Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1

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ABSTRACT
PON1 (paraoxonase-1) is an HDL (high-density lipoprotein)-associated enzyme capable of hydrolysing diverse substrates from OP (organophosphate) toxins to oxidized phospholipids. As such, it has been linked with both the prevention of OP poisoning and inhibition of atherosclerosis initiated by oxidatively modified LDL (low-density lipoprotein). Mice deficient in PON1 are more susceptible to OP poisoning and oxidative stress and more prone to develop atherosclerosis than their wild-type siblings. There are a number of polymorphisms in the PON1 gene which affect serum PON1 activity and concentration. Many (but not all) studies in human populations have suggested that these polymorphisms may be a risk factor for atherosclerosis. The serum concentration of PON1 across the general population is highly variable and there is some debate as to whether genotype or phenotype (i.e. the quantity or quality of the enzyme) is most accurately associated with risk of disease development. What is clear is that factors influencing serum levels of PON1, be they genetic or environmental, will, in turn, affect the capacity of HDL to protect LDL from oxidation and, consequently, may be linked to atherosclerosis. This review will focus on mechanisms which determine the serum concentration of PON1, including gene expression and genetic polymorphisms, protein secretion and association with HDL, pharmacological and environmental factors.

INTRODUCTION
PON1 (paraoxonase-1) is a 355 amino acid glycoprotein, which is synthesized in the liver and secreted into the blood, where it associates with HDL (high-density lipoprotein) [1]. It is a member of a three gene family consisting of PON1, PON2 and PON3 located on human chromosome 7 [2]. The PON proteins share 60% sequence identity [2]. The PON family are hydrolases with one of the broadest known substrate specificities. PON1 was the first of the proteins to be identified and is thus the most studied. It has a six bladed β-propeller structure reminiscent of DFPases (di-isopropylfluorophosphatases) with a unique active site lid [3]. Unlike PON2 and PON3, it is an efficient esterase towards many OP (organophosphate) compounds, including paraoxon (from which it takes its name), the insecticides parathion and chlorpyriphos as well as the nerve agents sarin and soman [4]. It also hydrolyses aliphatic lactones such as dihydrocoumarin, γ-butyrolactone and homocysteine thiolactone (Table 1) [5,6]. Its previously reported lactonase activity on lovastatin, simvastatin and...
Table 1 Kinetic analysis of substrate hydrolysis by purified human PON1isoforms

<table>
<thead>
<tr>
<th>Substrate</th>
<th>PON1&lt;sub&gt;192Q&lt;/sub&gt;</th>
<th>PON1&lt;sub&gt;192R&lt;/sub&gt;</th>
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<tr>
<td></td>
<td>( V_{\text{max}} ) (units/mg) ( K_m ) (mM) ( V_{\text{max}}/K_m )</td>
<td>( V_{\text{max}} ) (units/mg) ( K_m ) (mM) ( V_{\text{max}}/K_m )</td>
</tr>
<tr>
<td>Phenylacetate</td>
<td>845</td>
<td>0.69 1225</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>0.47</td>
<td>0.5 0.94 2.1</td>
</tr>
<tr>
<td>Diazoxon</td>
<td>222</td>
<td>2.98 75</td>
</tr>
<tr>
<td>Chlorpyrifos-oxon</td>
<td>82</td>
<td>0.54 152</td>
</tr>
<tr>
<td>Sarin</td>
<td>69</td>
<td>0.21 330</td>
</tr>
<tr>
<td>Soman</td>
<td>82</td>
<td>0.42 195</td>
</tr>
<tr>
<td>( \gamma )-Butyrolactone</td>
<td>420</td>
<td>15 23</td>
</tr>
<tr>
<td>Dihydrocoumarin</td>
<td>180</td>
<td>0.022 8200</td>
</tr>
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Spirinolactone [5] is now known to be due to small amounts of contaminating PON3 in purified preparations [7].

In vitro assays have shown that PON1 can inhibit LDL (low-density lipoprotein) lipid peroxidation and inactivate LDL-derived oxidized phospholipids. This could potentially reduce the serum content of the oxidized lipids involved in the initiation of atherosclerosis [8–10].

Atherosclerosis is the underlying cause of 50% of the mortality in the Western world, and OP use presents an environmental risk and a terrorist threat in today’s society. PON1 has been presented as a potential therapeutic agent against both OP poisoning and atherosclerosis development and as such has been the focus of intensive research.

The most convincing data to link PON1 with heart disease and poisoning comes from transgenic mouse studies. HDL from PON1 ‘knockout’ mice cannot prevent oxidation of LDL in a co-culture model simulating the artery wall and their macrophages contain more oxidized lipid and have an increased capacity to oxidize LDL. They are more prone to atherosclerosis than their wild-type siblings probably as a consequence of increased oxidative stress [11]. The apo (apolipoprotein)E knockout (apoE<sup>−/−</sup>) mouse is a well known model of atherosclerotic lesion development. The double knockout apoE<sup>−/−</sup>/PON1<sup>−/−</sup> has a susceptibility to atherosclerosis over and above that caused by the lack of apoE, suggesting a role for PON1 in the prevention of the disease [12]. In contrast, mice overexpressing PON1 are protected against atherosclerosis in both a wild-type and an apoE<sup>−/−</sup> background [13].

PON1<sup>−/−</sup> mice are also more susceptible to poisoning by the OP toxins chlorpyrifos-oxon and diazoxon, although interestingly not paraoxon [11]. Injection of purified PON1 into the mice restores OP resistance to normal levels [14].

Concentration and activity of PON1 are highly variable in human populations. The quantity and quality of the enzyme in serum is likely to be important in an individual’s response to OP poisoning or risk of developing vascular disease [15]. As such, it is vital to understand the factors that influence serum levels of PON1 in vivo, particularly if it is considered a target for therapeutic intervention. This review will focus on mechanisms which determine serum concentration and activity of PON1, including genetic polymorphisms and gene expression, protein secretion, association with HDL and environmental factors.

PON1 CODING REGION POLYMORPHISMS

There are two polymorphisms in the PON1 coding region at positions Gln<sup>192</sup> → Arg (Q<sup>192</sup>R) and Leu<sup>55</sup> → Met (L<sup>55</sup>M). Q<sup>192</sup>R has been more widely studied, because the two alloenzymes have different affinities and catalytic activities towards a number of substrates (Table 1). Paraoxon is hydrolysed six times faster by PON1<sup>192R</sup> than by PON1<sup>192Q</sup>, whereas the Q form is more active towards sarin, soman and diazoxon. For some substrates, there is no difference in hydrolytic rates, e.g. phenylacetate and dihydrocoumarin [4,5,14,16]. The dramatic alteration in enzyme activity caused by this single amino acid change is explained by the structure of the enzyme. Amino acid R<sup>192</sup> is an important active-site residue [3]. The difference in activity towards certain OPs is important as it affects susceptibility to some toxic substrates (e.g. chlorpyrifos-oxon) when injected into PON1 knockout mice [14] and may do the same in humans [17]. The Q<sup>192</sup>R polymorphism also alters the enzyme’s ability to protect LDL from oxidation in vitro with the Q form being the most protective [18,19].

The PON<sub>55M</sub> polymorphism does not affect the interaction of PON1 with its substrates, but is associated with lower serum activity and concentration of the enzyme [20]. Leviev et al. [21] found lower PON1 mRNA levels in individuals carrying the M allele. Subsequent analysis showed a strong linkage disequilibrium with the C(−108)T polymorphism in the promoter of the gene (see below). We found that this link did not completely
Modulating serum paraoxonase-1 activity

Figure 1  Coding regions of the PON1 gene
(A) Locations of the polymorphisms in the promoter and coding region of the PON1 gene. (B) Enlargement of the approx. 200 bp region of the promoter that is sufficient and necessary for transcription of the PON1 gene. The −162 and −108 polymorphisms and potential transcription factor binding sites are marked. NF-1, nuclear factor-1; NF-Y, nuclear factor-Y.

explain the effect of L55M, which remained significant when the −108 site was kept constant, suggesting a contribution of L55M that is independent of C(−108)T [22]. Brophy et al. [23] did not see this effect in a smaller population, but there was a trend towards higher serum PON1 in LL compared with MM genotypes when both were homozygous CC for the −108 site.

We have shown previously [24] the L55 isoform to be more stable and resistant to proteolysis, which may explain part of its association with higher serum PON1 levels. These data are supported by the recent publication of the PON1 crystal structure which shows a key role of L55 in the correct packing of the protein [3].

PON1 coding region polymorphisms and CHD (coronary heart disease)

Differences in the ability of the Q192R and L55M polymorphic forms to protect LDL from oxidation have led to numerous case-control studies aimed at determining their contribution to the risk of developing CHD. The results from these studies have not been conclusive. Many show an association between PON1192R or PON155M and CHD, but some do not (reviewed in [25]). Often these studies included small numbers of patients from different populations and/or used different genotyping methods, different sampling strategies and different endpoints, making the outcomes difficult to interpret. A recent meta-analysis using all 43 available studies of Q192R and L55M was unable to show a strong association between any of the polymorphisms and CHD [26]. This suggests that the link between PON1 genetic polymorphisms and CHD is, at best, weak. It is likely that other factors such as the large inter-individual variation in serum PON1 activity/concentration have a significant role to play in the potential protective effect of PON1 against CHD (see below).

PROMOTER POLYMORPHISMS

Sequencing of the promoter of the PON1 gene led to the discovery of at least five polymorphisms with varying degrees of influence over gene expression. These polymorphisms are located at positions −909/907 (C or G), −832/824 (A or G), −162 (A or G), −126 (C or G) and −108/−107 (C or T) (Figure 1A) [22,27,28]. Nomenclature differences are due to small variations in the sequences studied. For simplicity we refer to them herein as −909, −832 and −108.

Luciferase reporter gene experiments in HepG2 cells have shown that promoters containing the polymorphisms GAAC, as opposed to CGGT, at positions −909, −832, −162 and −108 respectively, are up to two times more active [22,27,28]. These variations in promoter activity were shown to be physiologically relevant as they correlated with significant differences in serum PON1 concentration and activity [22,23,28]. Polymorphisms have also been detected in the 3′ untranslated region of the PON1 gene, but their significance is as yet unknown [23].

Identification of clinically significant polymorphisms has been hampered by the fact that there is significant linkage disequilibrium between all the promoter polymorphisms. Haplotype analysis of two populations showed that the C(−108)T polymorphism was the main contributor to serum PON1 variation, accounting for 23–24% of the total variation [23,29]. Brophy et al. [23]...
also reported a slight contribution (1.1 % total variation) from the A(−162)G site. The sites at − 909 and − 832 made little or no difference to serum PON1 levels [23,29].

Reporter gene assays using promoter regions of varying lengths have shown that the approx. 200 bp region covering the −108 and −162 polymorphisms is sufficient for transcription of the PON1 gene [23,30,31]. Deleting this region completely abolishes promoter activity, indicating that it is an essential regulatory region of the PON1 promoter [31].

As the −108 site appeared the most significant contributor to PON1 serum variation, it has been the subject of further investigation. The polymorphism is located in the centre of a consensus binding site for the ubiquitous transcription factors Sp1 and Sp3. This consensus site is abolished by the presence of the −108T variant [22,27]. Binding of Sp1 to the −108 site is weaker in the presence of T than C, suggesting an effect of the polymorphism on Sp1 binding [29]. There are multiple Sp1 sites in this region of the PON1 promoter, so the effect of the polymorphism is likely to be positional. Co-transfection of the PON1 promoter with a plasmid expressing Sp1 resulted in strong up-regulation of promoter activity, supporting further the hypothesis that Sp1 is important in PON1 expression [32].

The −162 polymorphism lies over a potential NF-1 (nuclear factor-1) binding site, with the high-activity A variant forming the site and the low-activity G variant disrupting it [27]. This may explain the effect of the change at −162 on gene expression. Figure 1(B) shows the potential transcription factor binding sites located at the polymorphic sites on the PON1 promoter.

There is a significant linkage disequilibrium between the promoter polymorphisms and the coding region polymorphism at L99M discussed above). Brophy et al. [23] detected a linkage disequilibrium between −108C and the R192 coding region polymorphism, which is associated with a lower level of protection against CHD. Although Q99R is independently associated with variance in enzyme activity, it is possible that the −108C may partly compensate for the lower protection of PON192R, complicating the relationship between PON1 genotype and disease [23].

PON1 promoter polymorphisms and CHD
The role of the PON1 promoter polymorphisms in disease is not clear. A small number of epidemiological studies has investigated the effect of the PON C(−108)T polymorphism on the risk of developing vascular disease. A positive association between the low activity T allele and vascular disease has been observed, particularly in young populations [33–35] and patients with Type II diabetes [36]. In contrast, studies on other populations, e.g. hypercholesterolaemic patients and older populations, have not shown any association between PON1 promoter polymorphisms and disease [37,38]. A meta-analysis of the small number of studies available did not show any significant association of the −108 polymorphism with CHD [26].

PON1 STATUS
There is wide variation (up to 13-fold) in PON1 serum concentration and activity between individuals even within genotype groups [15]. In addition to genetic polymorphisms, PON1 levels can be modified by acquired factors such as diet, lifestyle and disease. It is likely to be the functionality of the enzyme and not simply the genotype that is important in the interaction of PON1 with CHD. Thus when carrying out studies into the association of PON1 with CHD and other diseases it is essential that PON1 serum concentration and/or activity are measured. The fact that the vast majority of published studies do not take into account that individual serum PON1 levels may be responsible for the lack of conclusive epidemiological evidence for a link between PON1 and CHD.

A small number of recent studies, including PON1 concentration and/or activity data, has found that PON1 levels are reduced in CHD, and that this effect is independent of PON1 genotype [37,39,40]. In a case-control study of carotid artery disease, Jarvik et al. [39] found no genotype effect unless PON1 activity was also considered. Results from these studies strongly suggest that future epidemiological studies should include a measure of PON1 quantity and quality designated ‘PON1 status’ in addition to data on genetic polymorphisms [15,40]. PON1 status can be determined using concentration and enzyme activity values or by using a two-dimensional substrate assay, such as developed by Richter and Furlong [15]. In this assay, rates of hydrolysis of diazoxon are plotted against hydrolysis of paraoxon at high salt concentration. This gives an accurate estimate of PON192 genotype as well as a measure of overall enzyme activity.

PON1 AND HDL
PON1 gene promoter polymorphisms account for approx. 25 % of variation in serum PON1 concentration, i.e. 75 % of this variation is attributable to other factors. HDL, the serum vector for PON1, is likely to be an important determinant of enzyme concentration. This is demonstrated by the fact that PON levels are reduced (although interestingly not abolished) in HDL-deficiency syndromes [41].

Early studies showed that PON1 is found in the HDL2 species of HDL in a particle enriched in triacylglycerols (triglycerides) [42]. A large proportion of PON1 is associated with HDL containing apoAI, although particles containing PON1, apoAI and apoAII
do exist [42]. There is also a subpopulation of HDL containing PON1 that is associated with apoJ or clusterin [42,43]. Approx. 30% of total PON1 is associated with apoJ and vice versa. The fact that PON1 tends to bind to larger sized species of HDL both in vivo [42] and in vitro [44] has implications for secretion of PON1 in certain diseases, e.g. diabetes in which HDL size is often reduced.

Association of PON1 with lipoproteins in experimental models

PON1 is known to associate with HDL. In contrast, it is unable to form complexes with LDL [44]. In vitro PON1 can bind to reconstituted HDL containing apoAI, apoAII or apoE and to protein free phospholipid micelles [44–46]. ApoAI is therefore not required for the association of PON1 with phospholipid-containing vesicles. This may offer an explanation for the presence of the enzyme, albeit diminished, in human HDL-deficiency syndromes and knockout mouse models [41,46]. Indeed, PON1 is found in the HDL fraction of apoAI knockout mice, confirming its ability to bind to HDL-like particles in vivo in the absence of ApoAI [46].

Serum PON1 activity from apoAI knockout mice is less stable than that of wild-type siblings [46]. In vitro experiments showed further that PON1 loses activity more rapidly when it is complexed with phospholipid micelles than when it is associated with protein-containing vesicles. Binding of PON1 to phospholipid increases PON1 specific activity, which is further enhanced when apoAI is present [44–46]. These observations suggest that, although PON1 binding to lipoproteins does not require apoAI, its presence is necessary to maintain optimum activity and stability of the enzyme.

Addition of free cholesterol to reconstituted HDL significantly lowered PON1 activity secreted from cells [44]. Free cholesterol is located in the outer lipid layer of HDL and has been shown to adversely affect lipoprotein function. The HDL2-cholesterol/free cholesterol ratio is substantially reduced in diabetic patients [47]. Consequently HDL from diabetic patients may create an unfavourable environment for optimum PON1 secretion and/or activity.

Mechanism of PON1 binding to HDL

PON1 is unusual in retaining its N-terminal hydrophobic signal sequence upon secretion from the cell [1]. An N-terminal PON1 mutant, in which the signal sequence is removed before secretion of the protein, was unable to bind to HDL [46]. This demonstrates that PON1 binds to HDL via its hydrophobic N-terminal signal sequence.

The recent publication of the three-dimensional crystal structure of PON1 has shed further light on its interaction with HDL. A secondary structure prediction of the N-terminal signal sequence shows that the entire region is compatible with a trans-membrane helix. The majority of the N-terminal is disordered and invisible in the tertiary structure, but its hydrophilic part forms a helix. The hydrophobic residues of a second helix, adjacent to the N-terminal helix, are oriented towards the solvent as are a number of hydrophobic residues in the loops that connect the helix to the main PON1 structure. These regions provide adjacent hydrophobic areas which would allow PON1 to bind to membranes and/or lipoproteins. The potential interface with HDL has an ‘aromatic belt’ rich in tryptophan and tyrosine residues, which has been described in a number of membrane-binding proteins (Figure 2) [3].

The N-terminal PON1 mutant described above has a lower \( K_m \) and \( V_{\text{max}} \) than wild-type PON1, suggesting a reduced affinity for lipophilic substrates [46]. PON1 substrates are generally hydrophobic and partition into the lipid phase. Association with HDL or phospholipid may therefore be important for enzymic function. Predictions from the three-dimensional structure indicate that PON1 binding to HDL may modify its active site, particularly as the HDL-anchoring region is part of an active site lid [3].

PON1 secretion and association with cell membranes

PON1 is synthesized in the liver and secreted into serum. The mechanism of PON1 secretion is important, because factors that modulate its release from the cell will, in turn, affect serum levels of the enzyme. We used a CHO (Chinese-hamster ovary) cell culture model to study PON1 secretion [44]. In the absence of lipoproteins, little PON1 was secreted. Addition of phospholipid micelles or HDL stimulated secretion, whereas LDL and lipid-free ApoAI had no effect. This suggests that PON1 requires an appropriate acceptor for release into serum. HDL appears to be the predominant physiological acceptor, but the presence of apoAI/apoAII are not essential [44].

PON1 can be competitively removed from HDL by phospholipid [46], thus it may be able to move between HDL and other phospholipid-rich areas, such as cell membranes and areas of lipid damage. Immunofluorescence studies showed that it is located at the external surface of transfected CHO and HuH-7 hepatocyte cell membranes [44]. Membrane-bound PON1 is active towards phenylacetate. In the presence of HDL, this activity is lost from the membrane [44], suggesting that HDL can actively remove PON1 from cell membranes. HDL-induced PON1 secretion is concentration dependent and saturable, indicating that it may be receptor mediated [44]. A possible candidate for this receptor is the scavenger receptor SR-B1. SR-B1 facilitates transient association of HDL with the cell surface. It has a loose ligand specificity and can bind apoAI- and...
Pharmacological Regulation of PON1

In recent years, the use of lipid-lowering drugs has increased dramatically. Although the main aim of these drugs is to reduce LDL-cholesterol levels, there has also been a great deal of interest in their ability to affect other aspects of lipid metabolism, including the elevation of HDL-cholesterol levels. A number of clinical studies has been carried out to examine the effect of lipid-lowering drugs (particularly fibrates and statins) on PON1 activity and concentration. Recently, more detailed studies of their potential role in the regulation of PON1 gene expression have been undertaken.

Fibrates

Fibrates have been shown to increase the level of HDL-cholesterol. Clinical studies of fibrate treatment in which PON1 activity and concentration were measured have given conflicting results. Balogh et al. [49] found that treatment with gemfibrozil for 3 months increased serum paraoxonase activity by 18%. In a separate study,
Modulating serum paraoxonase-1 activity

Figure 3 Proposed model for PON1 secretion, association with HDL and transfer to sites of lipid damage

(1) HDL is transiently bound to the cell surface via a receptor. (2) PON1 anchored in the cell membrane via its hydrophobic N-terminus is transferred to HDL under non-equilibrium conditions where it is stabilised by apoAI. (3) PON1 enters the intravascular space with HDL. (4) Under more static conditions favouring diffusion, PON1 could transfer to phospholipids in plasma membranes, possibly during receptor mediated recruitment of cholesterol from endothelial or smooth muscle cells. (5) PON1 may therefore have access to the interstitium and areas of LDL accumulation and oxidative damage where it could protect against adverse effects of oxidation. The retained hydrophobic N-terminal signal peptide is represented by a thick black line. EC, endothelial cell; SMC, smooth muscle cell; Mφ, macrophage; ECM, extracellular matrix; Ox-LDL, oxidised LDL. This Figure was adapted from R. C. Sorenson, C. L. Bisgaier, M. Aviram, C. Hsu, S. Billecke and B. N. La Du, Human serum paraoxonase/arylesterase’s retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: apolipoprotein A-I stabilizes activity. Arterioscler., Thromb., Vasc. Biol. 19 (9), 2214–2225 with permission.

Paragh et al. [50] showed an increase in PON1 specific activity in response to fenofibrate treatment. In contrast, Durrington et al. [51] found no effect of gemfibrozil or bezafibrate on PON1 activity, despite an increase in HDL-cholesterol and apoAI.

Gouédard et al. [30] showed that treatment of HuH7 cells with fenofibrate increased PON1 promoter activity by up to 70%. The effect on PON1 expression was limited to fenofibrate and, to a lesser extent, bezafibrate. Gemfibrozil which has had beneficial coronary effects in clinical studies had no effect on promoter activity [30].

Statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors)

Statins are often the drug of choice for treating elevated cholesterol. In addition to lowering LDL-cholesterol, a number of pleiotropic properties of the various statins have been identified, including an antioxidant effect. Several small clinical trials have investigated the ability of statins to influence PON1 activity and concentration. Tomas et al. [52] treated familial hypercholesterolaemic patients with simvastatin for 4 months and found a significant increase (approx. 12%) in serum PON1 activity, despite no effect on HDL-cholesterol. In a smaller study, we [31] also saw a significant increase (20%) in PON1 activity and concentration in patients treated with simvastatin for 6 weeks. Treatment with 20 mg of atorvastatin for 1 month resulted in an increase in PON1 activity by 53% in one study [53], but a larger study on patients with type IIA and type 1IB dyslipidaemia failed to show any effect over 4 months [54].

Assaying in vitro, we found that simvastatin up-regulated PON1 promoter activity up to 2.5-fold in HepG2 cells. The activation was dependent on a 127 bp region at the proximal end of the promoter containing the −108 promoter polymorphism [31]. This region was also required for up-regulation of the PON1 promoter by the transcription factor SREBP2 [SRE (sterol-regulatory-element)-binding protein 2]. SREBP2 is closely linked to cholesterol metabolism and is known to be up-regulated in HepG2 cells treated with statins [55]. It binds to DNA sequences with homology with the SRE, two of which are found in the statin-sensitive region of the PON1 promoter (Figure 1B). SREBP2 was able to bind to the
PON1 promoter only in combination with Sp1 [31]. This is important as the −108 polymorphism lies within an Sp1 site. Sp1 is a known co-activator of SREBP2 and an interaction between the two proteins has been demonstrated in vivo during transcription of the gene encoding the LDL-receptor [56]. The interaction between Sp1 and SREBP2 may be one way in which the C(−108)T polymorphism affects PON1 promoter activity [31].

In contrast with our findings, Gouédard et al. [30] showed that treatment of HuH-7 cells with simvastatin, pravastatin or fluvastatin resulted in an inhibition of PON1 promoter activity by up to 40%. PON1 mRNA levels were also reduced. These results are not in agreement with the clinical trials outlined above, however there are at least two studies in the rat which show a decrease in PON1 activity in response to statin treatment [57,58]. The directly contrasting results described above may be due to differences in the cell lines used or in the version of the PON1 promoter studied. Evidently more work is required to clarify the effect of statins on the expression of the PON1 gene.

REGULATION OF PON1 EXPRESSION BY INFLAMMATORY CYTOKINES

Inflammation is important in the atherosclerotic process. The acute-phase response is linked to atherosclerosis and causes changes to the HDL particle. Free cholesterol, SAA (serum amyloid A) and apoA are increased, whereas esterified cholesterol, apoAI, LCAT (lecithin: cholesterol acyltransferase), CETP (cholesteryl ester transfer protein) and hepatic lipase are decreased. HDL loses its ability to participate in reverse cholesterol transport and to protect LDL against oxidation. These pro-inflammatory changes in HDL proteins are often caused by down-regulation of hepatic mRNA [59].

Feingold et al. [59] investigated PON1 mRNA levels during the acute-phase response in Syrian hamsters. Treatment with LPS (lipopolysaccharide) as well as the administration of cytokines TNF (tumour necrosis factor) and IL-1 (interleukin-1) caused a fall in PON1 activity, corresponding to a decrease in PON1 mRNA in the liver. They concluded that PON1 is a negative acute-phase protein whose mRNA is rapidly down-regulated. Similar observations have been made in HepG2 cells using measurements of PON1 mRNA levels and promoter reporter gene assays [32,59,60].

Incubation of HepG2 cells with oxPAPC (oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine; oxidized phospholipid) and mmLDL (minimally modified LDL) down-regulated PON1 mRNA. IL-6 (interleukin-6) mimicked this effect, which was blocked by pretreatment with an anti-(IL-6) antibody. Injection of oxPAPC into mice caused a fall in hepatic PON1 mRNA. This reduction in PON1 mRNA was not seen when IL-6 knockout mice were used, suggesting an effect mediated through IL-6 [61]. Interestingly, the sequence surrounding the −162 promoter polymorphism has some homology with an IL-6 responsive element [23]. Further studies are required to determine if this region is involved in the regulation of PON1 expression by IL-6.

ENVIRONMENTAL FACTORS

Diet

In both rabbits and transgenic mouse models, a proatherogenic diet caused a significant fall in PON1 activity and/or mass, which correlated with a reduction in HDL-cholesterol [62–65]. In humans, the effect of the Western diet on PON1 activity is less well studied. Diets with a high trans-unsaturated fat content can reduce PON1 activity [66]. In contrast, oleic acid from olive oil is associated with increased activity [67,68]. Meals rich in red cooking fat, which contains a high content of oxidized lipids, were followed by a significant fall in PON1 activity when fed to healthy men [69]. This is in agreement with in vitro studies in which PON1 was inactivated by oxidized lipids and oxidized LDL [70].

PON1 is highly susceptible to inactivation by oxidation. In vitro, PON1 activity is protected by the antioxidant polyphenols quercetin and glabridin [70], suggesting that dietary antioxidants may play a similar role in vivo. Studies have shown that consumption of pomegranate juice, rich in polyphenols and other antioxidants, can raise PON1 activity up to 20% in both humans and apoE knockout mice [71]. Polyphenols extracted from red wine also increase PON1 activity in mice [72]. Recent work from Gouédard et al. [73] provides evidence that dietary polyphenols can influence PON1 gene expression. Quercetin and naringenin induced PON1 promoter activity in HuH-7 cells at concentrations consistent with those observed in vivo after a polyphenol-rich meal. Quercetin and naringenin act as ligands for the AhR (aryl hydrocarbon receptor). AhR is a ligand activated transcription factor which binds to the XRE (xenobiotic responsive element). Overexpression of AhR increased the induction of the PON1 gene by polyphenols, and AhR-targeted gene silencing abolished this effect. AhR specifically bound to an XRE-like sequence in the PON1 promoter (Figure 1B) shown to be necessary for polyphenol-induced expression [73]. This suggests an additional mechanism for the increase in PON1 activity observed after polyphenol consumption.

Clinical trials of the antioxidant vitamins C and E have, to date, been unsuccessful in showing a link between vitamin intake and CHD risk. Likewise, their effect, if any, on PON1 activity is not clear. Jarvik et al. [74] found that PON1 activity correlated positively with the quantity of vitamins C and E in the diet; however, another
study in which vitamin E was given to volunteers showed no change in PON1 activity [75].

**Alcohol consumption**

There has been much discussion on the potential benefits of moderate alcohol consumption. However, the mechanism by which alcohol may lower the risk of developing CHD is poorly understood. Van der Gaag et al. [76] showed that drinking 40 g/day of alcohol increased both PON1 activity and mass. There was no difference between red wine, beer or spirits, suggesting that it is not the red wine polyphenols alone that cause the effect. Similar results were obtained in a study that examined the effect of drinking alcoholic compared with non-alcoholic beer. Only alcoholic beer had a positive effect on PON1 activity [77]. Moderate drinking significantly increases serum HDL-cholesterol and apoAI concentrations, which may account for the observed increase in PON1 concentration [76,77]. Increased serum PON1 may be one of the factors underlying the reduced CHD risk in moderate drinkers.

**Smoking**

Cigarette smoke extract is known to inhibit PON1 activity in vitro [78], suggesting that smoking may be detrimental to enzyme activity in vivo. James et al. [79] showed that PON1 serum concentration and activity were reduced in smokers compared with non-smokers. Ex-smokers had activities and concentrations comparable with those of non-smokers, suggesting a reversible influence of smoking on PON1. This deleterious effect of smoking has been consistently confirmed by recent studies [80–83]. Interestingly, smokers who also drank moderately [82] or exercised regularly [81] had PON1 levels similar to those of non-smokers, suggesting that these activities can attenuate the effect of smoking on PON1.

**Age**

A number of studies have shown that PON1 activity decreases with age [79,84–86]. This correlates with an increased susceptibility of HDL to oxidation in elderly patients [85]. The PON1 Q192R polymorphism appears to play a role in the loss of activity due to aging. QQ homozygotes show a greater loss in enzyme activity with age [86]. PON1192Q has been associated with more advanced age [84], whereas a study of 308 centenarians found the R allele was more prevalent in individuals of advanced age compared with the general population [87].

**Environmental toxins**

PON1 can protect animals against OP poisoning [14]. Two recent publications [17,88] have suggested that exposure to environmental toxins can also have an impact on serum PON1 activity. Twenty-eight patients exposed to OP poisoning were found to have lower PON1 activity than controls. At 6 months after poisoning, the levels of eight patients had returned to normal, suggesting temporary inhibition of PON1 by the toxin [17]. In a second study, PON1 activity was 30% lower in workers exposed to radiation than in those not exposed [88]. In addition, personnel serving in the Gulf War, who may have been exposed to various OP toxins, had lower PON1 activity than military control groups [89].

**PON1 AND DISEASE**

**Diabetes**

Most studies have found that PON1 activity is reduced in Type I and Type II diabetic patients [90–95], with some dissension [96,97]. Boemi et al. [90] showed that activity is reduced to an extent that can alter antioxidant capacity in vitro, suggesting an association with the tendency towards increased oxidative stress in diabetics [93,94]. The effect on activity is probably independent of genotype [90,92,94,95]. However, PON155L has been associated with retinopathy, a frequent complication of diabetes [91,98,99], and PON1192R is more consistently associated with CHD in diabetics than in the general population [92,100,101]. The L55M polymorphism has also been linked with impaired glucose disposal [102], impaired β-cell function [102,103] and increased insulin resistance in healthy subjects [104].

The mechanism by which PON1 is reduced in diabetes is poorly understood, but may be associated with an increase in blood glucose concentration. Glycation can both inactivate PON1 and increase lipid peroxidation in HDL [105,106]. Glycated HDL also has a reduced ability to protect against oxidation [105]. In support of the above in vitro data, PON1 activity and concentration were decreased in studies of healthy subjects with elevated fasting glucose levels [34,91]. PON1 activity is also lower in patients with the metabolic syndrome, symptoms of which include abnormal fasting glucose levels and increased insulin resistance [107].

**Other diseases**

Oxidative stress is a risk factor for the development of dementia. PON1 activity is reportedly reduced in patients with vascular dementia and Alzheimer’s disease; however, it is not known if this is a cause or a consequence of increased oxidation [108,109]. The PON1192R and PON1107T variants have been linked with both vascular dementia and Alzheimer’s disease [110,111], although this is not a consistent observation [112,113].

Chronic renal failure is associated with elevated oxidative stress, and PON1 activity is consistently lower in patients suffering from renal failure [114–116]. In one study, PON1 activity was restored to normal levels after kidney transplantation, suggesting that the effect on
PON1 activity is a consequence of the disease and not an underlying cause [114]. Alterations in PON1 activity have been seen in a number of other disorders, including liver cirrhosis, chronic hepatitis, HDL deficiencies, Gulf War Syndrome and anxiety [41,89,117–120].

CONCLUSIONS

PON1 can protect against the oxidative modification involved in the onset of CHD and also prevent OP poisoning. Consequently, modification of its concentration and/or activity represents a potential therapeutic target to help combat these diverse illnesses.

Pharmacological modifications are the obvious avenue, and increased serum PON1 may be one side effect of statin therapy, although the effects of statins have to be more clearly defined. Increasing serum HDL concentrations appears a logical approach and, in this context, the effects of fibrates, which have a well-established HDL-raising effect, are encouraging, although the effect may be fibrate specific. A new generation of more powerful HDL-raising drugs is currently under development and their effects on PON1 await analysis.

Lifestyle modifications could also make a contribution to improved serum PON1. Interestingly, the lifestyle pattern that appears to favour increased PON1 corresponds to that given to patients to diminish coronary risk: no smoking, increased physical activity, greater fresh fruit intake and moderate alcohol consumption.

Finally, pioneering work by Aharoni et al. [121] has shown that PON1 is highly susceptible to the technique of directed evolution. Using an in vitro selection pressure, they were able to create a number of PON1 mutants which had altered enzyme activity and/or solubility. Thus there is the potential to create PON1 variants with increased affinity for oxidized lipids or with high activity against specific OP poisons.

Much work remains to be done if the evaluation and modulation of PON1 status is to become a useful clinical tool. In this respect emerging data continues to be encouraging.

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