism of HDL and its particle characterization such as size and zeta potential and can change the PON1 activity. So, the enhancing effect of fatty acids like DGLA on PON1 activity in this research may be the result of the changes in HDL size distribution toward the smaller particle size. However, more investigations are needed to evaluate the exact relationship between the type of HDL fatty acids with PON1 activity in different subclasses of HDL. These pieces of information may help to design therapeutic protocols toward beneficial modifications of HDL characteristics and its related enzyme, PON1.

Conflict of Interests

The authors of the study declare that they have no conflict of interests.

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