Paraoxonase 1 R/Q alleles are associated with differential accumulation of saturated versus 20:5n3 fatty acid in human adipose tissue

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Abstract Serum paraoxonase 1 (PON1) function has been associated with human cardiovascular disease. The projected mechanism postulates interaction of PON1 with lipoproteins and insulin signaling resulting in alterations in lipid homeostasis. Recently, PON2 was shown to directly regulate triglyceride accumulation in macrophages and PON1 was detected in the interstitial space of adipocytes. The aims of the present study were *a*) to examine the relationship of the PON1 function with serum parameters related to lipid homeostasis, and *b*) to examine a possible role of PON1 in the regulation of lipid composition in the human adipose tissue. Two important genetic variations with functional impact on PON1 activity in humans are the Q192R and the L55M. The present study evaluated the impact of the Q192R and the L55M polymorphisms in a cross-section of the population on the island of Crete, as regards to PON1 activity, plasma lipids/lipoproteins, parameters of the metabolic syndrome, and the fatty acid composition of the adipose tissue. We detected a significant association of the polymorphisms with blood pressure, fasting blood glucose, triglycerides, apolipoprotein B, serum iron, and homocysteine. Furthermore, a novel function is suggested for PON1 on the fatty acid composition in the adipose tissue through the positive association of the R allele with saturated fatty acid and of the Q allele with 20:5n3 fatty acid deposition.—Zafiropoulos, A., M. Linardakis, E. H. J. M. Jansen, A. M. Tsatsakis, A. Kafatos, and G. N. Tzanakakis. **Paraoxonase 1 R/Q alleles are associated with differential accumulation of saturated versus 20:5n3 fatty acid in human adipose tissue. *J. Lipid Res.* 2010.** 51: **1991–2000.**

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Serum paraoxonase I (PON1) belongs to the paraoxonase family, which has a total of three members, located in the chromosomal region 7q21.3-22.1 (1). All paraoxonase genes share a great sequence similarity (>60%). PON1 is synthesized in the liver where it enters the blood circulation and associates with the high-density serum lipoproteins. PON1 has been shown to have a protective role in cardiovascular disease (CVD), even though the exact mechanism of its action is still not known. The main hypothesis, however, suggests that PON1 is capable of hydrolyzing lipid peroxides in LDL (2), which prevents LDL uptake by macrophages during the atherogenesis process and reduces its intrinsic pro-inflamatory properties (3). Furthermore, PON1 has been shown to protect HDL from oxidation, thus preserving its atheroprotective function (4). The hypothesis for the PON1 protective effect in CVD is further supported by studies with PON1-deficient mice, which showed that these mice develop early atherosclerosis when fed with a high-fat/cholesterol diet (5). In fact, the overexpression of human paraoxonase gene cluster has been shown to repress atherogenesis and promote atherosclerotic plaque stability in transgenic ApoE-null mice (6).

Interestingly, significant interindividual variability of human serum PON1 activity has been described. This variability today is attributed partly to the presence of polymorphisms in the PON1 gene. The two most important genetic variations of PON1 that have been described to date are the Q192R and the L55M, both involving a single amino acid substitution (7, 8). Importantly, the R isoform demonstrates a 6-fold higher hydrolytic activity toward paraoxon as com-

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Abbreviations: Apo, apolipoprotein; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; DGLA, dihomo-γ linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PON, paraoxonase; SFA, saturated fatty acid; SBP, systolic blood pressure; TC, total cholesterol.

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pared with the Q isoform (8). Furthermore, the Q/R alleles differentially affect the PON1 hydrolytic activity toward a great number of additional substrates, thus significantly altering the PON1 role in different biological processes (9). On the other hand, the PON1 leucine/methionine substitution at the position 55 of the amino acid sequence seems to significantly affect the PON1 serum concentration (10).

A recent study demonstrated that PON2 can directly regulate triglyceride biosynthesis through modulation of diacylglycerol acyl transferase in mouse macrophages (11). Recently, PON1 presence was established in the interstitial space between unilocular fat cells of the adipose tissue (12). The actual PON1 function in human adipose tissue is unknown although its antioxidant function could contribute to the modulation of insulin-mediated signaling (13), which regulates lipid mobilization. The aims of the present study were *a*) to examine the relationship of PON1 function with serum parameters related to lipid homeostasis, and *b*) to examine a possible role of PON1 in the lipid composition of human adipose tissue. Our strategy involved impact analysis of the Q192R and L55M alleles, which significantly affect PON1 activity, in a defined cohort of human subjects (14, 15). In the process, we evaluated the previously debated associations of the polymorphisms with factors such as paraoxonase activity in serum, substrate specificity, lipoprotein profile, and other blood-derived measurements. A novel significant association of the rare R allele with the sequestering of saturated fatty acids and conversely, of the Q allele with 20:5n3 fatty acid in the human adipose tissue was identified.

SUBJECTS AND METHODS

Health assessment questionnaires

A total of 512 Greek men (228) and women (284) participated in the cross-sectional study carried out in Crete, Greece in 2004 (14, 15). Purpose-designed questionnaires were administered to ascertain biographical data, lifestyle behaviors on topics including cigarette smoking, and medical history. The physical activity and the food intake assessment tool are described in detail in previous publications of our group (14, 16). To validate the results, multiple cross-checked questions on the same topic were addressed to the participants. The Ethics Committee of the University of Crete approved this study and written informed consent was obtained from all donors.

Anthropometric measures

Body weight was assayed by a digital scale (Seca) with an accuracy of ±100 g. Subjects were weighed without shoes, in their underwear. Standing height was measured without shoes to the nearest 0.5 cm with the use of a commercial stadiometer with the shoulders in relaxed position and arms hanging freely. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2).

Blood measures

Early morning venous blood samples were drawn for biochemical screening tests following a 12 h overnight fast. The blood samples (10 ml) were transferred to the Nutritional Research Laboratory of the University of Crete in tanks containing ice packs so as to maintain a temperature of 3–4°C. Blood was centrifuged and 1.5 ml aliquots were pipetted into plastic Eppendorf tubes. One aliquot was used for blood analysis of triacylglycerol, total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) measurements on the same day of collection, whereas the other was stored (at −80°C) for determination of serum folate, vitamin B12, and total homocysteine. Serum folate and vitamin B12 concentrations were measured by a 96-well plate microbiological assay. Serum total homocysteine concentrations were determined fluorometrically and by HPLC.

DNA extraction and genotyping

The genotyping data were obtained in our parallel study (17). A brief description of the method that was used follows. DNA extraction was carried out according to previously published methods (18). All specimens were examined for the presence of amplifiable DNA. In each PCR reaction, two negative controls were employed to make sure that no contaminants were introduced in the initial PCR. The primers used in the study were: *CYP1A1*1A/*2A* Forward 5′-CAGTGAAGAGGTGTAGCCGCT-3′ and Reverse 5′-TAGGAGTCTTGTCTCATGCCT-3′, PON1 L/M Forward CCTGCAATAATAATATGAAACAACCTGT and Reverse CTGAAAGACT TAAACTGCCAGTC, PON1 Q/R Forward GAATGATATTGTTGCTGTGGGACCTG and Reverse CTTGCCATCGGGTGAA ATGTTGATT. The reaction mix consisted of PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 mM of each primer, and 1.2 units/reaction Taq DNA polymerase (Invitrogen). The reaction conditions were an initial denaturation at 94°C for 3 min, then 35 cycles of 92°C for 30 s, 60°C for 30 s, 72°C for 30 s, and finally, extension 72°C for 10 min. Approximately 2 μg of PCR product was digested with the appropriate restriction endonucleases at 37°C for 4 h. CYP1A1 was detected with MspI, PON1 L/M with NlaIII, and PON1 Q/R with AlwI (New England Biolabs). All PCR samples with an undigested or partial-digested result were submitted to redigestion with 20 units/reaction of enzyme and overnight incubation. The products of the digestion were visualized by agarose gel electrophoresis (2%) and ethidium bromide staining. Ten random DNA samples for each polymorphism were subjected to verification of the resulting genotype by sequencing both strands of the PCR product.

Adipose tissue measures

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan (19). The particular method has been reported to be rapid and safe and to cause no more discomfort than a routine venipuncture (19). Buttock adipose tissue samples can be safely stored for up to 1.5 years without changes in the component fatty acids. Samples were taken from the left upper outer quadrant of the gluteal area, through the use of a 10 ml vaccutaneous tube. Prior to aspiration, aspiration sites were sprayed with local anesthetic (ethyl chloride). Adipose tissue samples were stored at −80°C. Fatty acids analysis was carried out as previously described (20). The different adipose tissue n3 and n6 fatty acids assessed were C18:2n6, C18:3n6, C20:2n6, dihomo-γ linolenic acid C20:3n6 (DGLA), C20:4n6, C18:3n3, C20:3n3, eicosapentaenoic acid C20:5n3 (EPA), C22:5n3, and docosahexaenoic acid C22:6n3 (DHA). The presented value for each analyzed fatty acid represents a percentage in weight (g) of total tissue (g target/100g total tissue).

Arterial blood pressure

Blood pressure (BP) measurements were carried out by the same physician at all 6 sample collection time points according to the MONICA project protocol. Measurements were taken using a mercury sphygmomanometer (FOCAL-FC 110, JAPAN) with a bladder size sufficiently long to surround 50–75% of the upper arm. Triplicate BP measurements were taken at each visit on the subjects' right upper arm in the sitting posture after 10 min of

rest, with a 2 to 3 min interval between each reading [Korotkoff phase I for systolic blood pressure (SBP) and Korotkoff phase V for diastolic blood pressure (DBP)]. The mean value obtained from the second and third reading was used in the analysis.

Definitions. High-normal BP was defined as SBP > 130 mm Hg and/or DBP > 85 mm Hg and hypertension as SBP ≥ 140 mm Hg and/or a DBP ≥ 90 mm Hg. Compared with optimal BP, cut-offs for high-normal BP are associated with 1.6- to 2.5-fold risk of a CVD event (21) and they indicate a "prehypertensive" stage, which should instigate the adoption of health-promoting lifestyle changes so as to prevent gradual increase in BP and CVD (22).

PON1 activity measurements

Paraoxonase and diazoxonase activities were analyzed spectrophotometrically at the Laboratory for Toxicology, Pathology, and Genetics of the National Institute for Public Health and the Environment (Bilthoven, The Netherlands) using paraoxon and diazoxon as substrates in a Tris buffer (0.1 M, pH 8.5) containing 2 M NaCl and 2 mM CaCl2 (23, 24). After the addition of the serum sample (diluted 10-fold for paraoxonase and 20-fold for diazoxonase activity), the reaction was monitored on a microtiter plate for 5 min at 25°C. Arylesterase was measured using as a substrate phenylacetate (2 mM) in the buffer described above in the presence of 0.5% methanol. The samples were diluted 70-fold and measured in a kinetic mode at 37°C during 3 min at 270 nm in a microtiter plate reader. Paraoxonase, diazoxonase, and arylesterase activities are expressed as units per liter of serum, where 1 unit equals 1 μmol of substrate hydrolyzed per minute.

Statistical analyses

A statistical analysis program (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL) was used to analyze the data. ANOVA was used for comparing the demographic characteristics and the effect of genotype on the serum paraoxonase and diazoxonase activity between the genotype groups. Kruskal-Wallis analysis was used for the evaluation of the arylesterase activity. ANCOVA was performed to examine the changes in blood pressure, serum lipids, and adipose fatty acids adjusting for gender, age, and BMI. Heterogeneity was tested by the Levene's test in both methods. R-Pearson partial correlation coefficient was used to detect linear associations between paraoxonase, diazoxonase, and arylesterase activity in serum and nutrients intake and metabolic syndrome risk factors. Controlling variables were gender and age.

RESULTS

A total of 512 Greek men (n = 228) and women (n = 284) participated in the cross-sectional study carried out in Crete, Greece in 2004 (14, 15). A summary of the age, physical measurements, biochemical markers, blood pressure and subcutaneous fat analysis is shown in **Tables 1** and **2**. DNA was extracted from peripheral blood lymphocytes and the genotypes determined in our parallel study (17) as regards to the Q192R and the L55M alleles. We had observed 56.5% (n = 277) PON1 Q/Q homozygote, 38.5% (n = 189) heterozygote (Q/R) and 5% (n = 24) R/R homozygote individuals (17). The L55M analysis resulted in 36.8% (n = 163) L/L, 48.3% (n = 213) L/M and 14.9% (n = 66) M/M (17). The rare PON1 R allele frequency was 0.243 and the M allele was 0.39. Both polymorphisms were in equilibrium according to the Hardy-Weinberg analysis, demonstrating no bias of technical or evolutionary origin in the resulting genotype frequencies.

The PON1 alleles have been previously shown to affect the substrate specificity, serum enzymatic activity, and the total amount of PON1 protein in the serum (8). To check whether our genotypic analysis could identify such changes, we measured in the serum of the participants the enzymatic activity against paraoxon and diazoxon to assess enzyme specificity and arylesterase activity to asses the total amount of PON1 protein. Analysis of paraoxonase activity and correlation with genotype demonstrated that the R allele dose dependently increased the reactivity of PON1 toward the paraoxon substrate ($P < 0.001$) whereas the Q allele increased the reactivity of PON1 toward the diazoxonase ($P < 0.001$) (**Table 3**). Plotting the diazoxonase versus the paraoxonase activity in the total population visualizes the above result through the distinct grouping of the Q192R genotypes (**Fig. 1**). The L allele also increased the reactivity toward paraoxon ($P < 0.001$) but had no significant correlation with the diazoxon substrate. The Q allele also dose dependently increased the amount of arylesterase activity ($P < 0.002$) whereas the M/L alleles had no effect. All these finding are in complete agreement with previously published data demonstrating a solid genotyping database that can be used for further analysis. It is of note that because strong linkage disequilibrium has been reported between the polymorphism coding for leucine (L) at position 55 and arginine (R) at position 192, overlapping influences could account for some of the similar findings between the corresponding genotypes.

To address the main question of the present study, we assessed the relation of genotypes with all the available laboratory variables (described in detail in Tables 1 and 2). We assessed the impact of the genotypes independently as well as that of three genotype groupings. Only the statistically significant associations are presented in **Table 4**. The groupings were designed to assess the impact of the rare allele presence versus its absence, the impact of the wild-type allele versus its absence, and the impact of heterozygocity. No significant association was found with the respective genotypes or any other genotype grouping to the physical measurements (sex, age, physical activity, waist circumference, and BMI). The analysis of the combined effect of both genotypes was not considered due to the low allelic frequencies and the small sample size. When analyzing the blood pressure values, we identified a significant association of the L55M genotype with both the SBP ($P = 0.007$) and the DBP ($P = 0.003$). Analysis of the association in more detail with genotype groupings resulted that the MM genotype caused significantly higher SBP (132.5 vs. 125.9) and DBP (84.3 vs. 80.3). Analysis of the blood measurements resulted in significant associations for triglycerides, glucose, homocysteine, serum iron, and apolipoprotein (Apo)B. Specifically, serum triglyceride levels were found to be positively affected by the M allele in a dose dependent manner ($P = 0.071$, P for trend = 0.024) and negatively affected by the presence of the R allele ($P = 0.027$) and the Q/R heterozygocity ($P = 0.014$). Glucose was negatively affected by the Q allele in a dose dependent manner ($P = 0.037$). This relation, when ana-

TABLE 1. Age, physical measurements, blood pressure and biochemical markers

Parameters	Male	Female
	Mean ± SD (N)	
Age (yrs)	49.7 ± 13.2 (228)	49.5 ± 12.6 (284)
Physical Activity Level (16) *	2.5 ± 0.7 (225)	2.2 ± 0.6 (277)
Waist circ. (cm)	100.5 ± 11.5 (226)	102.8 ± 14.0 (284)
Body Mass Index (kg/m^2) *	29.3 ± 4.4 (226)	30.6 ± 5.5 (284)
Systolic blood pressure (mm Hg)	126.8 ± 15.3 (200)	127.3 ± 17.2 (262)
Diastolic blood pressure (mm Hg) *	82.4 ± 9.7 (200)	79.9 ± 8.0 (262)
Glucose (mg/dl)	96.3 ± 27.7 (211)	94.8 ± 27.8 (270)
Total cholesterol (mg/dl)	229.3 ± 46.0 (219)	226.4 ± 43.8 (281)
Triglycerides (mg/dl)	136.8 ± 78.2 (217)	124.3 ± 69.5 (277)
HDL-C (mg/dl) *	47.1 ± 9.2 (220)	53.5 ± 10.5 (281)
LDL-C (mg/dl) *	154.4 ± 39.1 (215)	147.4 ± 37.7 (276)
Total cholesterol: HDL-C ratio	5.0 ± 1.1 (219)	4.4 ± 1.0 (281)
Lipoprotein A (mg/dl)	23.4 ± 36.1 (193)	21.9 ± 33.1 (253)
Apo lipoprotein A1 (mg/dl) *	148.3 ± 21.9 (193)	160.3 ± 22.8 (253)
Apo lipoprotein B (mg/dl) *	114.8 ± 28.8 (185)	108.7 ± 27.3 (249)
Folate (ng/ml)	7.8 ± 4.2 (122)	8.7 ± 4.0 (150)
Serum vitamin B12 (pg/ml)	361.7 ± 118.5 (120)	378.8 ± 150.4 (148)
Serum iron (μg/dl) *	105.8 ± 44.6 (205)	87.8 ± 36.7 (262)
Homocysteine (μmol/l) *	16.4 ± 4.5 (121)	12.2 ± 2.8 (148)

ANOVA (* p-value<0.05; Heterogeneity was tested by Levene test and $\log_{10}$ transformations were used in physical activity level, waist circumference, body mass index, blood pressure, and homocysteine).

lyzed with genotype grouping, verified that the presence of the Q allele in homozygote or heterozygote form was responsible for the lower levels of glucose (93.6 vs. 107.0, $P = 0.029$). Homocysteine levels were found to be associated with the PON1 L55M genotype ($P = 0.037$) mainly due to the lower levels found in the heterozygotes (13.3 vs. 14.4, $P = 0.011$). Serum iron levels were found to be significantly higher in relation to the presence of the R allele ($P = 0.019$), whereas the ApoB levels were found significantly increased in L/M heterozygotes (114.5 vs. 108.3, $P = 0.021$).

Detailed analysis of the adipose tissue as regards to the fatty acid composition revealed two significant findings both related to the PON1 Q192R polymorphism. The deposition of SFAs was significantly higher in people carrying the R/R genotype (22 vs. 20.5, $P = 0.022$) whereas the deposition of 20:5n3 (EPA) fatty acids was higher in people carrying the Q/Q genotype (0.041 vs. 0.037, $P = 0.039$). The latter association was more pronounced when the heterozygocity was compared ($P = 0.015$). No other genotype association was identified for any of the fatty acids that were analyzed. ANCOVA on all the above associations, using as covariates gender, age, and BMI, did not change significantly the presented P-values. Heterogeneity was tested using Levene's test. Correlation of the amount of SFA and EPA in the food uptake with the deposition of the respective fatty acids in the adipose tissue resulted in a positive effect for SFA ($P < 0.001$). To determine whether the SFA uptake from food was responsible for the observed increased SFA presence in the adipose tissue, the SFA uptake was used as a covariate. The relation of genotype with SFA deposition when SFA uptake was used as a covariate resulted in increased statistical significance (20.5 ± 0.1 vs. 22.1 ± 0.5, $P = 0.004$). So the phenomenon is not due to SFA uptake from food.

The next step was to evaluate the relation of the PON1 serum quantity with the lipoprotein profile, other laboratory measurements, and the fatty acid profile of the adipose tissue. The most reliable method of assessing the PON1 quantity in human serum is to measure the arylesterase activity. The QQ, RR, and QR genotype

TABLE 2. Subcutaneous fat analysis

grams per 100 g of tissue	Male	Female
	Mean ± SD (N)	
Saturated fatty acids *	21.8 ± 2.5 (191)	19.5 ± 2.5 (225)
Mono-unsaturated fatty acids *	64.8 ± 3.6 (191)	66.7 ± 5.4 (225)
Poly-unsaturated fatty acids	12.0 ± 1.8 (191)	12.3 ± 1.7 (225)
Trans fatty acids	0.64 ± 0.19 (191)	0.87 ± 3.73 (225)
n3	1.11 ± 0.17 (191)	1.12 ± 0.19 (225)
n6	10.60 ± 1.83 (191)	10.81 ± 1.83 (225)
18:2n6 (LA)	0.05 ± 0.01 (191)	0.05 ± 0.01 (225)
18:3n3 (LNA) *	0.58 ± 0.08 (191)	0.56 ± 0.09 (225)
20:3n6 (dihomo-γ-linolenic) *	0.22 ± 0.06 (191)	0.28 ± 0.08 (225)
20:4n6 (arachidonic)	0.40 ± 0.11 (191)	0.41 ± 0.11 (225)
20:5n3 (EPA)	0.04 ± 0.02 (191)	0.04 ± 0.02 (225)
22:6n3 (DHA)	0.15 ± 0.06 (191)	0.16 ± 0.06 (225)

ANOVA (*p-value<0.05; Heterogeneity was tested by Levene test).

TABLE 3. The effect of PON1 genotype on the serum paraoxonase, diazoxonase and arylesterase activity

	Genotype	N	Paraoxonase[b]		Diazoxonase[b]		N	Arylesterase[b]	
PON1 Q/R	*Q/Q*	*237*	*343 ± 14*[a]	$P < 0.001$	10093 ± *198*	$P < 0.001$	94	8234 ± *174*	$P < 0.002$
	Q/R	*161*	*579 ± 21*		8024 ± *207*		68	*7676 ± 240*	
	R/R	*21*	*946 ± 56*		5686 ± *392*		11	*6152 ± 689*	
PON1 M/L	*L/L*	*155*	*593 ± 24*	$P < 0.001$	*9285 ± 270*	ns	52	*7734 ± 274*	ns
	M/L	*207*	*429 ± 18*		*8812 ± 212*		83	*7737 ± 203*	
	M/M	*63*	*223 ± 14*		*8912 ± 299*		28	*8017 ± 294*	

[a] Mean ± SE, ns: not statistically significant.
[b] Enzymatic activities are expressed as Units/L.

groups of the population, according to their well-documented difference in the paraoxonase/diazoxonase activity ratio (Fig. 1), define three distinct instances as regards to PON1 functional status. To alleviate possible effects from the differences in the substrate specificity, arylesterase was examined separately for each of the QQ, RR, and QR genotype groups (**Table 5**). The evaluation of the RR subset was not possible due to size restrictions posed by the statistical methodology. The analysis in the QR population subset, representing a balanced paraoxonase/diazoxonase ratio, identified a significant positive correlation of the arylesterase activity with TC ($P = 0.013$), low-density lipoprotein cholesterol (LDL-C) ($P = 0.043$) and ApoB ($P = 0.024$). Interestingly, no such association was identified in the QQ population subset. To further investigate this finding, we examined in the QQ population the distribution of diazoxonase activity, which is the predominant PON1 substrate specific activity in this subset. We identified a significant positive correlation of diazoxonase activity with TC ($P < 0.001$), LDL-C ($P = 0.033$), and ApoB ($P = 0.001$) as we previously reported for the QR subset. In addition, significant positive correlations were indentified with HDL-C ($P = 0.001$) and ApoA1 ($P = 0.004$). Summarizing these findings, it becomes plausible that in the situation where the paraoxonase/diazoxonase ratio is balanced, the PON1 function is directly related to the amount of LDL components (LDL-C, ApoB). Whereas in the situation where diazoxonase is the predominant PON1 function, it is related to both LDL (LDL-C, ApoB) and HDL particle components (HDL-C, Apo1). Finally, when assessing the relation of arylesterase to adipose tissue measurements, a negative correlation was identified in the QQ subset with 20:5n3 (EPA) ($P = 0.030$), indicative of a negative effect of the PON1 amount to 20:5n3 (EPA) sequestering. This finding corroborates our previously reported negative effect to the 20:5n3 (EPA) sequestering in the adipose tissue, related to the shift of PON1 specific activity toward paraoxonase (QR, RR subsets, Table 4).

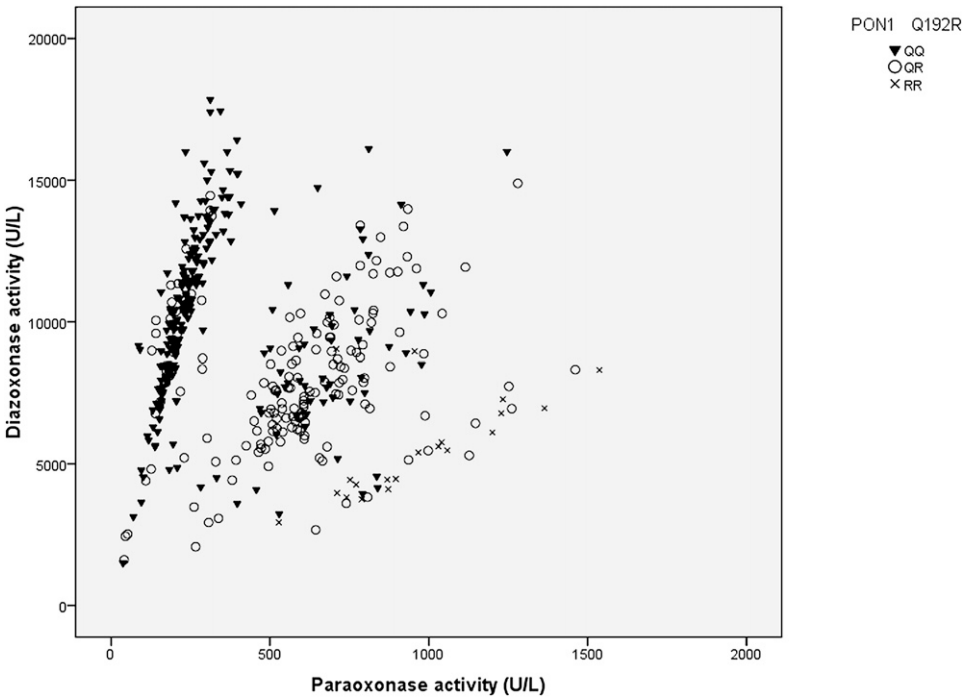

Fig. 1. Plot of the serum diazoxonase versus Paraxonosa enzymatic activity within the QQ, QR and RR genotypic subsets.

TABLE 4. Correlation of the PON1 L55M and Q192R polymorphisms with parameters derived from laboratory measurements (only statistically significant findings are presented)

Parameters	Genes	WW	WM	MM	*P*	P for trend	WW+WM	MM	*P*	WW	WM+MM	*P*	WW+MM	WM	*P*
		Mean ± SE (N)					Mean ± SE (N)			Mean ± SE (N)			Mean ± SE (N)		
Systolic BP (mm Hg)	PON1 L55M	125.3 ± 1.3 (146)	126.5 ± 0.8 (194)	132.5 ± 1.9 (58)	0.007	0.002	125.9 ± 0.8 (340)	132.5 ± 1.9 (58)	0.002						
Diastolic BP (mm Hg)	PON1 L55M	80.1 ± 0.7	80.4 ± 0.6	84.3 ± 1.1	0.003	0.001	80.3 ± 0.4	84.3 ± 1.1	0.001						
Triglycerides (mg/dl)	PON1 L55M	119.7 ± 5.8 (155)	129.9 ± 5.0 (204)	144.0 ± 9.1 (63)	0.071	0.024									
	PON1 Q192R									137.1 ± 4.6 (237)	121.6 ± 5.3 (182)	0.027	137.2 ± 4.4 (257)	119.6 ± 5.6 (162)	0.014
Glucose	PON1 Q192R	92.5 ± 1.6 (235)	93.9 ± 1.9 (157)	107.0 ± 5.9 (18)	0.039	0.018	93.6 ± 1.3 (392)	107.0 ± 5.9 (18)	0.029	92.5 ± 1.6 (235)	97.1 ± 1.9 (175)	0.050			
Homocysteine	PON1 L55M	14.3 ± 0.4 (82)	13.3 ± 0.3 (116)	14.6 ± 0.6 (37)	0.037	0.695							14.4 ± 0.3 (119)	13.3 ± 0.3 (116)	0.011
Serum Iron	PON1 Q192R									91.6 ± 2.6 (235)	101.0 ± 3.1 (162)	0.019			
Apo-B	PON1 L55M												108.3 ± 1.9 (197)	114.5 ± 1.9 (182)	0.021
Adipose tissue Saturated fat	PON1 Q192R						20.5 ± 0.1 (339)	22.0 ± 0.5 (18)	0.022						
20:5n3 (EPA)	PON1 Q192R									0.041 ± 0.001 (204)	0.037 ± 0.001 (153)	0.039	0.041 ± 0.001 (221)	0.037 ± 0.001 (136)	0.015

Analysis of covariance: covariates used were gender, age, and BMI. Heterogeneity was tested by Levene's test. W: wild-type allele, M: rare mutated allele (i.e., M for L55M is the allele M and for Q192R is the allele R).

TABLE 5. Correlation of the PON1 arylesterase serum activity with parameters derived from laboratory measurements within the QQ and QR genotypic subset

		Arylesterase	
		QQ	QR
Parameters		r-Pearson[a] (*p*) N	
Body measurements			
	Waist circumference	−0.009 (0.936) 89	0.116 (0.359) 63
	Body mass index	0.032 (0.766) 89	0.133 (0.291) 63
Blood pressure			
	Systolic blood pressure	0.032 (0.775) 82	0.099 (0.44) 60
	Diastolic blood pressure	0.007 (0.947) 82	−0.131 (0.311) 60
Glucose and biochemical indexes			
	Glucose	0.026 (0.812) 86	0.062 (0.618) 64
	Total cholesterol	0.114 (0.187) 90	0.307 (0.013) 63*
	Triglycerides	−0.006 (0.987) 88	0.240 (0.052) 64
	HDL-C	0.086 (0.413) 90	0.173 (0.164) 64
	LDL-C	0.108 (0.313) 87	0.252 (0.043) 63*
	Total cholesterol: HDL-C ratio	0.049 (0.646) 90	0.120 (0.341) 63
	Lipoprotein A	−0.096 (0.386) 82	0.060 (0.659) 55
	Apo lipoprotein A1	0.086 (0.436) 82	0.067 (0.619) 55
	Apo lipoprotein B	−0.051 (0.658) 77	0.313 (0.024) 50*
	Folate acid	0.198 (0.214) 39	0.100 (0.606) 27
	Serum vitamin B12	−0.290 (0.070) 38	0.185 (0.336) 27
	Serum iron	−0.012 (0.912) 84	−0.017 (0.907) 49
	Homocysteine	0.239 (0.137) 38	−0.230 (0.230) 27
Adipose tissue			
	Saturated fatty acids	0.119 (0.298) 76	−0.062 (0.625) 62
	Mono-unsaturated fatty acids	−0.131 (0.253) 76	0.115 (0.365) 62
	Poly-unsaturated fatty acids	0.100 (0.386) 76	−0.130 (0.306) 62
	Trans fatty acids	−0.037 (0.749) 76	−0.026 (0.836) 62
	n3	−0.153 (0.182) 76	−0.004 (0.975) 62
	n6	0.117 (0.307) 76	−0.132 (0.297) 62
	18:2n6 (LA)	0.131 (0.254) 76	−0.146 (0.249) 62
	18:3n3 (LNA)	−0.018 (0.879) 76	−0.052 (0.682) 62
	20:3n6 (dihomo-γ-linolenic)	−0.029 (0.501) 76	0.115 (0.365) 62
	20:4n6 (arachidonic)	−0.068 (0.557) 76	0.068 (0.593) 62
	20:5n3 (EPA)	-0.246 (0.030) 76*	−0.010 (0.935) 62
	22:6n3 (DHA)	−0.197 (0.084) 76	0.069 (0.588) 62

[a] Partial correlation coefficient. Controlling for age and gender. * $P < 0.05$, The RR subset was not evaluated due to size restrictions of the employed statistics.

DISCUSSION

The role of PON1 in the modulation of lipid metabolism is a research area under intense investigation due to its established relationship with human CVD (25). The PON1 polymorphisms L55M and Q192R are considered the major genetic determinants of the significant interindividual variability of serum PON1 quantity and activity (7, 8) in cooperation with environmental factors such as smoking, high-fat diet, and inflammation (26–28).

PON1 and plasma lipids/lipoproteins

The PON1 serum enzymatic activity has been previously associated with the concentration of serum ApoA-I, LDL-C, and HDL-C [for a comprehensive review see (29)]. The present study provided further insight by documenting two distinct instances: *a*) a situation where the paraoxonase/diazoxonase ratio is balanced (QR subset) and the PON1 function (arylesterase) is directly related to the amount of LDL components (LDL-C, ApoB), and *b*) a situation where diazoxonase is the predominant PON1 function (QQ subset) and it is related to both LDL (LDL-C, ApoB) and HDL particle components (HDL-C, Apo1). These findings offer new data about a possible novel differential function between the Q192R alleles of the PON1 molecule in addition to the well-documented effects on the enzyme substrate specificity. Specifically, it seems that it could be potentially implicated in changes of the enzyme's relative localization to HDL and LDL particles in the human serum. Nevertheless, it is necessary that this idea is further investigated in independent studies with a different model system.

Genetic epidemiology studies on the polymorphisms L55M and Q192R have previously reported conflicting data regarding effects on plasma lipoproteins (30–32). A specific search in the published genome wide association studies found no reported association of the PON1 genetic locus with lipids, BP, or any other of the reported parameters. Recently, the homozygotes of the PON1 Q192 polymorphism were reported to have lower levels of plasma ApoB related biochemical variables and lower ratios of TC and ApoB/ApoA-I (33). The present study did not find any such relationship. Nevertheless, we identified a significant association of the PON1 Q/R genotype with reduced serum triglycerides as well as an association of the PON1 R allele with increased levels of glucose. Interestingly, previous population studies have demonstrated a contribution of PON1 in pathways involving insulin resistance and diabetes (34–36). Regarding the PON1 L/M polymorphism, a previous study demon-

strated (37) a significant difference in the TC and LDL-C levels between subjects with the PON1 L/L and M/M genotype. Furthermore, increased baseline concentration of HDL (38) in the LL homozygotes and higher ApoA-I concentrations have also been reported. Another study (39) demonstrated that carriers of PON1 M55 have higher mean plasma concentrations of TC, LDL-C, and ApoB as compared with noncarriers. The present study identified significantly increased ApoB levels in the PON1 M/L heterozygotes whereas no association with the other plasma lipoproteins. Other previous studies have shown no association whatsoever between PON1 polymorphisms and plasma lipoproteins (30–32). Contradictory results from different ethnic groups can partly be explained by intrinsic differences in serum PON1 activity between populations. Nevertheless, analysis of the current data and bibliography demonstrates that the relationship of the PON1 genotypes with serum lipid and lipoprotein levels cannot be summarized in a unified model just yet.

PON1 gene polymorphisms and adipose tissue fatty acid content

The fatty acids are stored as triglycerides in the adipose tissue, which represents the major lipid storing site for mammals and birds. The amount of lipid storage at a given time point is the sum of lipid accumulation through synthesis and lipid mobilization through triglyceride breakdown (40). The relative rate of lipid deposition and removal is significantly affected by the nutritional state and endocrine factors (41, 42). Although fatty acid composition is believed to be in direct relative proportion to the dietary lipid intake, the PUFA stored in adipose tissue is always lower in proportion as compared with that in diet (43). This discrepancy can only be explained by the existence of a mechanism for preferential deposition or mobilization of specific fatty acids. Selectivity in the process of the fatty acid incorporation in adipose tissue has already been reported but the exact mechanism is still unknown. Measurement of the relative mobilization of different fatty acids demonstrated that the adipose tissue releases fatty acids with the following preference: 20:5n3 > 22:6n3 > 22:5n3 (44). Lipid mobilization experiments in humans, performed both in vitro (45) and in vivo (46), demonstrated that nonesterified fatty acids were preferentially enriched in PUFA and depleted in long-chain SFA and MUFA. The proposed models for the preferential lipid deposition/mobilization implicate parameters, which regulate the intracellular lipolysis performed by specific lipase enzymes. Interestingly, PON1 has been assigned with an inherent phospholipase A2-like activity toward phosphatidylcholine core aldehydes (47). The present study described a preferential reduction of SFA accumulation in adipose tissue associated with the PON1 Q allele and a reduction of 20:5n3 (EPA) fatty acids associated with the R allele. The Q/R alleles are responsible for the observed switch of the PON1 enzyme substrate specificity toward diazoxon/paraoxon respectfully. Furthermore, data from the analysis of PON1 serum total quantity in the QR, RR subsets documented an additional negative effect to the 20:5n3 (EPA) sequestering in the adipose tissue, related to the shift of PON1-specific activity toward paraoxonase. These results, which are independent of the dietary lipid intake, indicate that the paraoxonase 1 might be part of the elusive mechanism of preferential lipid deposition /mobilization. Previous studies have associated paraoxonase activity in the serum with diabetes and insulin resistance (34–36). Insulin signaling is linked to both lipid deposition in adipose tissue via activation of lipoprotein lipases and adipocyte lipolysis via suppression of the hormone sensitive lipase. The insulin dependent signaling via its receptors can be regulated by the oxidative stress in the environment surrounding the adipose tissue fat cells (13). Specifically, it has been established that oxidative stress can disrupt insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes (13). Recent data have established a direct link between paraoxonases and the mobilization of lipid biosynthesis through the activation of diacylglycerol acyl transferase (11). PON1 and 3 but not PON2 have been found to be present in the interstitial space between unilocular adipose tissue fat cells in mice (12) and thus, through their antioxidant activity, they could potentially participate in the modulation of the insulin-dependent lipid mobilization/deposition.

CONCLUSION

The present study evaluated the impact of the L55M and Q192R polymorphisms and PON1 activity on serum lipids and lipoproteins (CVD-related parameters) as well as on the adipose tissue fatty acid composition in a cross-section of the human population on the island of Crete (Greece). An association of the polymorphisms with blood pressure, glucose, triglycerides, and ApoB was established as well as a possible association of the enzymatic activity with differential localization to LDL/HDL components. Moreover, our results indicated a novel function for PON1 on the selectivity of fatty acid composition in the adipose tissue through the association of the R allele with SFAs and of the Q allele with 20:5n3 fatty acid deposition. Further studies are necessary to examine the exact role of PON1 in lipoprotein metabolism and lipid homeostasis.

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