Pharmacological and Lifestyle Factors Modulating Serum Paraoxonase-1 Activity

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Abstract: Paraoxonase-1 is a lactonase and an esterase and it plays a protective role in toxicity as well as in diseases involving oxidative stress. Recently, insights into how it may be modulated by environmental factors have acquired clinical relevance. This article reviews the state-of-the-art evidence regarding PON1 modulation by pharmacological products as well as nutritional and lifestyle factors.

Key Words: Apolipoprotein A-I, fibrates, high-density lipoproteins, lipid peroxidation, nutrition, paraoxonase-1, rosiglitazone, statins.

INTRODUCTION

Research into paraoxonase-1 (PON1) has flourished over the past few years. This enzyme belongs to a group currently containing 3 members: PON1, PON2 and PON3 the genes of which are located adjacent to each other on chromosome 7q21-22 [1, 2]. In humans, PON1 and PON3 genes are expressed, essentially, in the liver and kidney and their protein products are found in the circulation bound to high-density lipoprotein (HDL) [3-6]. Conversely, PON2 gene is expressed a variety of tissues. Its protein product is an intracellular enzyme which is not, however, found in plasma [7]. PON1 has esterase and lactonase activities [8]. It hydrolyses homocysteine thiolactone as well as the active metabolites of several organophosphate insecticides (paraoxon, chlorpyrifos oxon, and diazoxon) and the nerve agents sarin and soman [9]. PON2 and PON3 are not active against organophosphate substrates, but have lactonase activity [10]. All the three PON enzymes are able to retard low density lipoprotein (LDL) oxidation [11], while PON2 retards cellular oxidative stress and prevents apoptosis in vascular endothelial cells [12]. Existing evidence indicates that the PON enzyme family plays a protective role in several diseases involving oxidative stress, including cardiovascular diseases, Alzheimer's disease, diabetes, metabolic syndrome, and liver diseases [13, 14].

The main determinants of PON1 levels in the circulation are its gene polymorphisms. Many polymorphisms have been identified in the coding, intronic, and promoter regions of the PON1 gene [15, 16]. The polymorphisms in the coding region that have been identified to-date are: Arg/Gln substitution at position 192 (*PON1*$_{192}$ polymorphism with two alleles termed Q and R); and Leu/Met substitution at position 55 (*PON1*$_{55}$ polymorphism with two alleles termed L and M). Garin *et al.* [17] evaluated the influence of these polymorphisms on the enzyme's activity as well as its concentration, and observed important differences in relation to the *PON1*$_{55}$ genotype; individuals carrying the LL isoform having higher serum PON1 concentrations than those with MM at this position. In contrast, the *PON1*$_{192}$ polymorphism affected the enzymatic activity, but had little impact on the serum PON1 concentration. The QQ isoform hydrolyses paraoxon much less efficiently than does the RR isoform, while the opposite occurs for soman and sarin. The polymorphisms in the promoter region, *PON1*$_{-108}$, *PON1*$_{-909}$ and *PON1*$_{-1741}$, have also been reported to be significantly associated with changes in the serum enzyme concentration, or activity [18].

PHYSIOLOGICAL ROLE AND MECHANISM OF ACTION OF PON1

Mackness *et al.* reported, in 1991 [19], the first evidence that the physiological function of PON1 is to protect lipoproteins and cells from oxidative stress by hydrolysing lipid peroxides. These authors observed that purified PON1 prevented lipoperoxide generation during the process of LDL oxidation *in vitro*, and suggested that PON1 may be involved in the protective function of HDL. Subsequent studies from this group and others reached the conclusion that PON1 degrades specific oxidised cholesteryl esters and oxidised phospholipids contained in oxidised lipoproteins [20-26]. Experimental studies provided support for the data from the *in vitro* experiments. Probably the most conclusive data were generated in the PON1$^{(-/-)}$ mouse model and the human-PON1 transgenic mouse model [27-30]. Apolipoprotein E KO mice had lower lipoprotein oxidation and atherosclerosis than PON1 plus apolipoprotein E double KO mice [28]. HDL fractions isolated from PON1$^{(-/-)}$ mice were unable to prevent LDL oxidation in cultured arterial tissue, in contrast to the HDL obtained from control mice [28]. In agreement with these observations, over-expression of human PON1 in transgenic mice inhibits lipid peroxide formation in HDL, and protects the LDL structure and function [29]. Despite this experimental evidence, the precise biochemical mecha-

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nism of the enzyme activity that mediates these functions remains elusive, as does the identity of its endogenous substrate. Directed evolution and structure-function studies suggest that PON1 is a six-bladed beta propeller with a unique active site lid that is also involved in HDL binding (Fig. **1**), and that the primordial function of PON1 is that of a lipolactonase [31-35] which subsequently evolved new substrate specificities. These studies also established that the preferred substrates of PON1 are 5- and 6-membered ring lactones, typically with aliphatic side-chains [36]. A model has been proposed that links PON1 lactonase activity with its ability to degrade oxidised lipids [37,38] such that oxidised lipids containing hydroxyl groups at the 5' position could be lactonised by PON1 to yield lysophosphatidylcholine and δ-valerolactone products. According to this hypothesis, the ability of PON1 to degrade lipid peroxides is secondary to its lipolactonase activity.

A comprehensive Review on the biochemistry of PON1, its mechanism of action as well as assay methods and implications in disease, has been published recently [39]. Since serum PON1 deficiency has been associated with various common diseases, the possibility to modulate its activity seems to be an interesting, even if still unknown, new therapeutic option with several potential implications. In the present article we review the recent efforts directed towards increasing serum PON1 levels by means of pharmacological or nutritional interventions, and we comment upon some lifestyle habits that may have an impact on this enzyme.

INFLUENCE OF LIPID-LOWERING DRUGS ON PON1 STATUS

Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality in Western countries. Elevated LDL-cholesterol and triglycerides, and decreased HDL-cholesterol concentrations are important modifiable risk factors in the individual's predisposition to CVD. Over the last few decades several therapeutic strategies have been employed to improve the lipid profile of the at-risk individual and, in doing so, to prevent atherogenesis. Of these, the pharmaceutical agents most widely used have been statins and fibrates [40].

Statins are the inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. They compete with HMG-CoA for binding to the catalytic site of HMG-CoA reductase and, consequently, reduce the intracellular biosynthetic conversion of mevalonic acid to cholesterol [41]. A consequence is cellular upregulation of the LDL-cholesterol receptor, enhanced LDL uptake from the circulation and, finally, irreversible cholesterol catabolism mainly by the liver [42]. In addition, statins induce several beneficial effects independently of cholesterol regulation. These include improvement of endothelial function, increased nitric oxide bioavailability, and antioxidant and anti-inflammatory effects [43]. The chemical structures of the more important statins are shown in (Fig. **2**).

Over the last decade, there have been several clinical and experimental studies suggesting that the antioxidant effects of statins may be mediated, at least in part, by an increase of serum PON1 activity and/or concentration. Tomàs *et al.* [44] were to the first to report that simvastatin administration (20 mg/day for 4 months) increased serum PON1 activity in hypercholesterolaemic patients. The increases were modest (about 12% on average) and were accompanied by significant decreases in serum cholesterol and lipid peroxides, as well as LDL-cholesterol concentrations. They did not find any significant modulation associated with HDL-cholesterol levels or with $PON1_{192}$ and $PON1_{55}$ *DNA* polymorphisms. Harangi *et al.* [45] observed that atorvastatin (10 mg/day for 6 months) increased serum PON1 activity in hypercholesterolaemic patients, with changes in lipid profile and oxidative stress similar to those described by Tomàs *et al.* (described above). Kassai *et al.* [46] also confirmed that atorvastatin (20 mg/day for 3 months) increased serum PON1 activity. This statin has been shown to increase serum PON1 activities in experimental rabbits fed a high-cholesterol diet [47]. However, Bergheanu *et al.* [48] reported that atorvastatin (increasing doses up to 80 mg/day for

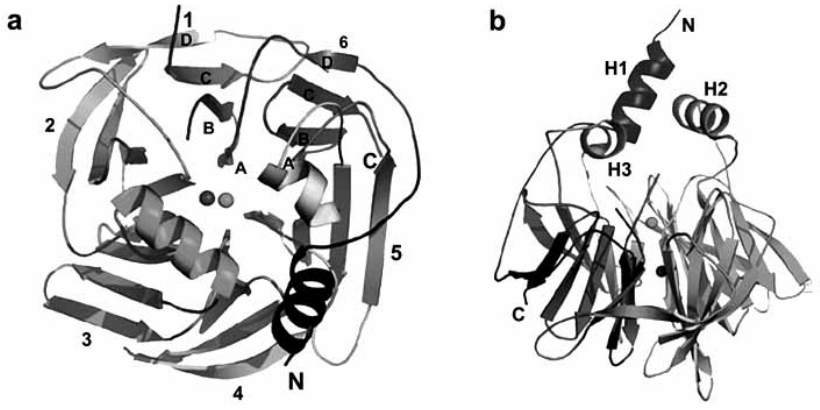

Fig. (1). Chemical structure of PON1.

(**a**) View from above of the six-bladed β-propeller configuration. The top of the propeller is, by convention, the face carrying the loops connecting the outer β-strand of each blade (strand D) with the inner strand of the next blade. Shown are the N and C termini and the two calcium atoms in the central tunnel of the propeller; (**b**) A side view of the propeller with the three helices at the top (H1-H3). This figure is reproduced from [31] with permission.

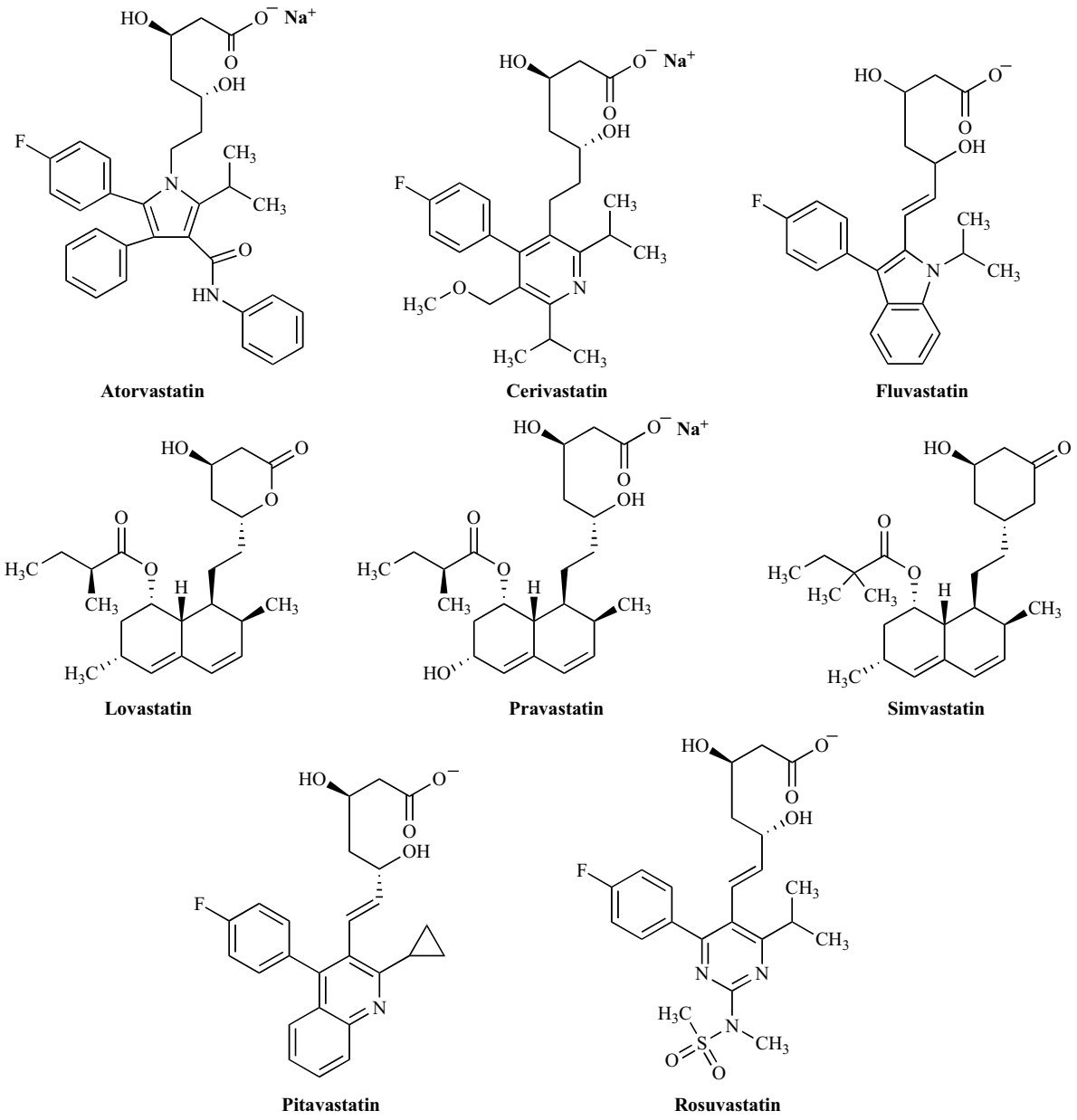

Fig. (2). Chemical structure of the clinically-most important statins currently in use.
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18 weeks) did not modify serum PON1 activity, although rosuvastatin administration (increasing doses up to 40 mg/day for the same period of time) was associated with a significant increase in serum PON1 activity. One of the most detailed clinical reports published to-date is that of Mirdamadi *et al.* [49]. The study was conducted in 164 hypercholesterolaemic patients subdivided into three groups to receive atorvastatin (10 mg/day, $n = 61$), simvastatin (10-20 mg/day, $n = 46$) or fluvastatin (80 mg/day, $n = 57$) for a period of 3 months. The results indicated that all three statins were able to increase serum PON1 activity, albeit moderately.

To-date, it is not absolutely clear whether the effect of statins on serum PON1 levels is secondary to the stimulation of *PON1* gene expression. Reporter gene assays showed that simvastatin up-regulated PON1 promoter activity in HepG2 and HEK293 cells, but the opposite results were obtained in HuH-7 cells [50]. Despite this controversy, Deakin *et al.* [51] were able to identify a statin responsive element at the proximal end of the *PON1* gene promoter region which contains the C(-108)T as well as the A(-162)G polymorphisms. They also found that, within the statin responsive element, there were two sequences with homology to the sterol regulatory element that binds the sterol regulatory element binding proteins. These proteins control cholesterol metabolism in HepG2 cells and are up-regulated by statins. These data suggest that the statin effect on PON1 may be mediated by increased interaction between the sterol regulatory element binding proteins and the *PON1* promoter.

The main therapeutic function of fibrates is to decrease serum triglyceride concentrations, and a mild increase in HDL-cholesterol concentration is also achieved. The fibrates act *via* activation of the peroxisome proliferator-activated receptor alpha (PPAR-α). PPARs are nuclear receptors that form heterodimers with another nuclear receptor termed the RXR and they bind to specific response elements in the promoter regions of their genes. PPARα activators induce the expression of apolipoprotein AI, the main apoprotein of HDL, and of the ATP-binding cassette of A1 (ABCA1); a transporter complex controlling cellular cholesterol efflux. [42]. Chemical structures of the more clinically-important fibrates are shown in (Fig. **3**).

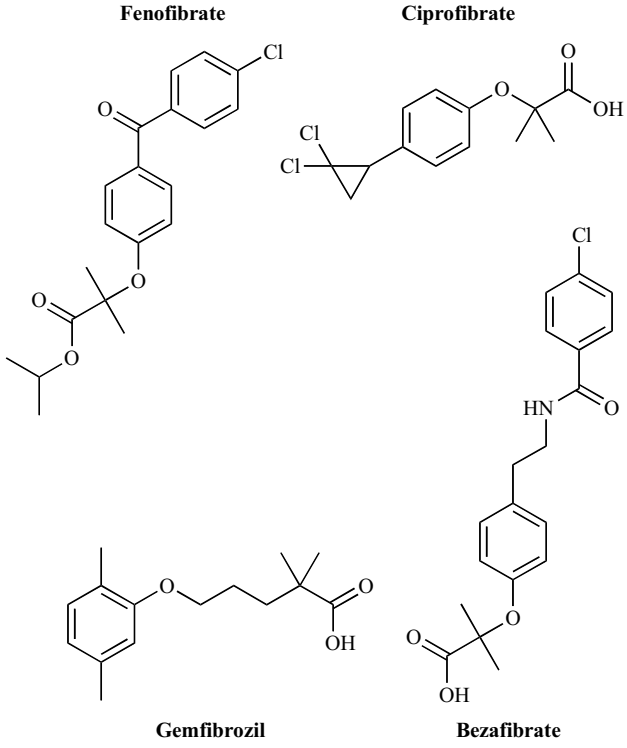

Fig. (3). Chemical structure of the most frequently used fibrates.

Reports on the influence of fibrate therapy on serum PON1 levels have been conflicting. The increase in enzyme activity appears to depend on the type, and perhaps the dosage, of fibrate employed. Durrington *et al.* [52] observed that bezafibrate and gemfibrozil, administered for 8 weeks, failed to influence serum PON1 activity in type IIb hyperlipidaemic patients. Tsimihodimos *et al.* [53] found that 3 months treatment with micronised fenofibrate did not influence PON1 levels in types IIa, IIb and IV dyslipidaemic patients. Conversely, Paragh *et al.* [54] observed that a 3 month administration of gemfibrozil increased serum PON1 activity in patients with hypertriglyceridaemia. This same research group found that ciprofibrate administration increased HDL-cholesterol concentration and serum PON1 activity in patients with the metabolic syndrome [55]. In rats receiving a fructose-enriched diet, an experimental model of liver steatosis and the metabolic syndrome, bezafibrate reduced oxidative stress and increased serum PON1 levels [56]. A recent report described that micronised fibrate increased the activity and concentration of PON1, and reduced oxidised LDL levels in dyslipidaemic patients with low HDL-cholesterol levels and, interestingly, this effect was independent of *PON1* gene polymorphisms [57]. There are several potential PPAR-α binding sites in the *PON1* gene promoter. However, Gouédard *et al.* [58] did not observe any increase in *PON1* gene expression after PPAR-α activation and this suggested that the mechanism of promoter activation induced by fibrates does not involve this nuclear receptor.

ORAL APOLIPOPROTEIN A-I MIMETIC PEPTIDES

An interesting line of research is that of the therapeutic effects of orally-administered apolipoprotein A-I mimetic peptides. The mimetic peptide 4F contains only 18 amino acids. It was constructed to contain a class of amphipathic helix with polar and non-polar faces which enable it to bind lipids in a similar manner to apolipoprotein A-I [59]. In several experimental models, 4F synthesized from D-amino acids (D-4F) and administered orally, induced the formation of anti-inflammatory HDL, increased PON1 activity, enhanced reverse cholesterol transport from macrophages, and reduced atherosclerosis. D-4F is an apolipoprotein A-I mimetic peptide and, like this apolipoprotein, binds and sequesters oxidised phospholipids [60-64]. Recently, this peptide has been administered in the clinical setting. The safety and pharmacokinetic evaluations were conducted in patients with coronary heart disease. The study indicated that oral D-4F administration did not produce any significant change in plasma lipoprotein levels, but improved the patient's HDL anti-inflammatory index [65].

OTHER PHARMACEUTICAL AGENTS

Rosiglitazone is a PPARγ agonist that improves insulin sensitivity and glycaemic control, stimulates reverse cholesterol transport and reduces inflammation in individuals with type 2 diabetes [66-68]. In a randomised, cross-over, placebo-controlled, double-blind clinical trial, rosiglitazone was shown to increase fasting PON1 activity, and to attenuate the post-prandial fall in PON1 activity; the serum PON1 concentration was observed not to change significantly [69]. A combination of rosiglitazone and metformin has been proposed to improve insulin resistance and fat distribution abnormalities (lipodystrophy) in patients infected with the human immunodeficiency virus (HIV) [70]. Both treatments increased fasting and post-prandial serum PON1 activity, and decreased plasma monocyte chemoattractant protein-1 concentrations in HIV-infected patients undergoing highly active antiretroviral (HAART) therapy [71]. The results of these studies indicated that plasma HDL-cholesterol concentrations did not significantly change. This suggested that the observed effects on PON1 were independent of HDL synthesis.

Increased atherogenesis and oxidative stress co-exist in obese individuals [72]. Orlistat is a gastrointestinal lipase inhibitor that enhances weight reduction in obese subjects, and improves post-prandial lipaemia in those with type 2 diabetes [73]. A recent longitudinal, multi-centre, randomised study demonstrated that orlistat administration significantly increased serum PON1 activity in obese patients [74]. The mechanisms underlying the effects of this drug on PON1

are not known, but possibly involve a decreased oxidative stress associated with weight loss.

Women have a considerably lower CVD risk to their male counterparts. This is particularly evident in pre-menopausal ages. Declining oestrogen production is an important contributory factor to the increased CVD risk in post-menopausal women. Menopause is associated with decreased HDL-cholesterol and PON1 levels together with increased LDL-cholesterol and insulin resistance [75,76]. Observational studies have shown that post-menopausal hormone replacement therapy (HRT) is associated with decreased CVD risk [77]. However, this is a controversial issue since oestrogens have been reported to enhance the thrombotic potential of blood [78]. Two studies in post-menopausal women observed a significant increase in serum PON1 activity following HRT (conjugated oestrogens + medroxyprogesterone). This increase was accompanied by an increase in HDL concentrations, and a decrease in oxidised LDL levels [79,80]. However, another study showed a slight increase in serum PON1 activity when oestrogen-alone HRT was prescribed, but a decrease was observed when the treatment consisted of oestrogens combined with several progestogens (desogestrel, medroxyprogesterone or norethisterone) [81].

Several other therapeutic agents have been assessed with respect to their effect on the stimulation of PON1 activity. The hypotensive drugs, amlodipine and captopril, have been shown to enhance the hepatic PON1 content in rats with experimentally-induced fatty liver [56]. The mechanisms of action are not known, but amlodipine has been reported to increase HDL-cholesterol concentrations [82]. Both drugs improve metabolic syndrome and, probably, decrease oxidative stress in hypertensive patients [82,83]. Exogenous erythropoietin-β has been reported to increase serum PON1 activity without changes in HDL levels, and to improve oxidative stress in pre-dialysis patients with chronic kidney disease and anaemia [84].

NUTRITIONAL AND LIFESTYLE FACTORS

Several studies have shown that diets relatively rich in fruits, vegetables and nuts and combined with a moderate intake of red meat and red wine (commonly described as the 'Mediterranean diet') are protective against CVD [85]. These diets have a high content of specific vitamins, minerals, phytochemicals, and oils. Many of these compounds have potent biological activities, including anti-oxidative and anti-inflammatory properties. Some epidemiological studies have analysed the effects of differences in dietary habits on PON1 activity. The results obtained have been inconclusive. For example, Jarvik *et al.* [86] reported that the intake of vitamins C and E directly correlate with PON1 activity in patients attending several American VA health centres. The subjects studied were middle-aged and elderly men (44 – 88 years) who were receiving prescription medications. However, Ferré *et al.* [87] did not find any significant association between vitamins C and E intake and PON1 in a Spanish population-based study. The participants in the study were healthy people with a wide age range (18 – 75 years of age) with an equal distribution with respect to gender. The possibility exists that the differences in dietary regimens between the American and the Spanish samples account for this discrepancy. Perhaps the higher amount of antioxidants present in the Mediterranean diet obscures the effects of vitamins among individuals exposed to a diet higher in saturated fats. Kleemola *et al.* [88], in a study conducted with a population of young Finnish women did not find any associations between these vitamins and PON1. However, they did observe an inverse relationship with the intake of β-carotenes. These conflicting results from epidemiological studies highlight the difficulties in reaching unambiguous conclusions on the influence of diets. Differences in the populations studied. or in the methods used for assessment of dietary intakes, may be important in these confounding variables.

Several reports have studied the effect of vegetable oils on serum PON1 activity. An *in vitro* study [89] reported that monoenoic acids ($C_{16:1} - C_{20:1}$) showed a high degree of protection of PON1 activity against oxidative stress. This was compared to saturated fatty acids ($C_6 - C_{18}$) which exhibited a modest protection, and polyenoic acids which showed no protection. Oleic acid, which is the dominant oil in olive oil, was the most effective. An epidemiological study in a Spanish population showed that a high intake of oleic acid was associated with an increase in serum PON1 activity, although only in $PON1_{192}$ homozygous RR individuals [90]. In a recent experimental study in apolipoprotein E-deficient mice that developed atherosclerosis, extra virgin olive oil administration (a dose equivalent to 25 ml of olive oil in humans per day) decreased the atherosclerosis lesion size, enhanced the cholesterol efflux from macrophages, and increased serum PON1 activity [91]. Other oils and fats commonly used in human feeding studies failed to show any effect on serum PON1 activity. Ferré *et al.* [87] did not observe any significant association between the intake of saturated fatty acids and tertiles of PON1 activity in a general Spanish population. In an intervention cross-over study, the intake of palm oil, canola oil, and soybean oil did not show any significant influence on serum PON1 activity in moderately hyperlipidaemic subjects [92]. In apolipoprotein E-deficient mice that developed atherosclerosis, the administration of a dietary formula of plant sterol esters of canola fatty acids, in a canola oil matrix containing 1,3-diacylglycerol, did not produce any significant effect on serum PON1 activity, despite a significant reduction in oxidative stress parameters [93].

Moderate alcohol intake is associated with a lower atherosclerosis risk [94]. The mechanisms of this effect involve an increase in HDL-cholesterol by enhancing the hepatic synthesis and transport rate of apolipoproteins A-I and A-II [95]. This increase is associated with an increased serum PON1 activity. However, the effect of ethanol *per se* is small. Sierksma *et al.* [5] demonstrated that an alcohol intake of 40 g/day in men and 30 g/day in women, increased serum HDL-cholesterol by 6.8% and PON1 by 3.7%. In contrast, heavy alcohol consumption decreased serum HDL-cholesterol and PON1 activity [96,97]. Red wine is well-documented to be one of the most cardio-protective alcoholic beverages. However, this property is not associated with ethanol, but with the high content of antioxidant molecules, essentially flavonoids, in the wine. The search of alternative sources of natural flavonoids that do not have the deleterious effects of ethanol has resulted in several studies assessing the consumption of fruit juices such as pomegranate. Pomegranate

juice has a high content of flavonoids including quercetin and ellagitannins, of which punicalagin is responsible for more than a half of the antioxidant activity [98]. A daily consumption of 50 ml of pomegranate juice for 1 year by patients with carotid artery stenosis induced an increased serum PON1 activity together with decreased levels of oxidised LDL and a decrease in the degree of atherosclerosis, as measured by the carotid intima-media thickness [99]. Similar results were obtained in apolipoprotein E-deficient mice when they had pomegranate juice or pure quercetin or catechin added to the daily fluid intake [100-102]. Another important flavonoid found in grapes and red wine is the phytoalexin resveratrol. This phytochemical increased PON1 gene expression in cultured liver cells [103,104] and, when administered to apolipoprotein E-deficient mice over a period of 20 weeks, increased the HDL-cholesterol levels and serum PON1 activity, and reduced LDL-cholesterol and oxidative stress [105].

Other lifestyle factors have been shown to have an impact on serum PON1 levels. Tobacco smoking significantly decreased the enzyme's activity and concentration, in healthy people as well as in patients with CVD [106-108]. Cigarette smoke is rich in acetaldehyde, formaldehyde, and α-β-unsaturated aldehydes that react with free thiol groups in proteins. Nishio and Watanabe [109] demonstrated that cigarette smoke extracts that included some of these aldehydes, decrease PON1 activity by modifying the enzyme's active site. The intake of re-used cooking fat, as is common in most fast-food restaurants involving deep-frying processes, reduces post-prandial serum PON1 activity. Fats that have been heated-reheated over protracted periods of time contain numerous compounds derived from the oxidation and breakdown of lipids. A meal that is rich in such re-used fat was sown to reduce serum PON1 levels by about 30% in healthy volunteers [110]. Physical exercise is known to be generally healthy and to be cardio-protective. Regular aerobic exercise was reported to improve measures of oxidative stress and insulin sensitivity in overweight subjects but, surprisingly, resulted in decreased serum PON1 activity [111]. However, this effect may be prevented by a dietary supplementation with α-tocopherol [112].

Table **1** summarises the possible modes of action of the different drugs and nutrients on serum PON1 activity, as described in the present review.

CONCLUSION AND PERSPECTIVES

Being able to modulate serum PON1 activity may have potential clinical benefits since this enzyme plays an important role in many diseases involving increased oxidative stress. It also protects against toxic effects of insecticides. The present article has highlighted some of the pharmacological and lifestyle interventions that could influence serum PON1 activity. However, a limitation of most of the studies to-date is that the increase in PON1 activity is moderate. Interesting lines of research include the administration of apolipoprotein A-I mimetic peptides. Nutritional interven-

Table 1. Suggested Mechanisms of Action of the Drugs and Nutrients on PON1, as Described in the Present Review

Compound	Increase in HDL-Cholesterol	Possible Mechanism of Action
Pharmacological		
Statins	Controversial	*PON1* gene up-regulation mediated by the sterol regulatory element binding proteins
Fibrates	Yes	PPAR-α activation and ABCA1 up-regulation
Apo A-I mimetic peptides	No (preliminary data)	Decreased oxidative stress. Improvement of HDL anti-inflammatory index
Rosiglitazone	No	Mechanism basically unknown, although it is a PPAR-γ activator
Orlistat	Controversial	Unknown. Effect on weight loss may induce a decreased oxidative stress
Oestrogens	Yes	HDL induction. Decreased oxidative stress
Amlodipine	Yes	Unknown. May be related to HDL induction or decreased oxidative stress
Captopril	No	Decreased oxidative stress
Erythropoietin-β	No	Decreased oxidative stress
Nutritional and lifestyle		
Vitamin C	No	Decreased oxidative stress
Vitamin E	No	Decreased oxidative stress
Monoenoic fatty acids	Yes	HDL induction
Polyphenols	Yes	*PON1* gene up-regulation mediated by the aryl hydrocarbon receptor
Alcohol (moderate)	Yes	HDL induction

tions with fruit juices such as pomegranate or other flavonoid-rich natural products have good outcomes in terms of PON1 stimulation. In the not-too-distant future, these and other new tools will be available for the conduct of well-designed multi-centred studies in large populations; the objective being to firmly establish their benefit as therapeutic agents.

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ABBREVIATIONS

ABCA1	=	ATP-binding cassette A1
CVD	=	Cardiovascular disease
HDL	=	High-density lipoprotein
HIV	=	Human immunodeficiency virus
HAART	=	Highly active antiretroviral therapy
HMG-CoA	=	3-hydroxy-3-methylglutaryl-coenzyme A
HRT	=	Hormone replacement therapy
LDL	=	Low-density lipoproteins
PON	=	Paraoxonase
PPAR	=	Peroxisome proliferator-activated receptor

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