RESEARCH ARTICLE

# Determinants of paraoxonase activity in healthy adults

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**Scope**: Paraoxonase-1 (PON-1), associated with HDL, is regarded as anti-atherogenic, attributed to its ability to hydrolyze oxidized lipids. Here, the impact of PON and apolipoprotein E genotypes, age, alcohol and HDL-cholesterol (HDL-C) on PON activity is examined.
**Methods and results**: In total, 104 healthy UK adults participated in the study, with basal (PONA) and stimulated (PONB) PON-1 activities and arylesterase activity determined in these individuals. In univariate and correlation analysis age, HDL-C, alcohol intake and both PON genotypes were significantly associated with PONA and PONB activities ($p < 0.05$). However, in the standard linear regression model, which explained 69% of the variability in both PONA ($p < 0.001$) and PONB activities ($p < 0.001$) only PON Q192R genotype emerged as a significant independent determinant, with four to fivefold higher levels in the RR *versus* wild-type QQ genotype groups. For PON arylesterase, HDL-C ($p = 0.030$), apolipoprotein E ($p = 0.023$) and PON Q192R ($p = 0.002$) and PON L55M ($p = 0.002$) genotypes collectively explained 14% of the total variability in the regression model.
**Conclusion:** Our results indicate that PON genotypes are stronger determinants of PON activity relative to the other potential modulators assessed. The relative impact of dietary components on PON activities remains to be established.

**Keywords:**
Age / apoE genotype / Paraoxonase / Paraoxonase L55M / Paraoxonase Q192R

## 1 Introduction

Paraoxonase (PON-1) is a HDL-associated glycoprotein which hydrolyzes organophosphates and lipid peroxides, included those derived from both LDL and HDL particles [1, 2]. It is therefore considered an important anti-atherogenic component [3, 4], preventing the accumulation of oxidized LDL (oxLDL) in the arterial intima. A missense mutation in the PON-1 gene, which results in a glutamine to arginine substitution at amino acid 192, greatly impacts PON activity [5, 6], and has been related to cardiovascular disease (CVD) risk, in some, but not all studies [6–9]. Furthermore, although not shown to be independent predictors of CVD, a number of additional coding and promoter gene variants have been shown to impact PON activity and circulating protein levels [10–12]. However, apart from the impact of PON genotypes, determinants of circulating PON-1 concentration and activity are poorly understood, with associations with age, HDL-cholesterol (HDL-C), alcohol and smoking status inconsistently reported in the literature [10, 12–16].

In addition to PON-1, lipoprotein-associated apolipoprotein E (apoE) is known to be a HDL-associated antioxidant. apoE genotype has been shown to impact plasma lipid oxidation with carriers of the $\varepsilon 4$ allele reported to have

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**Abbreviations: apoE,** apolipoprotein E; **CVD,** cardiovascular disease; **HDL-C,** HDL-cholesterol; **LDL-C,** LDL-cholesterol; **oxLDL,** oxidized LDL; **PA,** PON arylesterase; **PON,** paraoxonase

 

higher plasma oxLDL and F2-isoprostanes levels [17, 18]. Furthermore, apoE genotype is known to impact the lipoprotein distribution of apoE, with the E2 and E3 isoforms largely associated with HDL in the fasting state, in contrast to the E4 isoform which has a preference for the more lipid-rich very low density lipoprotein (VLDL) particles [19]. Although currently unknown, it is hypothesized that apoE genotype may influence PON-1 activity, through an impact on oxidative status and potential protein displacement on the HDL particle, which may in part explain the exaggerated CVD risk in carriers of the apoE4 genotype (25% Caucasians) [20–23].

In this study, the relative and independent impact of a number of potential physiological and behavioral determinants of PON-1 activity is investigated in healthy UK adults.

## 2 Materials and methods

### 2.1 Volunteers

One hundred and four healthy normolipidaemic males and females, 20–70 years, BMI 18.5–30 $kg/m^2$, were recruited. Exclusion criteria for participation in the study were diagnosed diabetes or CVD; fasting total cholesterol, triglycerides and glucose $>8.0$, 3.0 and 6.5 mmol/L, respectively; liver or other endocrine dysfunction; lipid lowering medication, consumption of fatty acid supplements or antioxidant supplements, or oily fish $>1$ time/week; on a weight-reducing diet. Trained athletes or those who participated in $>3$ h of organized exercise *per* week were also excluded. The study was approved by West Berkshire local research ethics committees (LREC, REC 111/02L) and all individuals provided informed written consent prior to participation.

### 2.2 Clinical visit protocol

On the day before their clinical visit, participants were asked to refrain from alcohol and organized exercise and to consume a low fat ($<10$ g fat) meal of their choice as their main evening meal in order to standardize short-term fat intake. Participants attended the unit in a 12 h fasted state. Height and weight were recorded and BMI was calculated. Blood was drawn from the antecubital vein into potassium-EDTA for lipid and apolipoprotein analysis and lithium heparin for PON activity quantification. Within 30 min of withdrawal, the blood was centrifuged at 3000 rpm for 10 min at 4°C and the plasma stored at −80°C awaiting analysis. Prior to the freezing of the plasma to be used for the determination of apoE, apoB and apoA1 concentrations, a protease inhibitor and antibiotic containing preservative were added (5% v/v) [24].

Habitual alcohol intake was established by a FFQ administered on two occasions (screening and study visit).

### 2.3 Biochemical analysis

Plasma total cholesterol and triglyceride concentrations were quantified on the Hitachi 717 analyzer using commercially available enzymatic colorimetric kits (Roche, Burgess Hill, UK). Plasma LDL-cholesterol (LDL-C) and HDL-C concentrations were determined according to the Lipid Research Clinics Program Manual of Laboratory Operations [25]. Plasma apoA1, apoB and apoE concentrations were determined by automated turbidimetric immunoassay kits (Alpha Laboratories, Eastleigh, UK) on an iLAB 600 autoanalyzer (Instrumentation Laboratory). oxLDL concentration was determined using a two-site ELISA (Mercodia AB, Uppsala, Sweden) which is based on the quantification of modified apoB in the sample.

### 2.4 PON activity measurement

Basal (PONA) and stimulated (PONB) PON and arylesterase (PA) activities were measured in plasma samples with paraoxon and phenylacetate as substrates [26, 27]. Hydrolysis rate of paraoxon (diethyl-*p*-nitrophenyl phosphate; Supelco) was measured by monitoring the increase of absorbance at 405 nm using 100 mmol/L Tris–HCl buffer (pH 8.0) with 1 mmol/L paraoxon and 2 mmol/L $CaCl_2$ without (basal) and with addition of 1 M NaCl (salt-stimulated activity). One unit of PON activity is defined as 1 nmol of 4-nitrophenol formed *per* minute under the above assay conditions (molar absorptivity coefficient 17 600 $M^{-1}\,cm^{-1}$). Phenylacetate was used as a substrate to measure the arylesterase activity in 20 mmol/L Tris/HCl buffer, pH 8.0, 4 mmol/L phenylacetate and 1 mmol/L $CaCl_2$. Enzymatic activity (PA) was calculated from the molar absorptivity coefficient of the released phenol (1310 $M^{-1}\,cm^{-1}$) with one unit of arylesterase activity defined as 1 μmol phenylacetate hydrolyzed/min and expressed as kU/L serum.

Although it is recognized that PON activity, as determined by its ability to hydrolyze these synthetic substrates, does not provide an exact measure of PON-1's ability to prevent lipid oxidation *in vivo* due to differences in active site requirement, it does represent a commonly used surrogate marker as there is considerable overlap in activities [28]. Paraoxon and phenylacetate hydrolytic activity provide measure of PON-1 activity and availability (as it is not affected by the activity of the catalytic site), respectively [12, 29].

### 2.5 *APOE* and *PON-1* genotyping

DNA was isolated from the buffy coat layer of 10 mL of EDTA blood using a Qiagen DNA Blood Mini Kit (Qiagen, Crawley, UK). *APOE* genotyping was determined using a derivation of the technique of Hixson and Vernier [30].

 

Following digestion with the *HhaI* restriction endonucleases, the resultant fragments were separated by gel electrophoresis on a 10% polyacrylamide gel (BioRad, Hemel Hempstead, UK) and *APOE* genotype identified from the fragment pattern as described previously [31].

Allelic discrimination of the two SNPs in the *PON-1* gene (rs662, rs854560) was performed with validated TaqMan SNP genotyping assays (Applied Biosystems, Darmstadt, Germany). SNP genotyping reactions were performed on a Rotorgene 3000 real-time thermal cycler (Corbett Research, Sydney, Australia) using standard cycling conditions. Control samples with known PON-1 genotypes were included in each PCR run. Data were analyzed using scatter analysis.

### 2.6 Statistical analysis

Data are presented as mean (SEM). Bivariate correlation analysis was used as a first step to examine associations between PON activities and age, HDL-C and alcohol intakes. The individual impact of apoE and PON genotypes, age, alcohol intakes and HDL-C on PON activities was subsequently determined using univariate ANOVA with age, alcohol intakes and HDL-C converted to categorical data as follows:

age: Group 1 $\leq$ 50 years, Group 2 $>$ 50 years
alcohol intake: Group 1 $\leq$ 3 units *per* week, Group 2 $>$ 3 units *per* week, with 1 unit equivalent to 8 g pure alcohol.
HDL-C: Group 1 $\leq$ 1.5 mmol/L, Group 2 $>$ 1.5 mmol/L

For the apoE genotype, individuals were categorized as apoE2 (E2/E2+E2/E3), apoE3 (E3/E3) or E4 (E3/E4+E4/E4), with E2/E4 individuals excluded from the analysis, as from a physiological perspective they cannot be categorized as E2 or E4 subgroups and cannot be considered as a individual subgroup due to small group size ($n = 2$). Standard linear regression was performed to examine the relative and independent impact of the variables on PON activity levels. All analyses were conducted using SPSS Version 15 (Chicago, IL, USA) and $p < 0.05$ was considered to be significant.

## 3 Results

Data from 104 individuals (58 females and 46 males) are included in the current analysis, with a group mean age (years), BMI (kg/m$^2$) LDL-C (mmol/L) and HDL-C (mmol/L) of 44.8 (1.3), 25.1 (0.4), 3.20 (0.09) and 1.51 (0.03), respectively. Complete data sets were available for 95 participants.

Although significant positive correlations between both alcohol intake and HDL-C and PON PON activities (PONA and PONB) ($r = 0.2$–$0.3$, $p < 0.05$) were evident (data not shown), in the ANOVA analysis no significant subgroup differences emerged (Table 1). In univariate analysis, age was significantly associated with PON activity, with 29% ($p = 0.024$) and 33% ($p = 0.012$) lower PONA and PONB activities in the older *versus* younger age groups (Table 1).

For the PON R192Q genotype, allele frequencies of 0.715 and 0.285 were evident for the Q and R alleles, with frequencies of 0.663 and 0.337 for the L and M allele of the L55M genotype. No significant impact of either genotype on LDL-C, HDL-C and apoA1 was evident (Table 2). A highly significant impact of PON Q192R genotype on both PONA ($p < 0.001$) and PONB ($p < 0.001$) activities emerged with

**Table 1.** PON activities according to age, apoE genotype, alcohol intake and HDL-C

| Variable | *n* | PONA (U/L) | PONB (U/L) | PA (kU/L) |
|---|---|---|---|---|
| **Age** | | | | |
| Younger ($\leq$50 years) | 63 | 118 (10) | 288 (26) | 186 (6) |
| Older ($>$50 years) | 41 | 84 (9) | 193 (22) | 184 (6) |
| $p^{a)}$ | | 0.024 | 0.012 | NS |
| **apoE genotype** | | | | |
| E2 | 37 | 100 (12) | 240 (31) | 176 (6) |
| E3 | 35 | 92 (11) | 220 (27) | 183 (8) |
| E4 | 32 | 124 (15) | 296 (38) | 198 (9) |
| $p^{a)}$ | | NS | NS | NS |
| **Alcohol intake** | | | | |
| Group 1 ($\leq$3 units/week) | 55 | 95 (10) | 233 (25) | 180 (6) |
| Group 2 ($>$3 units/week) | 49 | 115 (11) | 270 (27) | 191 (7) |
| $p^{a)}$ | | NS | NS | NS |
| **HDL-C** | | | | |
| Group 1 ($\leq$1.5 mmol/L) | 57 | 101 (9) | 243 (24) | 184 (7) |
| Group 2 ($>$1.5 mmol/L) | 43 | 108 (13) | 259 (30) | 190 (5) |
| $p^{a)}$ | | NS | NS | NS |

a) Values are means (SEM); HDL-C, HDL-cholesterol; apoE2 = E2/E2+E2/E3, E3 = E3/E3; apoE4 = E3/E4+E4/E4; PONA, unstimulated paraoxonase-1 activity; PONB, stimulated paraoxonase-1 activity.

 

**Table 2**. Paraoxonase activities according to PON Q192R and L55M genotypes[a)]

| Variable | PON Q192R genotype | | | $p$ | PON L55M genotype | | | $p^{a)}$ |
|---|---|---|---|---|---|---|---|---|
| | QQ ($n = 52$) | QR ($n = 33$) | RR ($n = 12$) | | LL ($n = 42$) | LM ($n = 45$) | MM ($n = 10$) | |
| LDL-C (mmol/L) | 3.27 (0.11) | 3.17 (0.17) | 3.00 (0.33) | NS | 3.20 (0.14) | 3.18 (0.13) | 3.28 (0.26) | NS |
| HDL-C (mmol/L) | 1.45 (0.04) | 1.57 (0.06) | 1.63 (0.10) | NS | 1.48 (0.05) | 1.56 (0.05) | 1.50 (0.11) | NS |
| apoA1 (μg/mL) | 1366 (29) | 1387 (36) | 1442 (57) | NS | 1349 (34) | 1414 (28) | 1388 (66) | NS |
| PONA (U/L) | 50 (4) | 148 (7) | 224 (21) | $< 0.001$ | 136 (12) | 90 (10) | 39 (7) | $< 0.001$ |
| PONA:LDL-C | 16 (1) | 52(4) | 82 (11) | 0.001 | 47 (5) | 31 (4) | 13 (3) | 0.001 |
| PONA:HDL-C | 37 (3) | 97 (5) | 141 (14) | $< 0.001$ | 92 (7) | 58 (6) | 28 (6) | $< 0.001$ |
| PONA:apoA1 | 0.038 (0.003) | 0.108 (0.005) | 0.156 (0.015) | $< 0.001$ | 0.100 (0.008) | 0.064 (0.007) | 0.029 (0.005) | $< 0.001$ |
| PONB (U/L) | 116 (11) | 356 (18) | 559 (54) | $< 0.001$ | 333 (31) | 215 (25) | 84 (8) | $< 0.001$ |
| PONB:LDL-C | 37 (3) | 126 (10) | 204 (26) | $< 0.001$ | 116 (13) | 73 (10) | 29 (6) | 0.001 |
| PONB:HDL-C | 84 (9) | 234 (13) | 353 (36) | $< 0.001$ | 223 (20) | 140 (16) | 58 (5) | $< 0.001$ |
| PONB:apoA1 | 0.087 (0.008) | 0.263 (0.014) | 0.391 (0.038) | $< 0.001$ | 0.245 (0.021) | 0.153 (0.018) | 0.061 (0.005) | $< 0.001$ |
| PA (kU/L) | 191 (5) | 185 (10) | 175 (12) | NS | 195 (7) | 183 (9) | 172 (13) | NS |
| PA:LDL-C | 62 (3) | 64 (5) | 70 (12) | NS | 67 (4) | 62 (3) | 59 (10) | NS |
| PA:HDL-C | 138 (6) | 123 (7) | 110 (9) | NS (0.053) | 137 (6) | 125 (7) | 118 (9) | NS |
| PA:apoA1 | 0.143 (0.005) | 0.136 (0.008) | 0.122 (0.007) | NS | 0.147 (0.005) | 0.133 (0.006) | 0.125 (0.009) | NS |
| oxLDL (U/L) | 59.8 (2.6) | 57.7 (3.4) | 59.4 (8.1) | NS | 58.7 (3.0) | 58.4 (2.6) | 63.8 (10.0) | NS |

a) Values are mean (SEM): apoA1, apolipoprotein A1; PONA, unstimulated paraoxonase-1 activity; PONB, stimulated paraoxonase-1 activity; PA, arylesterase activity.

four- to five-fold higher levels in the homozygous RR *versus* QQ groups (Fig. 1). In univariate ANOVA, the L55M genotype was also significantly associated with PONA and PONB with 70–75% lower PON activities and PON: LDL-C/HDL-C/apoA1 ratios in the homozygous rare group (MM) relative to the wild-type LL genotype (Table 2 and Fig. 1).

No significant impact of either PON genotype on PA was evident in univariate analysis (Table 2). Although there was a tendency for higher PON activities in the apoE4 *versus* E2 and E3 group, no significant genotype effect emerged in the ANOVA analysis.

In the linear regression model, the added variables collectively explained 69–70% of both PONA and PONB activities ($p < 0.001$), with only PON Q192R genotype emerging as a significant independent determinant. Collectively, the independent variables explained 14% of the total variation in PA activity, with the strongest determinants being the PON Q192R (standardized $\beta = -0.372$) and PON L55M genotypes (standardized $\beta = -0.362$), followed by apoE genotype (standardized $\beta = -0.222$) and HDL-C (standardized $\beta = 0.218$). Linear regression analysis conducted according to gender, indicated association between apoE genotype and PA activities in females (PONA ($p = 0.004$), PONB ($p = 0.016$) and PA ($p = 0.004$)) but not in males (Fig. 2). (Table 3)

## 4 Discussion

Low HDL-C levels are recognized as one of the most significant risk factors for CVD, with every 1% decrease in circulating levels associated with a 2–3% increase in risk [32]. There does not appear to be one single defining mechanism responsible, but undoubtedly the antioxidant properties of HDL-associated proteins and their impact of LDL oxidation and macrophage LDL scavenging are important in the inhibition of atherosclerosis initiation and progression. HDL-associated PON has emerged as a potentially important cardioprotective enzyme. In animal models, deletion of the PON-1 gene is associated with increased LDL oxidation, macrophage oxidative stress and lesion size, with mice over-expressing PON-1 exhibiting a decreased susceptibility to atherosclerosis [2, 33, 34]. The role of PON in cardiovascular and overall health in humans is demonstrated by the fact that a common SNP in the PON gene, the PON Q192R genotype which impacts PON activity, has been associated with CVD risk and longevity [6, 35–37]. In the recent Genebank prospective cohort, individuals with the QQ genotype had an adjusted hazard ratio (95% CI) of 2.05 (1.32–3.18) and 1.48 (1.09–2.03) for all-cause mortality and major adverse cardiac events relative to the combined QR and RR subgroup [6]. Lescai *et al.* have recently conducted a meta-analysis of 11 individual studies which have related PON Q192R to longevity [37]. The R allele was significantly over-represented in the older group (>65 years) with an OR (95% CI) of 1.17 (1.04–1.30).

 

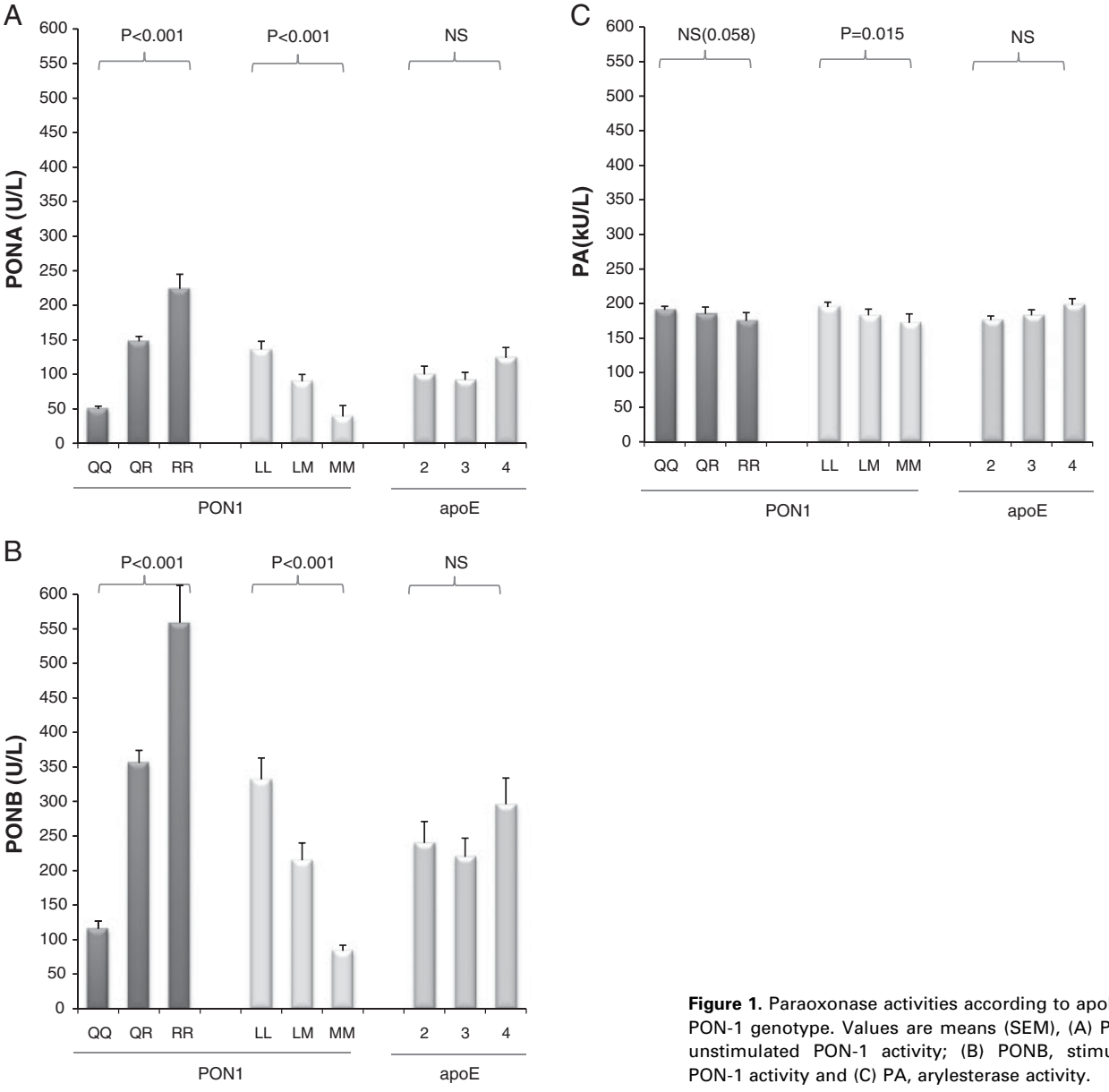


**Figure 1.** Paraoxonase activities according to apoE and PON-1 genotype. Values are means (SEM), (A) PONA, unstimulated PON-1 activity; (B) PONB, stimulated PON-1 activity and (C) PA, arylesterase activity.

The most important finding of this study is that relative to PON-1 genotype, age, alcohol intakes, HDL-C and apoE genotype have minimal impacts on PON PON activity, with genotype emerging as the only significant variable in our regression model which explained over 69% of the variation in both basal and stimulated activities. In line with the previous findings, four- to five-fold lower activities were observed in the QQ *versus* the RR subgroups [6, 12]. In a recent study, it was demonstrated that the amino acid at position 192 is critical in HDL-apoA1 binding and that the glutamine-containing alloenzyme (Q192) has threefold lower affinity for HDL relative to the arginine-containing isoform, which consequently results in lower PON stability and PON lipolactonase activity [38]. Although an association between the PON L55M genotype and the PON activity was evident in the univariate analysis, this genotype did not emerge as being independently associated, which is in agreement with other studies [29]. Its univariate association with PON activity may be attributable to its known linkage disequilibrium with the PON Q192R SNP [12, 39]. The PON Q192R SNP is also known to be in linkage disequilibrium with a number of SNPs in the gene regulatory region, and it may be that the apparent impact is in part attributable to these other potentially functional gene variants such as the C-107T variant, which have been shown to impact gene promoter activity and PON protein levels [11, 12, 29, 40].

The lower PON activity in PON QQ and MM groups was not reflected in higher circulating oxLDL levels as assessed

 

by modified apoB levels (which served as a biomarker for overall LDL oxidation status) which are consistent with the finding of van Himbergen, who examined associations in a group of familial hypercholesterolaemic individuals [29]. Plasma is considered a relatively antioxidant-rich environment relative to the arterial intima, with components such as uric acid acting as reducing agents. In the arterial intima, lower PON-1 activity would be predicted to have a greater impact on oxLDL concentrations, which may not be reflected in circulating oxLDL levels. On the contrary, in the Genebank study the PON Q192R genotype was associated with the concentration of a range of fatty acid oxidation products with often several fold higher levels in those with the QQ relative to the RR genotype [6].

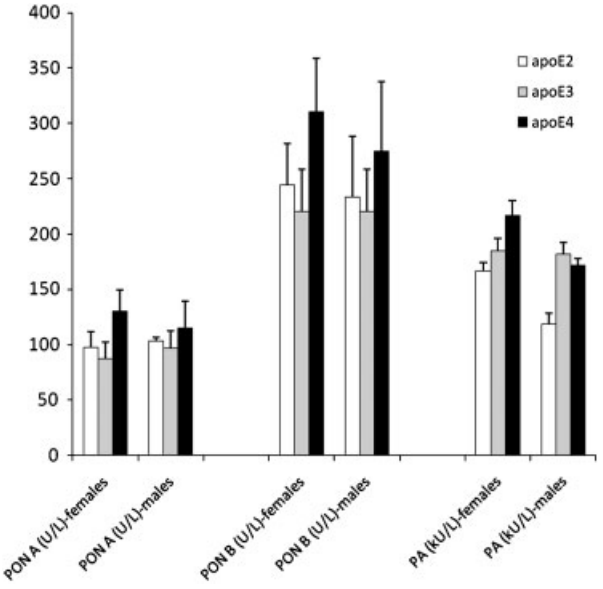


**Figure 2.** Paraoxonase activities according to apoE genotype in females and males. Values are means (SEM), PONA, unstimulated, PON-1 activity; PONB, stimulated PON-1 activity and PA, arylesterase activity. In linear regression analysis (with PON1 Q192R, PON1 L55M, apoE, age, alcohol and HDL-C added to the model) according to gender, in addition to PON1 genotype (Table 3), apoE genotype emerged as a significant determinant of PONA ($p = 0.004$) and PONB ($p = 0.016$) in females, but not in males. For PA, although a significant independent association with apoE genotype emerged in the group as a whole, the impact of genotype was significant in females ($p = 0.004$) but not in males ($p = 0.608$).

A lack of independent association of age with any measure of PON activity in the current analysis is consistent with a number of other studies [12, 14, 35]. In the largest of these ($n = 1527$), Roest *et al.* reported PON activities of 159, 166 and 151 U/L in subgroups with an age of $<53$, 53–60 and $>60$ years, respectively [12]. On the contrary, a small number of studies have reported a significant impact of age on PON activity [13, 15] with Cherki *et al.*, observing a 41% lower basal and salt-stimulated PON-1 activity in younger (20–30 years) compared with older (60–89 years) healthy individuals [13]. In our initial analysis of our cohort, using univariate ANOVA and correlation approaches, age did emerge as a significant determinant. In multivariate approaches, the significant association disappeared with a closer examination of the data indicating a higher prevalence of the QQ genotype in the older (65%) *versus* younger (46%) subgroups (Table 1), with the apparent effect of age actually reflecting a genotype effect. Given the large impact of genotype on PON activities, similar random differences in genotype prevalence, in particular in smaller cohorts, may be responsible for the reported impact of age where genotype was not determined and corrected for.

In line with the current findings, a number of observational studies have observed no significant independent

**Table 3.** Standard multiple regression model investigating the integrative impact of a number of variables on paraoxonase activities

| Dependent variable | $r^2$ (adjusted) | *p* | Predictors | β-coefficients (standardized) | *p* |
|---|---|---|---|---|---|
| PONA (U/L) | 0.694 | $<0.001$ | Age | 0.017 | NS |
| | | | Alcohol | 0.077 | NS |
| | | | HDL-C | 0.050 | NS |
| | | | apoE | 0.090 | NS |
| | | | PON Q192R | 0.764 | $<0.001$ |
| | | | PON L55M | −0.101 | NS |
| PONB (U/L) | 0.693 | $<0.001$ | Age | −0.022 | NS |
| | | | Alcohol | 0.040 | NS |
| | | | HDL-C | 0.054 | NS |
| | | | apoE | 0.086 | NS |
| | | | PON Q192R | 0.764 | $<0.001$ |
| | | | PON L55M | −0.096 | NS |
| PA (kU/L) | 0.142 | 0.003 | Age | −0.057 | NS |
| | | | Alcohol | 0.151 | NS |
| | | | HDL-C | 0.218 | 0.030 |
| | | | apoE | 0.222 | 0.023 |
| | | | PON Q192R | −0.372 | 0.002 |
| | | | PON L55M | −0.362 | 0.002 |

PONA, unstimulated paraoxonase-1 activity; PONB, stimulated paraoxonase-1 activity.

 

impact of alcohol intake on PON-1 activities [16, 41, 42]. On the contrary, some observation [12] and intervention studies [42, 43] have reported modestly (<15%) higher PON activities in moderate *versus* low/no alcohol intake. Mechanisms proposed for this PON activity raising effect include an indirect effect due to the known HDL-C raising impact of alcohol [44] (with an association between PON activity and HDL-C concentration frequently observed) or a direct impact on hepatic PON-1 mRNA [45]. Therefore, overall it appears that under particular conditions alcohol intake may modify PON activities but the effect size is likely to be modest relative to PON-1 Q192R genotype.

In addition to the Q192R genotype, the leucine (L) to methionine (M) amino acid change at position 55 in PON-1 was independently associated with arylesterase activity. An association between the M allele and reduced phenylacetate hydrolysis has been reported previously [11, 12, 29, 39, 46]. This impact of genotype has been proposed to be due to an effect on mRNA stability and PON-1 protein levels rather than an impact on the catalytic efficacy of the enzyme [12, 29, 47].

HDL-C was a significant, but relatively weak, determinant of PA, with no significant association with PON activity evident. Similar relatively weak associations have been reported previously [12]. As all circulating PON-1 is found in HDL particles, a stronger association may perhaps be predicted. A possible explanation is the fact that PON-1 is not distributed across the entire HDL spectrum, but is associated with a quantitatively minor fraction (10%), the characteristics of which remain somewhat ambiguous [48]. Therefore, total HDL-C may not represent a good overall biomarker for the subspecies of HDL which PON-1 is generally associated. Furthermore, as proposed by Roest *et al.* [12], the relative excess of HDL particles relative to PON molecules may mean that relatively minor fluctuations in HDL-C is unlikely to have a large overall impact on PON activities.

Here, we report for the first time that apoE genotype influences PON arylesterase activity. An apoE4 genotype has been associated with a 20–50% increased risk of CVD [49], which may be in part attributable to a compromised oxidative status associated with the *ε4* allele as reviewed by Jofre-Monseny *et al.* [50]. In the Framingham Offspring Study E4, smokers had approximately 30% higher oxLDL relative to non-E4 smoking group [18]. In a mixed smoking and nonsmoking group, those individuals with mild hypercholesterolemia had 30% higher circulating F2-isoprostanes relative to the E2 and E3 groups [17]. This study demonstrates that a detrimental effect of apoE genotype on PON-1 activity is not responsible for the lower oxidative status in E4 carriers, with evidence of higher PON activity in this subgroup. It may be speculated that any increase in this genotype group may be attributable to a compensatory effect for the lower oxidative status in E4, or due to a displacement of PON-1 by apoE on the HDL particle, due to the lower preference of E4 for HDL relative to the E2 and E3 isoforms [19].

In conclusion, the findings demonstrate a complex interplay between PON-1 gene variants and PON activities as measured by different substrates. Given the strong impact of the PON Q192R genotype on PON activities, and the impact this genotype has been shown to have on CVD risk and longevity, in some but not all studies, there is a need to identify strategies which effectively modify PON activity or its physiological response in carriers of the at risk Q allele. Although dietary flavonoids [51, 52], fat composition [53] vitamin C and vitamin E [54], smoking status [55], and a number of lipid modulatory drugs [10] have been identified as being potential modulators, findings thus far have been relatively inconsistent, and often limited to cell and animal models, with a need for confirmation in large human observational or intervention trials.

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*The authors have declared no conflict of interest.*

## 5 References

[1] Blatter, M. C., James, R. W., Messmer, S., Barja, F., Pometta, D., Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. *Eur. J. Biochem.* 1993, *211*, 871–879.

[2] Ng, C. J., Shih, D. M., Hama, S. Y., Villa, N. *et al.*, The paraoxonase gene family and atherosclerosis. *Free Radic. Biol. Med.* 2005, *38*, 153–163.

[3] Mackness, B., Durrington, P., McElduff, P., Yarnell, J. *et al.*, Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. *Circulation* 2003, *107*, 2775–2779.

[4] Soran, H., Younis, N. N., Charlton-Menys, V., Durrington, P. *et al.*, Variation in paraoxonase-1 activity and atherosclerosis. *Curr. Opin. Lipidol.* 2009, *20*, 265–274.

[5] Aviram, M., Hardak, E., Vaya, J., Mahmood, S. *et al.*, Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation* 2000, *101*, 2510–2517.

[6] Bhattacharyya, T., Nicholls, S. J., Topol, E. J., Zhang, R. *et al.*, Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *J. Am. Med. Assoc.* 2008, *299*, 1265–1276.

[7] Shih, D. M., Lusis, A. J., The roles of PON1 and PON2 in cardiovascular disease and innate immunity. *Curr. Opin. Lipidol.* 2009, *20*, 288–292.

 

[8] Lawlor, D. A., Day, I. N., Gaunt, T. R., Hinks, L. J. *et al.*, The association of the PON1 Q192R polymorphism with coronary heart disease: findings from the British Women's Heart and Health cohort study and a meta-analysis. *Biomed. Chromatogr. Genet.* 2004, *5*, 17.

[9] Mukamal, K. J., Pai, J. K., Jensen, M. K., Rimm, E. B., Paraoxonase 1 polymorphisms and risk of myocardial infarction in women and men. *Circ. J.* 2009, *73*, 1302–1307.

[10] Costa, L. G., Vitalone, A., Cole, T. B., Furlong, C. E., Modulation of paraoxonase (PON1) activity. *Biochem. Pharmacol.* 2005, *69*, 541–550.

[11] Phuntuwate, W., Suthisisang, C., Koanantakul, B., Mackness, M. I., Mackness, B., Paraoxonase 1 status in the Thai population. *J. Hum. Genet.* 2005, *50*, 293–300.

[12] Roest, M., van Himbergen, T. M., Barendrecht, A. B., Peeters, P. H. *et al.*, Genetic and environmental determinants of the PON-1 phenotype. *Eur. J. Clin. Invest.* 2007, *37*, 187–196.

[13] Cherki, M., Berrougui, H., Isabelle, M., Cloutier, M. *et al.*, Effect of PON1 polymorphism on HDL antioxidant potential is blunted with aging. *Exp. Gerontol.* 2007, *42*, 815–824.

[14] Saruhan, E., Olgun, A., Ozturk, K., Akman, S., Erbil, M. K., Age-related paraoxonase activity changes in Turkish population. *Ann. NY Acad. Sci.* 2007, *1100*, 218–222.

[15] Seres, I., Paragh, G., Deschene, E., Fulop Jr T., Khalil, A., Study of factors influencing the decreased HDL associated PON1 activity with aging. *Exp. Gerontol.* 2004, *39*, 59–66.

[16] Vincent-Viry, M., Sass, C., Bastien, S., Aguillon, D. *et al.*, PON1-192 phenotype and genotype assessments in 918 subjects of the Stanislas cohort study. *Clin. Chem. Lab. Med.* 2003, *41*, 535–540.

[17] Dietrich, M., Hu, Y., Block, G., Olano, E. *et al.*, Associations between apolipoprotein E genotype and circulating F2-isoprostane levels in humans. *Lipids* 2005, *40*, 329–334.

[18] Talmud, P. J., Stephens, J. W., Hawe, E., Demissie, S. *et al.*, The significant increase in cardiovascular disease risk in APOEepsilon4 carriers is evident only in men who smoke: potential relationship between reduced antioxidant status and ApoE4. *Ann. Hum. Genet.* 2005, *69*, 613–622.

[19] Hatters, D. M., Peters-Libeu, C. A., Weisgraber, K. H., Apolipoprotein E structure: insights into function. *Trends Biochem. Sci.* 2006, *31*, 445–454.

[20] Bennet, A. M., Di Angelantonio, E., Ye, Z., Wensley, F. *et al.*, Association of apolipoprotein E genotypes with lipid levels and coronary risk. *J. Am. Med. Assoc.* 2007, *298*, 1300–1311.

[21] Minihane, A. M., Jofre-Monseny, L., Olano-Martin, E., Rimbach, G. H., Apolipoprotein E genotype, cardiovascular risk and responsiveness to dietary fat manipulation. *Proc. Nutr. Soc.* 2007, *66*, 183–197.

[22] Song, Y., Stampfer, M. J., Liu, S., Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. *Ann. Intern. Med.* 2004, *141*, 137–147.

[23] Wilson, P. W., Schaefer, E. J., Larson, M. G., Ordovas, J. M., Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. *Arterioscler. Thromb. Vasc. Biol.* 1996, *16*, 1250–1255.

[24] Edelstein, C., Scanu, A. M., Precautionary measures for collecting blood destined for lipoprotein isolation. *Methods Enzymol.* 1986, *128*, 151–155.

[25] Lipid Research Clinics Program Manual of Laboratory Operations, DHEW Publications 1975.

[26] Charlton-Menys, V., Liu, Y., Durrington, P. N., Semi-automated method for determination of serum paraoxonase activity using paraoxon as substrate. *Clin. Chem.* 2006, *52*, 453–457.

[27] Browne, R. W., Koury, S. T., Marion, S., Wilding, G. *et al.*, Accuracy and biological variation of human serum paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay. *Clin. Chem.* 2007, *53*, 310–317.

[28] Aviram, M., Does paraoxonase play a role in susceptibility to cardiovascular disease? *Mol. Med. Today* 1999, *5*, 381–386.

[29] van Himbergen, T. M., Roest, M., de Graaf, J., Jansen, E. H. *et al.*, Indications that paraoxonase-1 contributes to plasma high density lipoprotein levels in familial hypercholesterolemia. *J. Lipid Res.* 2005, *46*, 445–451.

[30] Hixson, J. E., Vernier, D. T., Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hhal. *J. Lipid Res.* 1990, *31*, 545–548.

[31] Minihane, A. M., Khan, S., Leigh-Firbank, E. C., Talmud, P. *et al.*, ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. *Arterioscler. Thromb. Vasc. Biol.* 2000, *20*, 1990–1997.

[32] Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D. *et al.*, High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989, *79*, 8–15.

[33] Shih, D. M., Gu, L., Xia, Y. R., Navab, M. *et al.*, Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 1998, *394*, 284–287.

[34] Tward, A., Xia, Y. R., Wang, X. P., Shi, Y. S. *et al.*, Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. *Circulation* 2002, *106*, 484–490.

[35] Can Demirdogen, B., Turkanoglu, A., Bek, S., Sanisoglu, Y. *et al.*, Paraoxonase/arylesterase ratio, PON1 192Q/R polymorphism and PON1 status are associated with increased risk of ischemic stroke. *Clin. Biochem.* 2008, *41*, 1–9.

[36] Wheeler, J. G., Keavney, B. D., Watkins, H., Collins, R., Danesh, J., Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. *Lancet* 2004, *363*, 689–695.

[37] Lescai, F., Marchegiani, F., Franceschi, C., PON1 is a longevity gene: results of a meta-analysis. *Ageing Res. Rev.* 2009, *8*, 277–284.

[38] Gaidukov, L., Rosenblat, M., Aviram, M., Tawfik, D. S., The 192R/Q polymorphs of serum paraoxonase PON1 differ in HDL binding, lipolactonase stimulation, and cholesterol efflux. *J. Lipid Res.* 2006, *47*, 2492–2502.

[39] Brophy, V. H., Jarvik, G. P., Richter, R. J., Rozek, L. S. *et al.*, Analysis of paraoxonase (PON1) L55M status requires both

 

genotype and phenotype. *Pharmacogenetics* 2000, *10*, 453–460.

[40] Deakin, S., Leviev, I., Brulhart-Meynet, M. C., James, R. W., Paraoxonase-1 promoter haplotypes and serum paraoxonase: a predominant role for polymorphic position –107, implicating the Sp1 transcription factor. *Biochem. J.* 2003, *372*, 643–649.

[41] Ferre, N., Camps, J., Fernandez-Ballart, J., Arija, V. *et al.*, Regulation of serum paraoxonase activity by genetic, nutritional, and lifestyle factors in the general population. *Clin. Chem.* 2003, *49*, 1491–1497.

[42] van der Gaag, M. S., van Tol, A., Scheek, L. M., James, R. W. *et al.*, Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. *Atherosclerosis* 1999, *147*, 405–410.

[43] Sierksma, A., van der Gaag, M. S., van Tol, A., James, R. W., Hendriks, H. F., Kinetics of HDL cholesterol and paraoxonase activity in moderate alcohol consumers. *Alcohol Clin. Exp. Res.* 2002, *26*, 1430–1435.

[44] Rimm, E. B., Williams, P., Fosher, K., Criqui, M., Stampfer, M. J., Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *Br. Med. J.* 1999, *319*, 1523–1528.

[45] Rao, M. N., Marmillot, P., Gong, M., Palmer, D. A. *et al.*, Light, but not heavy alcohol drinking, stimulates paraoxonase by upregulating liver mRNA in rats and humans. *Metabolism* 2003, *52*, 1287–1294.

[46] Mackness, B., Mackness, M. I., Arrol, S., Turkie, W. *et al.*, Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. *Atherosclerosis* 1998, *139*, 341–349.

[47] Leviev, I., Negro, F., James, R. W., Two alleles of the human paraoxonase gene produce different amounts of mRNA. An explanation for differences in serum concentrations of paraoxonase associated with the (Leu-Met54) polymorphism. *Arterioscler. Thromb. Vasc. Biol.* 1997, *17*, 2935–2939.

[48] James, R. W., Deakin, S. P., The importance of high-density lipoproteins for paraoxonase-1 secretion, stability and activity. *Free Radic. Biol. Med.* 2004, *37*, 1986–1994.

[49] Minihane, A. M., Jofre-Monseny, L., Olano-Martin, E., Rimbach, G., ApoE genotype, cardiovascular risk and responsiveness to dietary fat manipulation. *Proc. Nutr. Soc.* 2007, *66*, 183–197.

[50] Jofre-Monseny, L., Minihane, A. M., Rimbach, G., Impact of apoE genotype on oxidative stress, inflammation and disease risk. *Mol. Nutr. Food Res.* 2008, *52*, 131–145.

[51] Aviram, M., Dornfeld, L., Rosenblat, M., Volkova, N. *et al.*, Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am. J. Clin. Nutr.* 2000, *71*, 1062–1076.

[52] Hayek, T., Fuhrman, B., Vaya, J., Rosenblat, M. *et al.*, Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arterioscler. Thromb. Vasc. Biol.* 1997, *17*, 2744–2752.

[53] de Roos, N. M., Schouten, E. G., Scheek, L. M., van Tol, A., Katan, M. B., Replacement of dietary saturated fat with trans fat reduces serum paraoxonase activity in healthy men and women. *Metabolism* 2002, *51*, 1534–1537.

[54] Jarvik, G. P., Tsai, N. T., McKinstry, L. A., Wani, R. *et al.*, Vitamin C and E intake is associated with increased paraoxonase activity. *Arterioscler. Thromb. Vasc. Biol.* 2002, *22*, 1329–1333.

[55] James, R. W., Leviev, I., Righetti, A., Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. *Circulation* 2000, *101*, 2252–2257.

 