Arylesterase Activity and Antioxidant Status Depend on PON1-Q192R and PON1-L55M Polymorphisms in Subjects with Increased Risk of Cardiovascular Disease Consuming Walnut-Enriched Meat\textsuperscript{1,2}

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Abstract

Human paraoxonase (PON1) exists in 2 major polymorphic forms and has been shown to protect LDL and HDL against oxidation. The aim of this study was to assess the differences between subjects at increased risk of cardiovascular disease (CVD), taking into account the effects of PON1-Q192R and PON1-L55M polymorphisms on: 1) basal serum arylesterase activity, lipid peroxidation (LPO), and LDL-cholesterol (LDL-C), HDL-C, total cholesterol (TC), and oxidized-LDL (ox-LDL) concentrations; 2) the relations between arylesterase activity and lipid variables; and 3) the effect of walnut-enriched meat (WM) consumption on arylesterase activity and lipid variables. Twenty-three Caucasians at increased risk of CVD were randomly assigned to diet order groups in a crossover, nonblinded, placebo-controlled trial, consisting of two 5-wk experimental periods [WM and control meat (CM)]. Significant PON1-L55M × PON1-Q192R interactions affected basal serum HDL-C (\(P = 0.019\)), LDL-C (\(P = 0.028\)) and TC (\(P = 0.022\)) and tended to affect arylesterase activity (\(P = 0.083\)). Basal arylesterase activity was positively correlated with basal HDL-C (\(r = 0.53\); \(P < 0.05\)) and TC (\(r = 0.43\); \(P < 0.05\)) and negatively correlated with LPO (\(r = -0.70\); \(P < 0.01\)) and the ox-LDL:LDL ratio (\(r = -0.63\); \(P < 0.01\)). WM decreased arylesterase activity in PON1-55M carriers (\(P = 0.012\)) but not in PON1-L55 individuals, and decreased LPO concentrations in PON1-192R carriers (\(P = 0.031\)) but not in PON1-Q192 subjects. To conclude, serum TC, HDL-C, and LDL-C concentrations and arylesterase activity depend on the interaction of PON1-L55M and PON1-Q192R polymorphisms. However, the PON1-Q192R polymorphism is more closely related to antioxidant status. Both polymorphisms modulate the effect of WM consumption on CVD biomarkers. J. Nutr. 137: 1783–1788, 2007.

Introduction

HDL are reported to have antioxidant properties and the ability to inhibit oxidative modifications of LDL (1,2). Some authors suggest that the HDL-bound paraoxonase-1 (PON1)\textsuperscript{6} enzyme may be involved in lipoprotein-phospholipid metabolism, and they hypothesize that this enzyme could inhibit lipid peroxide generation in LDL (3,4).

Major PON 1 polymorphisms include the replacement of glutamine (Q or A) by arginine (R or B) at position 192 (5), and that of leucine (L) by methionine (M) at position 55 (6). PON1-192R and PON1-L55 carriers hydrolyze paraoxon faster than noncarriers (7,8). However, it has been suggested that the arylesterase activity of both allozymes toward phenylacetate (PA) is similar (9). The ability of HDL to protect LDL against peroxidation in vitro is significantly lower in HDL particles containing PON1-192R (10) than in those with PON1-Q192.

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\textsuperscript{6} Abbreviations used: CVD, cardiovascular disease; C, cholesterol; CM, control meat; LPO, lipid peroxidation; LL, (QR + RR), subjects homoygous for leucine at position 55 and with almost one allele encoding for arginine at position 192; PM + MM, subjects homoygous for methionine at position 55 and homoyoys for glutamine at position 192; MM, subjects homoygous for methionine at position 55; ox-LDL, oxidized-LDL; PON1, paraoxonase; PA, phenylacetate; PON1-L55, subjects homoygous for leucine at position 55; PON1-55M, subjects carrying at least one allele encoding for methionine at position 55; PON1-Q192, subjects homoygous for glutamine at position 192; PON1-192R, subjects carrying arginine at least in one allele of position 192; SBF, simulated body fluid; TC, total cholesterol; WM, walnut-enriched meat.

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Studies evaluating the interaction of diet and PON1 polymorphisms on PON1 activity and lipoprotein metabolism are scarce (11–13). Most of the studies focus on measuring paraoxonase activity (11–13) but not that of arylesterase (14). Intake of different vegetable-based foods has been found to reduce lipid peroxidation in PON1-192R carriers (14) and to diminish paraoxonase activity in PON1-L55 and PON1-192R subjects (13). Walnuts, which are rich in PUFA and antioxidant substances, protect against cardiovascular disease (CVD) (15–17). Thus, we hypothesized that frequent walnut intake, in the form of walnut-enriched meat (WM), might decrease arylesterase activity and improve lipoprotein profile in individuals at increased risk of CVD by raising their PUFA and antioxidant intake. Furthermore, we hypothesized that those changes might be related to the effects and interaction of the PON1-Q192R and L55M polymorphisms.

The objectives of the present study were to assess differences between subjects at increased risk for CVD, taking into account PON1-Q192R and PON1-L55M polymorphisms, with regard to: 1) basal arylesterase activity and lipid peroxidation (LPO), LDL cholesterol (LDL-C), HDL-C, total cholesterol (TC) and oxidized LDL (ox-LDL) concentrations; 2) the relations between arylesterase activity and lipid variables; and 3) the effect of WM consumption on arylesterase activity and lipid variables.

Materials and Methods

Subjects. Twenty-three Caucasian subjects at increased risk of CVD were included in this study. Candidates were recruited in Madrid (Spain) through announcements. Volunteers had to fulfill the following eligibility criteria: 1) high meat consumption (>5 times/wk), 2) men aged ≥45 y and postmenopausal women ≥50 y, and 3) BMI ≥25 and <35 kg/m². In addition, at least one of the following criteria was required: serum TC ≥5.69 mmol/L, smoking habit of ≥10 cigarettes/day, and/or hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg). Exclusion criteria included: volunteers with familiar hypercholesterolemia and/or type I or type II diabetes; those taking any hypolipemiant, antihypertensive, or antiinflammatory drugs, and those receiving substitutive hormonal therapy. The basal characteristics of the subjects before dietary intervention are presented in Table 1. Procedures followed were in accordance with the Ethics Committee standards of the Puerta de Hierro University Hospital (Madrid, Spain) and the Helsinki Declaration. Participants provided informed consent before the start of the study.

After an overnight fast, blood was collected from the subjects between 0800 and 1000 h by venipuncture into Vacuette tubes (Labolan). Serum was separated by centrifugation at 1500 × g for 15 min at 4°C, and stored at 4°C for up to 24 h before the measurement of HDL-C and TC or stored at −20°C until arylesterase activity, LPO, and ox-LDL were assayed. For DNA analysis, aliquots of whole blood were deposited in tubes containing EDTA and stored at −80°C until extraction.

Study design. Subjects were randomly assigned to diet-order groups in a nonblinded, crossover, placebo-controlled study, consisting of two 5-wk (18) experimental periods: WM and control meat (CM). This time of treatment has been effective in identifying lipid changes in response to dietary nut intervention in humans (18). Both periods were separated by a 4- to 6-wk washout interval during which subjects consumed normal diets. During the intervention period, volunteers consumed four 150 g/wk WM steaks and a 150 g/wk ration of WM sausages, all containing 20% walnut paste (Table 2). During the control meat (CM) period, volunteers consumed identical amounts of restructured steaks and sausages that did not include walnut paste (Table 2). Volunteers were firmly advised to avoid consuming all other meats and meat derivatives during both periods.

Dietary control and compliance. Subjects received frozen control and walnut meat weekly. Compliance with study CM and WM consumption was evaluated by subject interview and a review of the dietary surveys they were required to complete at wk 0, 1, 2, 3, 4, and 5 of both periods. All volunteers complied strictly with meat-consumption stipulations. Compliance was defined as consumption of 100% of the scheduled servings during both studied periods. Compliance was also assessed by measuring plasma γ-tocopherol concentrations after each experimental period. As previously published (20), at the end of the WM period, plasma γ-tocopherol was ~20% greater than the basal concentration, whereas no change occurred during the CM period. Energy and nutrient intakes were calculated with DIAL 2005 hardware, using Food Composition Tables for the foodstuff raw weights (21) (Table 3).

Paraoxonase genotyping. DNA was extracted from peripheral blood cells using the Ultraclean Bloodspin kit (MoBio Laboratories). PON1 genotyping was carried out by a multiplex PCR assay (22). Amplification of 111 and 144 pb fragments was carried out by PCR for PON1-Q192R and PON1-L55M, respectively (19).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Basal characteristics of participants at study entry 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Value</td>
</tr>
<tr>
<td>Males, n</td>
<td>14</td>
</tr>
<tr>
<td>Females, n</td>
<td>9</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.8 ± 8.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.0 ± 12.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.6 ± 3.4</td>
</tr>
<tr>
<td>25–29.99 kg/m², %</td>
<td>60</td>
</tr>
<tr>
<td>≥30 kg/m², %</td>
<td>40</td>
</tr>
<tr>
<td>Smoking &gt;10 cigarettes/day, %</td>
<td>20</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>142.0 ± 22.0</td>
</tr>
<tr>
<td>&gt;140 mm Hg, %</td>
<td>64</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>98.3 ± 25.8</td>
</tr>
<tr>
<td>&gt;90 mm Hg, %</td>
<td>72</td>
</tr>
<tr>
<td>Serum TG, mmol/L</td>
<td>1.70 ± 0.90</td>
</tr>
<tr>
<td>≥1.69 mmol/L, %</td>
<td>36</td>
</tr>
<tr>
<td>Serum glucose, mmol/L</td>
<td>5.85 ± 0.66</td>
</tr>
<tr>
<td>≥6.1 mmol/L, %</td>
<td>22.7</td>
</tr>
<tr>
<td>Serum TC, mmol/L</td>
<td>5.64 ± 1.12</td>
</tr>
<tr>
<td>&gt;5.69 mmol/L, %</td>
<td>60</td>
</tr>
<tr>
<td>PON1-L55M, %</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>35</td>
</tr>
<tr>
<td>LM</td>
<td>52</td>
</tr>
<tr>
<td>MM</td>
<td>13</td>
</tr>
<tr>
<td>PON1-Q192R, %</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>52</td>
</tr>
<tr>
<td>GR</td>
<td>30</td>
</tr>
<tr>
<td>RR</td>
<td>17</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 23 or %.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Proximate composition and energy content of control and 20% walnut-enriched beef steaks 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>CM</td>
</tr>
<tr>
<td>Moisture, g/100 g fresh matter</td>
<td>74.7</td>
</tr>
<tr>
<td>Protein, g/100 g fresh matter</td>
<td>20.6</td>
</tr>
<tr>
<td>Fat, g/100 g fresh matter</td>
<td>1.6</td>
</tr>
<tr>
<td>Ash, g/100 g fresh matter</td>
<td>3.1</td>
</tr>
<tr>
<td>SFA, g/100 g total fatty acids</td>
<td>42.1</td>
</tr>
<tr>
<td>MUFA 2, g/100 g total fatty acids</td>
<td>38.0</td>
</tr>
<tr>
<td>PUFA, g/100 g total fatty acids</td>
<td>19.8</td>
</tr>
<tr>
<td>Energy, kJ/100 g</td>
<td>403.3</td>
</tr>
</tbody>
</table>

1 Adapted from Serrano et al. (19).
2 MUFA, monounsaturated fatty acid.
and PON1-L55M, respectively. Reagents were purchased from Promega and PCR was performed using a DNA thermocycler (Mastercycler-ep380, Eppendorf).

**Measurement of arylesterase activity.** Arylesterase activity was measured with simulated body fluid (SBF) as buffer and PA as substrate at pH 7.34–7.4 and 37°C (23). Reaction rates of arylesterase were followed at 270 nm in thermostatically controlled 10-mm lightpath quartz cuvettes using a Shimadzu UV-2401PC spectrophotometer. The final reaction volume in the cuvettes was 2.0 mL and the total time was 3 min. One unit of arylesterase activity is equal to 1 mol of PA hydrolyzed/(L·min).

**Analytical measurements.** Serum TC and HDL-C concentrations were measured by an enzymatic colorimetric method (CHOD-PAP, Boehringer Mannheim). LDL-C was calculated using the formula by Friedewald et al. (24).

Measurement of LPO in erythrocytes, based on the determination of malondialdehyde (MDA) and 4-hydroxyalkenals, was performed using the Bioxytech LPO-586 kit (Oxis Research).

Serum ox-LDL was measured by ELISA (Mercodia AB). The ox-LDL:LDL-C ratio was calculated to obtain an estimate of the percentage of ox-LDL particles (25).

**Statistics.** Values are presented as means ± SD. The Pearson product moment correlation coefficient was used to evaluate the association between 2 continuous variables. Chi-square analysis was used to determine whether the genotype distribution was in Hardy-Weinberg equilibrium and to compare categorical variables. Student’s t tests were used to assess differences in macronutrient intakes during the WM and CM periods.

This study was designed to have a power of 80% to detect a 50% relative difference between basal arylesterase activity in subjects carrying different PON1-L55M and PON1-Q192R polymorphisms, assuming a pooled SD of 35% for arylesterase activity. Two-way ANOVA was used to assess the effect of the different polymorphisms on basal arylesterase activity, lipid variables, and LPO concentrations, considering BMI, smoking habit, sex, and age as covariables. When an interaction of both polymorphisms was observed, [LL, (QR+RR)], and [(LM+MM), QQ] groups were compared using Student’s t tests. Groups [LL, QQ] and [(LM+MM), (QR+RR)] were not included in the analysis to avoid a Type II error. Placebo-controlled dietary study data were analyzed by 4-factor repeated-measures ANOVA, with time and treatment as intrasubject factors and PON1 polymorphisms as intersubject factors. Significant interactions between intra- and/or intersubject effects were analyzed with 2-way ANOVA. Statistics were performed with the SPSS 13.0 statistical package and SIMFIT 5.4 (26). Significance was determined at P < 0.05.

**Results**

Genotype frequencies at both restriction enzyme sites (55 and 192) were in Hardy-Weinberg equilibrium (chi-square < 3.84; P > 0.05) (Table 1).

**Influence of PON1-L55M and PON1-Q192R polymorphisms.** PON1-192Q homozygous subjects had higher basal ox-LDL:LDL ratio (P = 0.042) and lower basal serum TC (P = 0.042), HDL-C (P = 0.047), and LDL-C (P = 0.050) concentrations and arylesterase activity (P = 0.036) than PON1-192R carriers (Table 4). PON1-L55 homozygous individuals had higher basal arylesterase activity than PON1-55M carriers (Table 4). We found significant PON1-L55M × PON1-Q192R interactions for basal serum TC, HDL-C, and LDL-C concentrations (P < 0.05) and a marginal interaction for arylesterase activity (P < 0.1) were found (Table 4).

Subjects with the [(LM+MM), QQ] genotype had a lower basal serum TC concentration (P = 0.037) and arylesterase activity (P = 0.012) than [LL, (QR+RR)] subjects (Table 4).

**Associations with arylesterase activity before the dietary intervention.** Basal arylesterase activities were positively correlated with basal serum HDL-C and TC concentrations, and negatively correlated with basal LPO concentration and the ox-LDL:LDL-C ratio (Table 5). When stratified by PON1-L55M, basal arylesterase activity was significantly correlated with basal

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**Table 3** Daily energy and nutrient intakes of the subjects at increased risk of CVD during WM and CM periods\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>WM</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ</td>
<td>7721 ± 1244</td>
<td>7419 ± 1202</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>148.1 ± 38.9</td>
<td>153.7 ± 27.0</td>
</tr>
<tr>
<td>Protein, g</td>
<td>84.9 ± 14.3</td>
<td>84.7 ± 14.6</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>97.0 ± 14.1</td>
<td>79.4 ± 15.0*</td>
</tr>
<tr>
<td>SFA, g</td>
<td>25.8 ± 5.7</td>
<td>31.9 ± 11.6*</td>
</tr>
<tr>
<td>MUFA, g</td>
<td>39.4 ± 9.8</td>
<td>39.2 ± 12.0</td>
</tr>
<tr>
<td>PUFA, g</td>
<td>26.7 ± 5.5</td>
<td>14.2 ± 6.9*</td>
</tr>
<tr>
<td>Alcohol, g</td>
<td>1.8 ± 5.5</td>
<td>1.3 ± 2.3</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>371 ± 152.1</td>
<td>360 ± 138.1</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>13.4 ± 5.8</td>
<td>13.4 ± 4.7</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SD, n = 23. *Different from WM, P < 0.05.

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**Table 4** Basal serum cholesterol concentrations and arylesterase activities in subjects at risk of CVD according to PON1 genotypes\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Arylesterase</th>
<th>TC</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>LPO</th>
<th>Ox-LDL</th>
<th>Ox-LDLDLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1-Q192 (QQ)</td>
<td>13</td>
<td>20.8 ± 17.8</td>
<td>5.0 ± 1.1</td>
<td>1.1 ± 0.3</td>
<td>3.2 ± 0.8</td>
<td>3.1 ± 2.1</td>
<td>45.2 ± 11.7</td>
</tr>
<tr>
<td>PON1-Q192 (QR+RR)</td>
<td>10</td>
<td>36.1 ± 11.2</td>
<td>6.2 ± 1.1</td>
<td>1.4 ± 0.3</td>
<td>4.0 ± 0.9</td>
<td>4.1 ± 1.1</td>
<td>44.7 ± 13.0</td>
</tr>
<tr>
<td>P-value (Q192 vs. 192)</td>
<td>(0.036)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PON1-L55 (LL)</td>
<td>9</td>
<td>37.8 ± 7.9</td>
<td>6.1 ± 1.0</td>
<td>1.3 ± 0.3</td>
<td>3.9 ± 0.8</td>
<td>3.5 ± 1.3</td>
<td>44.2 ± 12.5</td>
</tr>
<tr>
<td>PON1-55M (LM+MM)</td>
<td>14</td>
<td>20.9 ± 17.9</td>
<td>5.2 ± 1.3</td>
<td>1.2 ± 0.4</td>
<td>3.4 ± 1.0</td>
<td>3.7 ± 2.1</td>
<td>45.4 ± 12.2</td>
</tr>
<tr>
<td>P-value (L55 vs. 55M)</td>
<td>(0.022)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL QQ</td>
<td>3</td>
<td>43.0 ± 9.7</td>
<td>6.5 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>3.4 ± 0.3</td>
<td>2.3 ± 0.8</td>
<td>48.6 ± 7.2</td>
</tr>
<tr>
<td>LL (QR+RR)</td>
<td>6</td>
<td>36.0 ± 7.3*</td>
<td>5.9 ± 1.1*</td>
<td>1.3 ± 0.3</td>
<td>3.4 ± 0.9</td>
<td>4.0 ± 1.1</td>
<td>42.8 ± 14.1</td>
</tr>
<tr>
<td>[LM+MM], QQ</td>
<td>10</td>
<td>16.4 ± 15.6</td>
<td>4.7 ± 0.9</td>
<td>1.0 ± 0.2</td>
<td>3.0 ± 0.7</td>
<td>3.4 ± 2.4</td>
<td>44.4 ± 12.7</td>
</tr>
<tr>
<td>[LM+MM], (QR+RR)</td>
<td>4</td>
<td>36.2 ± 19.1</td>
<td>6.7 ± 1.1</td>
<td>1.6 ± 0.3</td>
<td>4.6 ± 0.7</td>
<td>4.3 ± 1.3</td>
<td>44.9 ± 12.0</td>
</tr>
<tr>
<td>P-value (L55M effect)</td>
<td>(0.076)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>P-value (Q192R effect)</td>
<td>(0.394)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>P-value (L55M × Q192R)</td>
<td>(0.083)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SD, *Different from (LM+MM), QQ, P < 0.05 (Student’s t test).
HDL-C concentration and significantly negatively correlated with the basal ox-LDL:LDL ratio in PON1-55M carriers. When stratified by PON1-Q192R, basal arylesterase activity was significantly correlated with basal HDL-C concentrations in all genotypes (Table 5), but not in PON1-192R carriers (data not shown). Basal arylesterase activity and basal LPO levels were negatively correlated in all genotypes (Table 5).

Basal arylesterase activity of PON1-192Q homozygous individuals and PON1-55M carriers was significantly correlated with basal serum HDL-C and was significantly negatively correlated with basal ox-LDL and LPO concentrations. Basal arylesterase activity of PON1-192R carriers and PON1-55L homozygous subjects was significantly correlated with the basal ox-LDL:LDL ratio (Table 5).

### TABLE 5: Correlations between basal arylesterase activity (U/L) and other basal lipid variables in subjects at increased risk of CVD

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>r</th>
<th>P-value</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>In total population</td>
<td>23</td>
<td>0.43</td>
<td>0.046</td>
<td>y = 5.753x - 2.026</td>
</tr>
<tr>
<td>TC</td>
<td>0.53</td>
<td>0.011</td>
<td>22.795x + 1.980</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.38</td>
<td>0.062</td>
<td>0.3867x + 0.444</td>
<td></td>
</tr>
<tr>
<td>Ox-LDL:LDL</td>
<td>0.63</td>
<td>0.004</td>
<td>-0.708x + 84.75</td>
<td></td>
</tr>
<tr>
<td>LPO</td>
<td>0.70</td>
<td>0.003</td>
<td>-0.6203x + 53.54</td>
<td></td>
</tr>
</tbody>
</table>

In PON1-55M carriers | 14 | 0.78 | 0.005 | y = 41.282x - 25.70 |
| HDL-C | 0.66 | 0.027 | -0.6512x + 59.51 |
| LPO | 0.71 | 0.033 | -0.6331x + 50.37 |

In PON1-L55 homozygous | 9 | 0.77 | 0.042 | y = -4.896x + 53.70 |
| HDL-C | 0.84 | 0.003 | y = 60.527x - 43.56 |
| Ox-LDL:LDL | 0.86 | 0.036 | -0.2677x + 62.02 |
| LPO | 0.86 | 0.006 | y = -7.369x + 50.57 |

In PON1-192R carriers | 10 | 0.74 | 0.037 | y = -7.703x + 66.26 |
| HDL-C | 0.74 | 0.035 | y = 60.888x - 44.18 |
| Ox-LDL | 0.68 | 0.042 | -0.828x + 55.26 |
| LPO | 0.88 | 0.045 | -0.6525x + 44.70 |

In PON1-L55 and 192R | 10 | 0.83 | 0.040 | y = 4.206x - 11.16 |
| Ox-LDL:LDL | 0.8 | 0.042 | y = 0.829x + 55.26 |

1 HDL-C, LDL-C and TC are expressed as mmol/L, LPO as μmol/L, and Ox-LDL as mg/L.

### Discussion

The PON1 enzyme has attracted considerable interest due to the protection it affords against LDL oxidation and thus for its potential role in preventing the development of atherosclerosis (1–4).

Because the present study was performed using subjects at increased risk of CVD, the genotypic distribution of the sample studied for PON1-Q192R and PON1-L55M (Table 1) was the same as that reported by Tomás et al. (27) in controls and hypercholesterolemic individuals.

In accordance with the study by Sentí et al. (28), PON1-Q192 individuals in our study had lower HDL-C levels than PON1-192R carriers. To our knowledge, our data are the first to show that low levels of LDL-C, TC, and arylesterase activity are also associated with the PON1-Q192 genotype. Furthermore, we conclude that differences in TC, HDL-C, and LDL-C concentrations and arylesterase activity are increased by the interaction of PON1-192 and PON1-55 polymorphisms. We also demonstrated that PON1-L55 and PON1-192R are associated with higher activity toward PA than PON1-55M and PON1-Q192. These results could be due to the use of SBF, instead of Tris/HCl (29), to measure arylesterase activity (23). SBF seems to be a more suitable reagent than Tris/HCl for the enzyme activity assessment, especially in samples with very low arylesterase activity.
Bub et al. (14) suggested that PON1 of 192Q healthy homozygous subjects is more effective against LDL peroxidation, measured as LDL oxidation lag time, than PON1 of their 192R counterparts; however, carriers of these 2 polymorphisms did not differ in MDA concentrations. We did not find significant differences in LPO and ox-LDL levels between carriers of both PON1-Q192R polymorphisms at increased risk of CVD. PON1-192Q individuals had a higher estimated percentage of ox-LDL particles than PON1-192R carriers. This was more evident in \([LM+MM], QQ\) subjects, who also had the lowest arylerase activity, explaining, at least in part, the highest estimated percentage of ox-LDL in those volunteers. Because the ox-LDL:LDL-C ratio was considered a marker of oxidative stress (25), and \([LM+MM], QQ\) subjects had a 36% higher ox-LDL:LDL-C ratio than \([LL, (QR+RR)]\), we hypothesized that \([LM+MM], QQ\) individuals had lower arylerase activity primarily because they were more susceptible to oxidative stress than \([LL, (QR+RR)]\) carriers.

We found a higher correlation between arylerase activity and HDL-C than that reported elsewhere (30). The negative correlation between arylerase activity and LPO, ox-LDL concentration and the ox-LDL:LDL ratio supports the protective effect of PON1 on LDL oxidation as well as the susceptibility of PON1 to oxidative stress. These correlations were more pronounced in subjects with PON1-Q192 and PON1-55M polymorphisms. We have shown, to our knowledge for the first time, that arylerase activity is correlated with TC.

Previous studies reported that supplementation with vitamin C and E increases paraoxonase activity (31), whereas intake of vegetables rich in antioxidants reduces it, especially in PON1-192R carriers and PON1-L55 homozygous individuals (13). In our study, the effect of WM consumption was also regulated genetically. The unique composition of walnuts rich in retinol, \(\beta\)-carotenes, vitamin E, folic acid, and vitamin C (32) could explain the significant decrease in arylerase activity in PON1-55M carriers, whose arylerase activity was the lowest in the study.

The second most important finding of our study was that WM consumption reduced LPO levels in individuals with a risk of CVD with at least 1 allele encoding the PON1-192R genotype, thus contributing to a decrease of their CVD risk. Bub et al. (12,14) found that intake of tomato but not carrot juice reduced plasma MDA in young and old healthy, male, PON1-192R carriers.

In conclusion, TC, HDL-C, and LDL-C concentrations and arylerase activity depend on the interaction of PON1-L55M and PON1-Q192R polymorphisms. Nevertheless, the PON1-Q192R polymorphism appears to be more related to antioxidant status than does the PON1-L55M polymorphism. WM consumption diminishes arylerase activity in PON1-55M carriers, and reduces LPO in PON1-192R carriers. More studies are needed to assess the importance of the interaction of PON1 polymorphisms on arylerase activity in high- and low-risk CVD subjects. Moreover, additional studies are also necessary to evaluate the effects of long-term WM consumption.

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**Literature Cited**


