Paraoxonase Status in Coronary Heart Disease
Are Activity and Concentration More Important Than Genotype?

Bharti Mackness, Gershan K. Davies, Wajdi Turkie, Evelyn Lee, David H. Roberts, Elizabeth Hill, Chris Roberts, Paul N. Durrington, Michael I. Mackness

Abstract—Human serum paraoxonase (PON1) hydrolyzes oxidized lipids in low density lipoprotein (LDL) and could therefore retard the development of atherosclerosis. In keeping with this hypothesis, several case-control studies have shown a relationship between the presence of coronary heart disease (CHD) and polymorphisms at amino acid positions 55 and 192 of PON1, which we associated with a decreased capacity of PON1 to protect LDL against the accumulation of lipid peroxides, but some other studies have not. However, the PON1 polymorphisms are only 1 factor in determining the activity and concentration of the enzyme. Only 3 of the previous 18 studies directly determined PON1 activity and concentration. Therefore, we studied PON1 activity, concentration, and gene distribution in 417 subjects with angiographically proven CHD and in 282 control subjects. We found that PON1 activity and concentration were significantly lower in subjects with CHD than in control subjects (activity to paraoxon 122.8 [3.3 to 802.8] versus 214.6 [26.3 to 620.8] nmol · min⁻¹ · mL⁻¹, P<0.001; concentration 71.6 [11.4 to 489.3] versus 89.1 [16.8 to 527.4] μg/mL, P<0.001). There were no differences in the PON1-55 and -192 polymorphisms or clusterin concentration between patients with CHD and control subjects. These results indicate that lower PON1 activity and concentration and, therefore, the reduced ability to prevent LDL lipid peroxidation may be more important in determining the presence of CHD than paraoxonase genetic polymorphisms. (Arterioscler Thromb Vasc Biol. 2001;21:1451-1457.)

Key Words: paraoxonase ■ oxidation ■ coronary heart disease ■ genetic polymorphisms

Paraoxonase (EC.3.1.8.1, aryldialkylphosphatase) has been extensively studied in the field of toxicology.1,2 Paraoxonaxole hydroxyls organophosphate compounds, which are widely used as insecticides and nerve gases.3,4 Human serum paraoxonase (PON1) is synthesized in the liver and is physically associated with HDL, on which it is almost exclusively located. The serum concentration of HDL has long been known to have an inverse correlation with the development of atherosclerosis.5 The mechanism by which HDL renders its protective effect against atherosclerosis continues to be the subject of considerable debate. The initial focus of attention was on the role of HDL in reverse-cholesterol transport. However, recent studies have suggested more diversity in the role of HDL in atherogenesis. Several laboratories have reported that HDL protects against LDL oxidative modification,6–9 which is believed to be central to the initiation and progression of atherosclerosis.10 We have previously shown that the antioxidant activity of HDL may relate, at least in part, to the enzymes associated with HDL.11 Further studies have indicated that PON1 can prevent lipid peroxide accumulation on LDL in vitro and in vivo.12–14 Studies have shown that serum PON1 activity is reduced in diabetes and familial hypercholesterolemia,15,16 diseases that are associated with accelerated atherogenesis.

PON1 activity is in part genetically determined. In this regard, most investigations have focused on an amino acid substitution at position 192 (Q⇒R), giving rise to 2 allozymes.3,17,18 This PON1 activity polymorphism is substrate dependent. Some substrates, such as paraoxon and fenitroxon, are hydrolyzed faster by the R allozyme, whereas other substrates, such as phenyl acetate, are hydrolyzed at the same rate by both allozymes, and yet others, such as diazoxon and the nerve gases soman and sarin, are hydrolyzed more rapidly by the Q allozyme.19 A second polymorphism of the PON1 gene is present at the amino acid at position 55 (L⇒M). This polymorphism has also been shown to have an effect on PON1 activity. Although this is much smaller than that of the 192 polymorphism,20,21 it is independent of the 192 polymorphism.

We have recently shown that the PON1 R allozyme is less efficient at retarding the oxidation of LDL than is the Q allozyme because of the decreased hydrolysis of lipid peroxides by the R allozyme.22,23 This has been confirmed by other workers24 and indicates that the efficacy of the 2 allozymes toward lipid peroxides is opposite that toward paraoxon, the substrate most commonly used to assay PON1 activity.22–24 These findings may explain why in some case-control studies

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1451
the paraoxonase R allele has been found to be present at an increased frequency in coronary heart disease (CHD), leading to the hypothesis that the PON1-192 polymorphism might be a risk factor for atherosclerosis. However, some studies have failed to find such a relationship, but in none has the R allele been less common than in patients with CHD (see Discussion). Unfortunately, the majority of these studies were exclusively genetic, with no measurements of PON1 activity or mass. Only 3 of the 18 previous studies directly measured PON1 activity or mass.25,30,41

The Fogelman group (Watson et al14) has reported that PON1 in HDL may block inflammatory responses by preventing the oxidation of LDL. The same group went on to demonstrate that during an acute-phase reaction, there is a significant loss of the PON1 activity, thus accounting for the failure of HDL to protect LDL from oxidation during acute-phase reactions.20 More recently, the Fogelman group (Navab et al35) reported a failure of HDL to protect LDL from oxidation in patients with coronary atherosclerosis, which they proposed was due to their low serum PON1 activity. We found a decrease in serum PON1 activity and concentration in myocardial infarction within 2 hours of the onset of symptoms; PON1 did not change up to 42 days after the myocardial infarction, long after the acute phase had passed, strongly suggesting that a decrease in its activity may have preceded the acute event.44 The decrease was substantially greater than could be accounted for by any differences in the prevalence of PON1 polymorphisms between patients developing CHD and control subjects.

Therefore, we hypothesized that in CHD the status of PON1 (ie, its activity and concentration) would be more important than the PON1 genotype. It has been suggested that an increased ratio of apoJ to PON1 may be a better indicator of atherosclerosis than the ratio of total cholesterol to HDL cholesterol (HDL-C).41 To test these hypotheses, we analyzed these parameters and apoJ in a population of >400 people with angiographically proven CHD and a population without CHD.

Methods

Subjects

Patients included in the present study were all outpatients attending the Cardiology Departments at either Manchester Royal Infirmary, UK, or the Royal Victoria Hospital, Blackpool, UK. All patients had angiographically proven CHD, which was visually assessed. All patients had stenosis that was severe enough to require intervention either by coronary angioplasty or surgery. No patient had sustained a myocardial infarction within 6 months before taking part in the study. Patients with diabetes or renal or hepatic disease were excluded from the study. Four hundred seventeen sequential patients who fulfilled the inclusion criteria were studied. Fully informed consent was obtained, and the study was approved by the Central Manchester Healthcare NHS Trust Research Ethical Committee. One hundred four patients were receiving a β-adrenoceptor–blocking drug, 89 were taking a calcium channel blocker, and 154 were on lipid-lowering medication (151 received statins, and 3 received fibrates). All patients were receiving aspirin (75 or 150 mg daily).

The control population consisted of 282 (147 men) healthy subjects, who either attended a routine health check at a general practice or at their place of work. Lack of coronary artery disease in the control population was assessed by use of a health questionnaire, and all had no history suggestive of coronary artery disease. Subjects with diabetes or renal or hepatic disease were excluded. The demographic details of the patients and controls are given in Table 1.

### Table 1. Demographic Details and Lipid and Lipoprotein Concentrations in the Study Populations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (male/female), n</td>
<td>282 (147/135)</td>
<td>417 (302/115)</td>
</tr>
<tr>
<td>Age, y</td>
<td>42.2±12.2</td>
<td>58.5±10.2*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.9±3.7</td>
<td>27.6±4.5*</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>5.8±1.20</td>
<td>5.0±1.20*</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.35</td>
<td>1.65†</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.44±0.46</td>
<td>1.49±0.50</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>92.4±26.1</td>
<td>90.6±25.5</td>
</tr>
<tr>
<td>ApoAI, mg/dL</td>
<td>114.5±30.3</td>
<td>84.5±23.3*</td>
</tr>
</tbody>
</table>

Values are mean±SD, unless indicated otherwise. Serum triglycerides are median (range).

*P<0.001 and †P<0.05 vs control.

Venous blood was collected from all subjects between 9:00 and 11:00 AM after fasting from 10:00 PM the previous day. Serum and plasma were isolated by low-speed centrifugation. White cells were removed from the buffy coat of the plasma tube. HDL was isolated immediately by precipitating LDLs with heparin-Mn²⁺. Other samples were stored at −20°C for no more than 2 months before analysis.

Serum Lipids

Serum cholesterol and HDL-C were determined by the CHOD-PAP method (Cobas Mira, Roche). Serum triglycerides were measured by the enzymatic GPO-PAP method (Cobas Mira, Roche). ApoA-I and apoB were determined by immunoturbidimetry on the Cobas Mira by using reagents, standards, and controls provided by the manufacturer. LDL cholesterol was estimated by the Friedewald formula:

\[
LDL \text{ cholesterol} = \text{total serum cholesterol} - (\text{HDL cholesterol} + \text{triglyceride}/2.2) \text{ mmol/L}
\]

Analysis of PON1 Activity

PON1 activity was measured by adding serum to Tris buffer (100 mmol/L, pH 8.0) containing 2 mmol/L CaCl₂ and 5.5 mmol/L paraoxon (O,O-diethyl-O-p-nitrophenylphosphate, Sigma Chemical Co). The rate of generation of p-nitrophenol was determined at 405 nm, 25°C, with the use of a continuously recording spectrophotometer (Beckman DU-68) as described previously.15

Determination of PON1 Concentration

PON1 concentration was determined by using our in-house competitive ELISA with rabbit anti-human PON1 monospecific antibodies used as described previously.16

Determination of PON1 Genotype

DNA was extracted from the white cells, and PON1 genotype for the 192 and 55 polymorphisms was determined by polymerase chain reaction amplification and restriction enzyme digestion as described.17,18

Determination of ApoJ (Clusterin) Concentration

ApoJ concentration was determined by ELISA with monoclonal antibodies to human clusterin and pure clusterin used as standards (both were purchased from Quidel) as described previously.43 Interassay and intra-assay coefficients of variation were 7.2% and 4.2%, respectively.

Statistical Analysis

The Wilcoxon signed rank test was used to test for differences in variables with a non gaussian frequency distribution, namely, PON1 activity toward paraoxon and PON1 concentration and triglyceride concentration. The Student unpaired t test was used for total cholesterol, LDL, HDL, apoA-I, and apoB estimations. A value of
A meta-analysis of previously published studies was carried out to examine the relationship between the PON1 R allele and CHD by estimating a pooled odds ratio by first comparing alleles per genotype RR and QR against QQ and then alleles RR against QR and QQ. In meta-analyses, it is important to examine whether there is heterogeneity of effect between studies. When there is a random effects model and when there is evidence of significant heterogeneity of effect between studies and sample size. When there is evidence of a large effect in studies with a smaller sample size, this may indicate publication bias that is due to the lack of publication of smaller nonsignificant studies. Metaregression techniques were used to examine any difference associated with race. Statistical analysis was carried out by using the Stata Statistical Software Package (StataCorp 1999, Stata Statistical Software, Release 6).

### Results

Compared with the control group, the study population had a greater proportion of males, was significantly older, and had a larger average body mass index (Table 1). The CHD group also had significantly higher serum triglyceride levels and lower total cholesterol and apoA-I levels than did the control group. However, there were no differences in HDL-C or apoB between the groups (Table 1).

There were no significant differences between the populations in the gene frequencies of either PON1-55 or PON1-192. Genotype frequencies for the Q192R polymorphism were 55.3% QQ, 35.1% QR, and 8.5% RR (Q = 0.74, R = 0.26) in the control subjects and 50.1% QQ, 41.5% QR, and 7.7% RR (Q = 0.71, R = 0.29) in the CHD population. Genotype frequencies for the L55M polymorphism were 36.9% LL, 52.8% LM, and 9.2% MM in the control subjects (L = 0.64, M = 0.36) and 40.3% LL, 47.7% LM, and 11.3% MM (L = 0.65, M = 0.35) in the CHD population. However, paraoxon hydrolysis was 50% less in the CHD population than in the control group ($P < 0.001$). PON1 concentration was also significantly reduced (Table 2). The serum clusterin concentration was the same in the 2 populations. The apoA-I/PON1 and HDL-C/PON1 ratios were both higher in the CHD population. The difference in PON1 activity between the control and CHD populations was tested for independence of other variables by multiple regression analysis. The model included sex, age, body mass index, total cholesterol, triglycerides, HDL, apoB, apoA-I, clusterin, PON1 concentration, and the PON1-55 and -192 polymorphisms (Table 3).

### Table 2. PON1 Parameters and Clusterin in the Study Populations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 Concentration, µg/mL</td>
<td>89.1 (16.8–527.4)</td>
<td>71.6* (11.4–489.3)</td>
</tr>
<tr>
<td>Paraoxon hydrolysis, nmol·min⁻¹·mL serum⁻¹</td>
<td>214.6 (26.3–620.8)</td>
<td>122.8* (3.3–802.8)</td>
</tr>
<tr>
<td>Specific activity, nmol·min⁻¹·µg⁻¹</td>
<td>2.10 (0.22–20.2)</td>
<td>1.70* (0.1–26.4)</td>
</tr>
<tr>
<td>Clusterin, µg/mL</td>
<td>115.3±32</td>
<td>111.0±62</td>
</tr>
<tr>
<td>Clusterin/PON1 activity</td>
<td>0.54 (0.03–4.87)</td>
<td>0.85 (0.01–15.22)</td>
</tr>
<tr>
<td>ApoAI/PON1 activity</td>
<td>0.62 (0.13–3.79)</td>
<td>0.67 (0.02–6.47)</td>
</tr>
<tr>
<td>HDL-C/PON1 activity</td>
<td>7.2×10⁻¹±4.1×10⁻³</td>
<td>12.1×10⁻¹±5.3×10⁻³</td>
</tr>
<tr>
<td>Clusterin/PON1 concentration</td>
<td>1.48±0.06</td>
<td>1.85±0.08</td>
</tr>
<tr>
<td>ApoAI/PON1 concentration</td>
<td>1.49±0.07</td>
<td>1.36±0.05</td>
</tr>
<tr>
<td>HDL-C/PON1 concentration</td>
<td>18.0×10⁻³±0.07×10⁻³</td>
<td>24.2×10⁻³±0.09×10⁻³</td>
</tr>
</tbody>
</table>

Values are mean±SD or median (range).

*P<0.001 vs control.

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**TABLE 3. Multiple Regression Analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficient</th>
<th>95% CI</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>−0.038</td>
<td>−0.026</td>
<td>−0.171 to 0.095</td>
<td>−0.562</td>
<td>0.574</td>
</tr>
<tr>
<td>Age</td>
<td>−0.005</td>
<td>−0.017</td>
<td>−0.011 to −6.93×10⁻⁵</td>
<td>1.991</td>
<td>0.047</td>
</tr>
<tr>
<td>Body mass index</td>
<td>−0.004</td>
<td>−0.028</td>
<td>−0.019 to 0.010</td>
<td>−0.593</td>
<td>0.554</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.085</td>
<td>0.157</td>
<td>3.45×10⁻³ to 0.170</td>
<td>1.966</td>
<td>0.049</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.004</td>
<td>−0.007</td>
<td>−0.060 to 0.051</td>
<td>−0.156</td>
<td>0.876</td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.099</td>
<td>−0.066</td>
<td>−0.261 to 0.060</td>
<td>−1.223</td>
<td>0.222</td>
</tr>
<tr>
<td>ApoB</td>
<td>−0.003</td>
<td>−0.013</td>
<td>−0.007 to 3.21×10⁻⁴</td>
<td>−1.799</td>
<td>0.073</td>
</tr>
<tr>
<td>Clusterin</td>
<td>−3.33×10⁻⁵</td>
<td>−0.024</td>
<td>−0.001 to 8.36×10⁻⁴</td>
<td>−0.561</td>
<td>0.575</td>
</tr>
<tr>
<td>PON1 mass</td>
<td>0.001</td>
<td>0.129</td>
<td>4.28×10⁻⁴ to 0.002</td>
<td>2.978</td>
<td>0.003</td>
</tr>
<tr>
<td>Q192R</td>
<td>−0.005</td>
<td>−0.031</td>
<td>−0.040 to 0.009</td>
<td>−1.845</td>
<td>0.068</td>
</tr>
<tr>
<td>M55L</td>
<td>−0.002</td>
<td>−0.006</td>
<td>−0.071 to 0.060</td>
<td>−0.228</td>
<td>0.714</td>
</tr>
</tbody>
</table>
The difference in PON1 activity was found to be dependent on differences in age ($P < 0.047$), total cholesterol ($P < 0.008$), HDL cholesterol ($P < 0.025$), and PON1 concentration ($P = 0.0031$).

Total cholesterol was lower in those CHD subjects taking statins than in those not taking statins (4.7 ± 1.1 versus 5.2 ± 0.9, respectively; $P < 0.01$), and HDL-C was significantly higher (1.55 ± 0.42 versus 1.46 ± 0.50, respectively; $P < 0.05$). However, there were no significant differences in triglycerides, apoA-I, apoB, paraoxon hydrolysis, PON1 concentration, or clusterin between those taking statins and those not taking statins (result not shown). The reason(s) for the different responses of apoA-I and apoB to total cholesterol is not known, but it may be due to the mode of action of statin drugs.

The effect of the PON1-55 and -192 polymorphisms on paraoxon hydrolysis was similar to that reported in previous studies from our laboratory. Thus, paraoxon hydrolysis was similar to that reported in previous studies. To summarize the meta-analysis, there was evidence of a large effect in small studies ($P = 0.008$). This can be seen in the top panel of the Figure; smaller studies having a smaller marker (presented as a square) and wider CI tend to show a larger effect. This suggests that smaller nonsignificant studies may have been missed because of a lack of publication or publication in more obscure journals, which could have been missed from our search, sometimes referred to as publication bias.

When allele RR was compared with alleles QR and QQ, the results were similar but less strong. There was again evidence of heterogeneity of the odds ratio between studies ($P = 0.065$). In a random effect meta-analysis, the pooled estimate of the odds ratio was 1.162 (95% CI 1.00 to 1.35), which is just significantly different from 1 at conventional levels ($P = 0.05$). There was again no evidence of a race effect ($P = 0.237$), and there was some suggestion of publication bias ($P = 0.103$).

### Discussion

Previous studies that have investigated the relationship between the PON1-192 genetic polymorphism and CHD have produced inconsistent results. Some studies have shown the PON1-192R genotype to be present at a higher frequency in CHD, leading to the hypothesis that the PON1-192 polymorphism might be a risk factor for atherosclerosis, but some studies have failed to find such a relationship. However, no study has found the R allele to be less common in CHD. We have carried out a meta-analysis on all studies published (at the time of writing this article) on the PON1-192 genotype in CHD and found that overall there is an increase in the frequency of the PON1-192R allele in CHD (Figure). Given the suggestion of publication bias, ie, evidence of a large effect in smaller studies, which is possibly due to the lack of publication of studies with a small nonsignificant effect, any conclusion must be treated with caution. There was also evidence of heterogeneity between studies. To summarize the meta-analysis, there was evidence...
that the R allele was associated with increased risk of CHD. There was no evidence of a difference associated with race. Far fewer studies have been conducted into the relationship between the PON1-55 polymorphism with CHD, but again, inconsistent results have been produced.49 However, the vast majority of the studies described above did not measure the quality of PON1, ie, its activity and mass in the serum of the individuals studied, which in the present study were lower in the CHD population than in the control subjects regardless of PON1 genotype. The lack of data on PON1 activity and concentration may well have been a cause of the variation in the studies that was due to a normal PON1 activity/mass in those studies showing no relationship between PON1 polymorphisms and CHD. Differences in PON1 activity and concentration between populations of the same ethnic group are well known,50,51 which, if not taken into account, could have affected the results of the case-control studies. In addition, proatherogenic diets have been shown to reduce PON1 in mice, rabbits, and humans.52–54 Lipid peroxides, which are substrates for PON1 and which have been shown to be raised in people with CHD,55 are inhibitors of PON1.56 Thus, acquired factors that are prevalent in CHD may be responsible for the lower PON1 found in CHD rather than genetic factors. However, genetic factors other than the 55 and 192 polymorphisms should not be entirely dismissed. Recent evidence has indicated that polymorphisms in the promoter region of the PON1 gene are important in determining levels of PON1,57 and a low expressor promoter polymorphism has been associated with increased CHD in a diabetic population.58 Whether the promoter polymorphism determining low PON1 is more prevalent in nondiabetic CHD has yet to be established.

As we have shown, however, it is likely that PON1 activity and mass are more important determinants of susceptibility to CHD than are the PON1-55 and PON1-192 genotypes. In a previous study, we showed that PON1 activity and concentration were significantly reduced within 2 hours of the onset of myocardial infarction. PON1 did not change up to 42 days after the myocardial infarction, long after the acute phase had passed, strongly suggesting that it was lower before the event.44 In the present study, we have shown that PON1 activity and mass are reduced independently of the PON1 genotype in people with established CHD. Interestingly, a recent, although smaller, study, reached the same conclusion, finding the measurement of PON1 activity to be a better prediction of carotid artery disease than either the PON1-55 or PON1-192 genotype.59 Low or absent PON1 has been associated with an inability of HDL to prevent the oxidation of LDL in humans and in animal models.53,60–62 Low PON1 has been shown to reduce the capacity of HDL to prevent the oxidation of LDL63,64 and may, therefore, lead to CHD.

The results of an earlier study have suggested that the ratio of clusterin to PON1 may be a more accurate predictor of CHD than the ratio of total cholesterol to HDL-C.43 However, in the present investigation, we found no differences in the serum clusterin concentration between the CHD and control populations. The reason for this difference between two studies is unclear but could be due to sample size, which was very low in the previously reported study.43 In the present study, the ratio of PON1 to either clusterin, apolA-I, or HDL-C appears largely to reflect the low PON1 in the CHD population.

In summary, we have shown that PON1 activities toward paraoxon and PON1 concentrations are lower in subjects with CHD than in control subjects regardless of the PON1 genotype. This would suggest that the quality of the PON1 enzyme is a more important factor in CHD than is the PON1 gene. At the present time, the effect of the deranged enzyme activity appears to be substrate dependent, and further studies investigating the hydrolysis of lipid peroxides by PON1 in CHD are warranted. We, along with other authors,59,65 would strongly suggest that all further epidemiological studies into the role of PON1 and disease should include a measurement of the enzyme itself in addition to the genetic polymorphisms. In the absence of a routine assay based on the hydrolysis of lipid peroxides, this measurement should include either the hydrolysis of paraoxon and/or diazoxon or the concentration of the enzyme.

Meta-analysis of studies investigating the relationship between the PON1-192 genotype and CHD.

that the R allele was associated with increased risk of CHD. There was no evidence of a difference associated with race. Far fewer studies have been conducted into the relationship between the PON1-55 polymorphism with CHD, but again, inconsistent results have been produced.49 However, the vast majority of the studies described above did not measure the quality of PON1, ie, its activity and mass in the serum of the individuals studied, which in the present study were lower in the CHD population than in the control subjects regardless of PON1 genotype. The lack of data on PON1 activity and concentration may well have been a cause of the variation in the studies that was due to a normal PON1 activity/mass in those studies showing no relationship between PON1 polymorphisms and CHD. Differences in PON1 activity and concentration between populations of the same ethnic group are well known,50,51 which, if not taken into account, could have affected the results of the case-control studies. In addition, proatherogenic diets have been shown to reduce PON1 in mice, rabbits, and humans.52–54 Lipid peroxides, which are substrates for PON1 and which have been shown to be raised in people with CHD,55 are inhibitors of PON1.56 Thus, acquired factors that are prevalent in CHD may be responsible for the lower PON1 found in CHD rather than genetic factors. However, genetic factors other than the 55 and 192 polymorphisms should not be entirely dismissed. Recent evidence has indicated that polymorphisms in the promoter region of the PON1 gene are important in determining levels of PON1,57 and a low expressor promoter polymorphism has been associated with increased CHD in a diabetic population.58 Whether the promoter polymorphism determining low PON1 is more prevalent in nondiabetic CHD has yet to be established.

As we have shown, however, it is likely that PON1 activity and mass are more important determinants of susceptibility to CHD than are the PON1-55 and PON1-192 genotypes. In a previous study, we showed that PON1 activity and concentration were significantly reduced within 2 hours of the onset of myocardial infarction. PON1 did not change up to 42 days after the myocardial infarction, long after the acute phase had passed, strongly suggesting that it was lower before the event.44 In the present study, we have shown that PON1 activity and mass are reduced independently of the PON1 genotype in people with established CHD. Interestingly, a recent, although smaller, study, reached the same conclusion, finding the measurement of PON1 activity to be a better prediction of carotid artery disease than either the PON1-55 or PON1-192 genotype.59 Low or absent PON1 has been associated with an inability of HDL to prevent the oxidation of LDL in humans and in animal models.53,60–62 Low PON1 has been shown to reduce the capacity of HDL to prevent the oxidation of LDL63,64 and may, therefore, lead to CHD.

The results of an earlier study have suggested that the ratio of clusterin to PON1 may be a more accurate predictor of CHD than the ratio of total cholesterol to HDL-C.43 However, in the present investigation, we found no differences in the serum clusterin concentration between the CHD and control populations. The reason for this difference between two studies is unclear but could be due to sample size, which was very low in the previously reported study.43 In the present study, the ratio of PON1 to either clusterin, apolA-I, or HDL-C appears largely to reflect the low PON1 in the CHD population.

In summary, we have shown that PON1 activities toward paraoxon and PON1 concentrations are lower in subjects with CHD than in control subjects regardless of the PON1 genotype. This would suggest that the quality of the PON1 enzyme is a more important factor in CHD than is the PON1 gene. At the present time, the effect of the deranged enzyme activity appears to be substrate dependent, and further studies investigating the hydrolysis of lipid peroxides by PON1 in CHD are warranted. We, along with other authors,59,65 would strongly suggest that all further epidemiological studies into the role of PON1 and disease should include a measurement of the enzyme itself in addition to the genetic polymorphisms. In the absence of a routine assay based on the hydrolysis of lipid peroxides, this measurement should include either the hydrolysis of paraoxon and/or diazoxon or the concentration of the enzyme.

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