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Review

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The role of genetic (*PON1* polymorphism) and environmental factors, especially physical activity, in antioxidant function of paraoxonase*

Udział czynników genetycznych (polimorfizm PON1) i środowiskowych, a zwłaszcza aktywności fizycznej, w procesie antyoksydacyjnego działania paraoksynazy

Aneta Otocka-Kmiecik, Monika Orłowska-Majdak

Department of Experimental Physiology, Chair of Experimental and Clinical Physiology, Medical University of Lodz, Lodz, Poland

Summary

Paraoxonase 1 (*PON1*) is a member of a three-gene family (*PON1*, *PON2*, and *PON3*). PON1 activity dominates in human plasma. It is secreted from hepatic cells and is found in the circulation bound to high-density lipoproteins (HDLs). For many years it has been known only for its ability to hydrolyze organophosphate derivatives. More recently, PON1's antioxidant activity draws attention as the enzyme was described to prevent oxidation of lipoproteins by reactive oxygen species formed during oxidative stress. PON1 was also shown to hydrolyze atherogenic products of oxidative lipid modification such as phospholipid peroxides and cholesterol ester hydroperoxides. Some studies indicate that the enzyme presents a lipolactonase activity and hydrolyzes homocysteine thiolactone (HCTL). There is growing evidence as to PON1's protective role in atherosclerosis. Genetic (PON1 polymorphism) and environmental factors and lifestyle may influence PON1 blood concentration and biological activity. Among the many recognized factors accounting for lifestyle, physical activity plays an important role. Various, often opposite, effects on PON1 status are observed in regular training and single physical activities. The results of different studies are often contradictory. It may depend on the time, intensity, and frequency of physical activity. Additionally, it seems that the effects of physical activity on *PON1* blood concentration and activity are modified by environmental and lifestyle factors as well as *PON1* polymorphism.

Key words: **paraoxonase • *PON1* polymorphism • antioxidant activity • atherosclerosis • physical activity**

Streszczenie

Paraoksonaza 1 (PON1) należy do trójgenowej rodziny enzymów (PON1, PON2 i PON3). W osoczu człowieka dominuje aktywność PON1. Enzym ten wydzielany jest przez hepatocyty wątroby do krwi, gdzie wiąże się z lipoproteiną o dużej gęstości optycznej (HDL). Przez wiele lat znano tylko jedną jego funkcję polegającą na hydrolizowaniu związków fosforoorganicznych. Ostatnio zainteresowano się PON1 w związku z jego antyoksydacyjną aktywnością, gdyż okazało się, że zapobiega on utlenianiu lipoprotein przez reaktywne formy tlenu powstające w warunkach stresu oksydacyjnego. Ponadto PON1 hydrolizuje związki wywołujące miażdżycę, powstające w pro-

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cesie utleniania lipoprotein, takie jak nadtlenki fosfolipidów i wodoronadtlenki estrów cholesterolu. Stwierdzono również, że enzym ten ma aktywność lipolaktonazową polegająca na hydrolizowaniu tiolaktonu homocysteiny (HCTL). Coraz więcej danych wskazuje na ochronne działanie PON1 w procesie arteriosklerozy. Stężenie PON1 we krwi i jej aktywność biologiczna modyfikowana jest przez czynniki genetyczne (polimorfizm), środowiskowe i styl życia człowieka. Spośród wielu znanych już czynników składających się na styl życia wymienia się aktywność fizyczną człowieka. Stwierdzono odmienne zachowania PON1 w odpowiedzi na regularny i jednorazowy wysiłek fizyczny. Uzyskiwane w różnych doświadczeniach wyniki były często przeciwstawne. Może to zależeć od czasu trwania, natężenia i częstotliwości stosowanego wysiłku. Ponadto wydaje się, że wpływ wysiłku fizycznego na stężenie i aktywność PON1 we krwi jest związany z jednoczesnym modulującym działaniem polimorfizmu PON1 i wszystkich wymienionych w treści pracy czynników środowiska i stylu życia człowieka.

Słowa kluczowe: **paraoksonaza • polimorfizm PON1 • aktywność antyoksydacyjna • miażdżyca • aktywność fizyczna**

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Author's address: Assoc. Prof Monika Orlowska-Majdak, MS, Ph.D., Chief of the Department of Experimental Physiology, Chair of Experimental and Clinical Physiology, Medical University of Lodz, ul. Mazowiecka 6/8, 92-215 Lodz, Poland; e-mail: monika.orlowska-majdak@umed.lodz.pl

Background

Paraoxonase 1 (PON1, aryldialkylphosphatase, E.C.3.1.8.1) is a calcium-dependent enzyme which functions as an esterase and lactonase. The enzyme was first described in 1946 by A. Mazur. Little was known about its physiological role at that time. The activity of the enzyme was described as the capacity of tissues to hydrolyze organophosphate derivatives [54]; therefore it became of great interest in the field of toxicology. Organophosphate derivatives inhibit the action of acetyl cholinesterase [50], causing cholinergic syndrome and leading to chronic polyneuropathy [46]. The substrates for paraoxonase are triesters of phosphoric acid [18]. Those of greatest importance include paraoxon and diazoxon, metabolites of the highly toxic insecticides parathion and diazinon, respectively. Paraoxonase also hydrolyzes nerve agents such as sarin and soman as well as aromatic esters such as phenyl acetate and naphtyl acetate [43].

It was not until the early 1990's that a possibility to define PON1's biological role was finally revealed. Mackness et al. [49] hypothesized that the enzyme may be able to prevent or limit oxidation of low-density lipoproteins (LDLs). PON1 appeared for the first time as an enzyme having an antioxidant activity. That was a turning point for the acknowledged clinical importance of the enzyme, considering that the relationship between oxidative stress, LDL oxidation, and atherosclerosis was a major cause of mortality and morbidity in developed countries. Nowadays, growing attention is drawn to the enzyme because of very convincing evidence that PON1 plays a role in protection from the development of atherosclerosis.

The paraoxonase gene family

Primo-Parmo et al. [62] discovered that *PON1* is a member of a three-gene family: *PON2*, *PON3*, and *PON1*, located in this order on the long arm of human chromosome 7 (q21.22). The three PONs share about 70% identity in their nucleotide and amino-acid sequences. *PON1* is the best studied and characterized member of the family. The paraoxonase family most likely arose by the duplication of one common ancestral gene (probably *PON2*). This would explain the sequence homology and adjacent location on chromosome 7. The paraoxonase gene family is most likely evolutionarily linked to lactonases, for there are sequence similarities reported for these two enzyme genes [41].

The *PON* gene family members are expressed in different tissues in the human organism [62]. The *PON3* and *PON1* genes are expressed and synthesized exclusively in the liver and *PON2* gene is expressed in various tissues (brain, liver, kidney, and testis) [58]. PON1 and PON3 enzymes are secreted from liver cells and found in the circulation bound to high-density lipoproteins (HDLs), but in human serum, PON1 activity predominates [12]. It is anchored to the HDL particle by the hydrophobic N-terminal leader sequence [82]. PON2 enzyme resides in many tissues and is not released from the cells. It is located in the cell membrane with its active side exposed to the outer side of the cell. PON1 is similarly orientated in the cell membrane before it is excreted to the serum and bound to HDL [21].

The polymorphisms of *PON1*

The cloning of the gene in 1993 resulted in the identification of over 200 single-nucleotide polymorphisms (SNPs) in *PON1* in different regions of the gene [32]. Further studies were conducted to identify the impact of these polymorphisms on the activity and protein level of the enzyme. Researchers' attention has focused on SNPs of the coding region at positions 192, 55, and the -108 promoter region. Glutamine (Q)/arginine (R) substitution at codon 192 results in different hydrolytic activity of the alleles towards various substrates [34]. The Q allele is less efficient in hydrolyzing paraoxon, but more efficient towards diazoxon, soman, and sarin [19] as well as towards oxidized high- and low-density lipoproteins (ox-HDL and ox-LDL) than the R allele [3]. There is no influence of the SNP at 192 position on the hydrolysis rate of phenyl acetate.

The fact that the Q alloenzyme was proven to be more efficient in protecting lipoproteins from oxidation resulted in research studying the relationship of *PON1* polymorphism to the risk of coronary artery disease (CAD). Most studies have reported that either the Q192 polymorphism is associated with a lower CAD risk than the R polymorphism or that there is no association with either of the *PON1*-192 alleles [25].

It seems even more contributory to evaluate PON1 phenotype instead of genotype as there is emerging evidence that there are other factors, apart from genetic predisposition, which influence the activity of PON1 and its relation to cardiovascular disease (CVD). Therefore, PON1 status, i.e. the activity and concentration of PON1 enzyme, seems to be a better predictor of CVD than *PON1* polymorphism [47]. This issue requires further research because the results of another study indicate that only PON1 genotype is a surrogate marker for PON 1 status [69]. The possibility of determining *PON1* polymorphism on the basis of the paraoxonase-to-arylesterase activity quotient appears to be particularly useful [57]. According to this method, individuals may be assigned to the QQ, QR, and RR phenotype.

The leucine (L)/metionine (M) substitution at position 55 results in different plasma PON1 protein levels. The M allele is associated with low PON1 plasma protein level. Fortunato et al. [27] found that the M55L *PON1* polymorphism is independently associated with the early formation of atherosclerotic plaques. Similar results suggesting that the *PON1* LL genotype is a significant predictor of CAD were obtained by Watzinger et al. [90]. Martinelli et al. [53], in contrast, found no association between *PON1* polymorphisms and CAD or between *PON1* polymorphisms and myocardial infarction. There are five polymorphisms described in the promoter region. The C-108T/C substitution appears to have the highest influence on plasma PON1 protein levels. As a matter of fact, the low plasma PON1 protein level in PON1M55 seems to result from linkage disequilibrium with the C-108T allele. The C-108C allele provides PON1 levels which are twice as high as the C-108T allele [15].

Paraoxonase structure

Human paraoxonase is a glycoprotein composed of 354 amino acids and its molecular weight is 43 kDa. It is a six-bladed β-propeller and each blade contains four strands. In the central tunnel of the PON propeller are two calcium ions, 7.4 Å apart. The calcium ion in the central section is referred to as structural, because its dissociation leads to irreversible denaturation of the protein. The calcium in the upper section is probably the catalytic calcium. Its removal from PON1 or the addition of a metal ion chelator (EDTA) results in inactivation of the Ca^{2+}-dependent PON1 activities towards paraoxon and phenyl acetate, but does not affect the ability of PON1 to protect LDL from oxidation. These findings led to the conclusion that there may be separate active sites on PON1 for paraoxonase/arylesterase activities and for protection against LDL oxidation [1].

There is only one free sulfhydryl group, at position 284. It is essential for the enzyme's activity and the ability of PON1 to protect LDL from oxidation. Blockage of the PON1 free sulfhydryl group at Cys284 affected not only arylesterase activity, but its ability to protect LDL from oxidation as well. Moreover, replacing the PON1 sulfhydryl group with either Ala or Ser in recombinant PON1 mutants caused partial reduction of its paraoxonase/arylesterase activity and complete inhibition of its ability to protect LDL from oxidation [1]. The active site contains histidine dyad formed by His 115 and His134, which potentially cooperate in serving as a base, deprotonating a water molecule and generating the attacking hydroxide ion that produces the hydrolysis of phenyl acetate [32].

PON1 forms complexes with HDL, but not with LDL. Moreover, HDL seems to be an appropriate physiological acceptor of PON1 secreted from the liver into serum. The activity and stability of the enzyme binding to the HDL particle depends on the composition of the particle, especially the presence of apoAI; apoAI enhances PON1 activity [20].

Biological substrates for PON1

There is still uncertainty as to the exact biological substrate for paraoxonase. It seems that the enzyme serves several roles and may have a number of substrates. Most evidence that PON1 inhibits oxidized lipid formation comes from general measurements of lipid oxidation, such as thiobarbituric acid-reactive substances (TBARS), lipoperoxides, and conjugated diene formation [63].

Watson et al. [89] reported that PON1 purified from human HDL catalyzes the hydrolysis of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (Ox-PAPC). Aviram et al. [5] proposed that the substrate for PON1 is cholesterol linoleate hydroperoxide, while van Himbergen et al. [88] postulated that the substrate is oxidized linoleic acid. According to some opinions, human serum paraoxonase is also responsible for the hydrolysis of platelet-activating factor (PAF) [68]. However, further research showed that this activity, initially assigned to PON1, was probably caused by small amounts of PAF acetylhydrolase, which is located in the same lipoprotein particle as PON1. As the purification was incomplete, PAF acetylhydrolase contaminated and altered the apparent enzymatic activity of PON1 [52]. Jakubowski et al. [36] indicated that another substrate for PON1 is homocysteine thiolactone (HCTL).

It seems that PON1 has a wide range of biological substrate specificity. However, the variety of lipids postulated as substrates for PON1 may also stem from the technical difficulties in identifying and analyzing specific lipid oxidation products, as they are often unstable and depend on the type and length of the oxidation process. In the absence of an acknowledged biological substrate for PON1, its activity is measured through its degrading function towards artificial substrates, paraoxon and phenyl acetate. Recent studies indicate that arylesterase activity best reflects the antioxidant activity of PON1, although it is not directly responsible for it [71]. Furthermore, this hydrolytic activity correlates well with serum PON1 concentrations measured by ELISA [69].

The protective role of HDL-associated PON1 in atherosclerosis

Traditional risk factors of CAD include age, sex, hypercholesterolemia, arterial hypertension, diabetes, and smoking. Other risk factors, such as homocysteine, inflammatory cytokines, ox-LDL, and small dense LDL were recently added to the list [44]. A low concentration of plasma HDL-cholesterol is one of the strongest risk factors for CAD [55]. The role of HDL-associated PON1 as a potential inverse risk factor of CAD has yet to be determined. The results are so far conflicting, in part because they are based on different research methods, some being exclusively genetic, while others rely on the measurement of PON1 activity and PON1 protein mass. In the presence of increased oxidative stress, HDL may act as a shield protecting LDL and cell membranes from oxidative damage. This function of HDL may be assigned to the chemical composition of HDL, the presence of liposoluble antioxidants associated with the particle, enzymes such as platelet-activating factor acetylhydrolase (PAF-AH), lecithin/cholesterol acyl transferase (LCAT), as well as PON1 [13].

Animal studies on transgenic mice bring very convincing evidence that PON1 has an inhibitory effect on the formation of atherosclerosis. PON1-knockout mice are more prone to develop atherosclerotic lesions [81], while mice over-expressing PON1 present decreased atherosclerotic lesion development [86].

It was described in some studies that serum PON1 activity in young patients with CAD is lower than in control groups [47,80]. Sarkar et al. [76] observed low PON1 activity without decreased HDL concentration in Asian Indians suffering from premature CAD. However, Rahamani et al. [64] described a lack of significant difference in *PON1* activity between patients with CAD and controls in Iranian subjects. These differences in results may be explained by ethnic variability in *PON1* polymorphism. Finally, prospective studies gave strong evidence that paraoxonase level is an independent risk factor for atherosclerosis. The results of the Caerphilly Prospective Study on 1353 participants over a mean period of 15 years indicate that low serum PON1 activity towards paraoxon, but not towards phenyl acetate or its concentration, is a predictive risk factor for subsequent CAD events independent of all coronary risk factors [48]. Even though a certain correlation between HDL cholesterol and PON1 was noted, it was not strong enough to explain the relationship between PON1 and CAD. PON1 activity was a stronger predictor of a new coronary event in men who were at the highest quintile of risk measured by the Framingham risk equation and in those who already had clinical evidence of CAD at admission to the study. Similarly, a study by Bhattacharyya et al. on 1399 patients followed up for four years provides evidence of a link between paraoxonase and, additionally, arylesterase plasma activity as well as its functional polymorphism (Q192R) and risk of development of CAD and its acute complications [10]. But most studies indicate that PON1 activity, not its genotype inversely relates to CAD risk. Two mechanisms are currently proposed in which PON1 is thought to participate in atherosclerosis prevention: antioxidant protection and hydrolysis of homocysteine thiolactone.

Antioxidant function of PON1

There is evidence in some studies that PON1's antioxidant function begins at the level of lipoprotein (LDL and HDL) protection against oxidative modification by reactive oxygen species [5]. HDL from PON1-knockout mice could not protect LDL from oxidation, while HDL from wild-type mice caused inhibition of lipid peroxide formation [81]. Subjects with metabolic syndrome present low PON1 activity and high concentrations of lipid peroxides [78]. Navab et al. [56] showed that HDL with low PON1 activity was unable to protect LDL from oxidation. It was observed that PON1 can also prevent lipid peroxide accumulation on LDL *in vitro* and *in vivo* [49]. By hydrolyzing lipid peroxides in ox-LDL and preventing lipid peroxide accumulation on LDL, PON1 deprives them of atherogenic properties, as only ox-LDL, not native LDL, are bound to scavenger receptors and taken up by macrophages at an enhanced rate, leading to the formation of foam cells. Rozenberg et al. [72] demonstrated that PON1 decreases the accumulation of cholesterol in macrophages by inhibiting macrophage cholesterol biosynthesis. Additionally, PON1 may hydrolyze oxidized fatty acids from phospholipids in the membranes of various cells, including macrophages.

It was also described that PON1 protects not only LDL from oxidation, but also HDL in order to preserve its role in cholesterol efflux [5]. The search for possible mechanisms of PON1-induced inhibition of HDL oxidation revealed PON1-mediated hydrolysis of phospholipid peroxides and cholesterol ester hydroperoxides in oxidized HDL (esterase activity) [3]. The enzyme also reduces lipid hydroperoxides to hydroxides and presents a peroxidase-like activity, as PON1 was shown to degrade hydrogen peroxide (H_2O_2), a major reactive oxygen species produced under oxidative stress [5]. PON1 bound to HDL is additionally capable of generating lysophosphatidylcholine (LPC), which besides its inhibitory effect on cholesterol biosynthesis, also stimulates the binding of HDL to macrophages and cholesterol efflux from the cells. Lower amounts of cholesterol are accumulated in macrophages and the process of foam cell formation is retarded.

Hydrolysis of homocysteine thiolactone (HCTL)

Billecke et al. [11] showed that human serum PON1 is able to hydrolyze lactones and cyclic carbonate esters. HCTL is formed from homocysteine due to its nonspecific acti-

vation by methionyl-tRNA synthetase. The conversion of homocysteine to HCTL takes place in the cells of all tissues. Homocysteine thiolactonase is an enzyme inhibiting this reaction by hydrolyzing thiolactone back to homocysteine. Homocysteine thiolactonase was shown to have an identical amino-acid sequence as PON1 [40]. HCTL hydrolysis is Ca^{2+} dependent, like the paraoxonase/arylesterase activities of PON1. The serum of PON1-knockout mice was not able to degrade HCTL. In contrast, the serum of rabbits, known for high PON1 activity, hydrolyzed HCTL rapidly [8].

These findings suggest that the anti-atherogenic function of PON1 is more complex and is not only restricted to inhibition of LDL oxidation. The enzyme may play a protective role towards proteins, as the highly reactive HCTL is responsible for the acylation of free amino groups of protein lysine residues (protein N-homocysteinylation). This process results in the incorporation of additional thiol groups into the protein molecule, which damages its structure and impairs its physiological activities [8]. In particular, homocysteinylation of LDLs increases their susceptibility to oxidation and facilitates their uptake by macrophages. Additionally, homocysteinylated LDLs elicit an autoimmune response and increase vascular inflammation, which are known modulators of atherosclerosis. Clots formed from homocysteinylated fibrinogen present a higher resistance to lysis, contributing to a higher risk of thrombosis and vascular disease. The hypothesis of homocysteine thiolactone's pathophysiological role in atherogenesis was widely discussed by H. Jakubowski in a recent review article [37]. In humans, the homocysteine thiolactonase activity of PON1 inversely correlates with homocysteine concentration and predicts CAD [23]. Thus, perhaps the most important anti-atherogenic function of PON1 is attributable to its ability to hydrolyze HCTL, a considered risk factor of cardiovascular disease [36].

Factors influencing the level of PON1 activity

Research is being conducted to identify the environmental and lifestyle factors which may influence PON1's antioxidant activity. Several factors have been investigated. As mentioned earlier, PON1 activity is associated with its gene's polymorphisms; therefore the distribution of the *PON1*-192 polymorphism was studied in different ethnic populations. The Q genotype predominates in Caucasian populations [43], while in Africans and Asians the R genotype is more common [24]. Age also influences PON1 activity, which is lower in children [17], reaches its highest level in young adults, and then declines in older people and in women after menopause [79]. Goldhammer et al. [30] obtained a significant effect of gender on PON1 activity, with higher mean paraoxonase levels among women.

Oxidative stress status is known to have a major impact on PON1 activity. Increased oxidative stress can lead to enzyme inactivation [4]. Oxidized phospholipids in LDL and HDL particles reduce the activity of PON1. The PON1R isoform appears to be more sensitive to inactivation by these oxidized lipoproteins than the PON1Q isoform [39]. Factors that diminish oxidative stress are therefore potentially capable of improving PON1 activity.

Antioxidant flavonoids present in pomegranate juice protect PON1 in humans and atherosclerotic apolipoprotein E-deficient mice from loss of activity due to Cu^{2+}-induced oxidation [2]. Administration of pomegranate juice to a group of patients with carotid artery stenosis for one year resulted in a significant increase (by 83%) in PON1 activity, accompanied by a significant 90% reduction in basal and Cu^{2+}-induced LDL oxidation [63]. Consumption of red wine or its flavonoids quercetin or cathechin by atherosclerotic apolipoprotein E-deficient mice reduced oxidative stress and contributed to PON1's hydrolytic activity on lipid peroxides in oxidized LDL and in atherosclerotic lesions [28,33].

A beneficial effect of monoenoic acids, especially oleic acid, and their phospholipid derivatives on PON1 was described by Nguyen et al. [59]. Rats administered monounsaturated fatty acids presented higher serum PON1 activity than those administered saturated or highly polyunsaturated fatty acids [42]. Postprandial hypertriglyceridemia was found to transiently modulate serum PON1 activity and concentration [7].

Moderate alcohol intake [82] causes a 395% increase in PON1 activity, while the serum of alcoholics presents a 45% decrease in PON1 activity compared with nonalcoholics. Light alcohol intake stimulates paraoxonase by upregulating liver mRNA in rats and humans. Heavy alcohol intake inhibits gene expression and PON1 activity, independently of the PON1 polymorphism. The beneficial influence of light alcohol intake on PON1 activity is probably also caused by its ability to increase the concentration of HDL in serum [65].

Senti et al. [77] showed that *PON1* activity levels were lower in smokers than in non- or ex-smokers as a consequence of increased oxidative stress and modification of the enzyme's activity through interaction between the enzyme-free sulfhydryl group and oxidized lipids formed during LDL oxidation. Cigarette smoking is considered a classical risk factor for CVD. The LDL particles of smokers are more susceptible to oxidation and metabolism by macrophages than those of nonsmokers [31]. James et al. [38] reported that smoking is associated with reduced serum paraoxonase activity and concentration in patients with CAD.

The question has arisen about the possibility of serum PON1 activity modification by pharmacological intervention. Research was initiated on drugs capable of altering the lipoprotein metabolism, i.e. statins and fibrates. The metabolites of atorvastatin and gemfibrozil serve an antioxidant action against lipoprotein peroxidation. Therefore, they may preserve PON1 activity. After administration of atorvastatin [29], subjects had a significantly improved serum antioxidant status and increased PON1 activity. However, fenofibrate administration to rats resulted in decreased plasma levels of lipid peroxidation products and decreased PON1 activity [9]. Studies *in vitro* on cell lines delivered conflicting data [20].

Increasing evidence indicates that physical activity is one of the factors serving a protective role against CVD. In one study, serum PON1 activity was postulated as a marker for studying sedentary lifestyles because the values of

PON1 were lower in sedentary than in active individuals, showing the greatest percentage of variation between these two groups than did other metabolic syndrome, anthropometric, and biochemical marker indices [16]. Various, often opposite, effects on the cardiovascular system and plasma oxidative status are observed in regular training and in single physical activities.

Influence of physical activity on lipid profile and PON1 activity

Effects of regular physical activity

Regular physical activity has many positive effects on the cardiovascular system. It causes loss of excessive weight, increases insulin sensitivity, decreases plasma fibrinogen concentration, and lowers blood pressure. Its role in improving lipid serum profile appears mainly as an increase of HDL cholesterol and a decrease in very-low-density lipoprotein and triglyceride concentrations. There is no significant change in the concentration of LDL cholesterol in trained subjects compared with sedentary controls, but according to Sanchez-Quesada et al. [75], these particles show an increased resistance to oxidative modification. Additionally, the susceptibility of LDL to *in vitro* oxidation in trained subjects participating in a marathon run was lower than in sedentary controls [45].

As mentioned above, there is some evidence that HDL particles serve a protective role against LDL oxidation as they seem to be preferentially oxidized instead of LDL lipids. Brites et al. [13] measured HDL's ability to inhibit LDL oxidation in a group of sportsmen and a sedentary control group. In the overall population there was no significant difference between these two groups. However, when the studied population was divided according to *PON1* genotype, sportsmen belonging to the QR subgroup showed significantly increased HDL capacity to inhibit LDL oxidation than controls. Additionally, this subgroup presented higher HDL oxidizability and increased PON1 activity towards phenyl acetate, contributing to HDL protective action. In the QQ group, LDL and HDL susceptibility to oxidation, HDL antioxidant capacity, and arylesterase activity did not differ significantly between the groups. HDL particles from the *PON1*-QR genotype carriers showed a more efficient antioxidant defense against the increased oxidative stress caused by regular physical exercise which, according to the authors, could be an adaptive response to regular exercise. Somewhat different results were obtained in a retrospective cohort study performed by Manresa et al. [51] on 651 women. The authors showed that *PON1*-192 polymorphism could modulate the effect of physical activity on HDL concentration and triglyceride-rich lipoprotein catabolism as the HDL cholesterol concentration increases and the log triglyceride-to-HDL-cholesterol ratio (a marker of hyperinsulinemia among nondiabetic adults) decreases with increasing physical activity level exclusively in RR homozygous non-menopausal women. Tomas et al. [84] obtained interesting results by measuring PON1 activity after classification of the subjects according to *PON1* polymorphisms. They reported no significant change in PON1 activity due to training in the overall study group or in subjects stratified by *PON1*-55 genotypes. However, when classified according to *PON1*-192 genotypes, QQ subjects showed significantly increased PON1 activity levels after training, while a significant decrease was found in R carriers. Despite the differences in activity level of PON1 depending on the *PON1*-192 genotype, oxidized LDL decreased in all groups after training.

There are some contradictory data as to whether PON1 levels are higher in subjects who are physically active than in those leading a sedentary lifestyle. In a study by Senti et al. [77], physically active subjects had increased PON1 levels (measured as paraoxonase activity) compared with those who were inactive. They also presented lower lipid peroxide concentrations. They also measured the influence of cigarette smoking on PON1 activity depending on physical activity level. They reported that increased physical activity diminished the deleterious effects of cigarette smoking on PON1 activity. Similarly, Cabrera de Leon et al. describes higher paraoxonase activity in physically active versus sedentary subjects [16]. Evelson et al. [26] showed that aerobic physical exercise increases PON1 activity measured through arylesterase activity. Goldhammer et al. [30] described a 16.7% increase in PON1 activity following a 12-week aerobic exercise program in patients with ischemic heart disease. None of the available data explain why regular physical activity has such an impact on PON1 activity level. It seems reasonable to assume that stimulation of the endogenous antioxidant systems by repeated physical activity results in reduced oxidative stress status leading to augmentation of PON1 activity [26]. At the gene level, regular physical activity causes a repeated increase in the release of free radicals as a result of each exercise session, which hypothetically acts as a transcription inducer of endogenous antioxidant genes, particularly *PON1* [83].

However, some studies failed to show an increase in PON1 activity in subjects who practiced regular physical activity. Richter et al. [66] reported that the activity towards phenyl acetate was not significantly influenced by 12-week endurance training in a group of patients with elevated cardiovascular risk. Aerobic exercise training did not modify PON1 activity in a group of patients suffering of diabetes mellitus type 2 nor healthy subjects. Nevertheless, the plasma lipid peroxides were reduced and HDL antioxidant efficiency improved [35]. In another study, overweight/obese men with cardiovascular risk factors underwent a residential program of daily aerobic exercise and a high-fiber low-fat diet. This three-week program did not affect PON1 activity or PON1 and PON3 protein content. Although there were no changes regarding PON1 and PON3, a large reduction in LDL and lipid hydroperoxides was again noted, suggesting that the existing PON activity may protect LDL against oxidation better [67]. In a study on rats by Romani et al. [70], 10-week moderate training was shown to increase arylesterase activity and PON3 but not PON1 level. The authors found the upregulation of PON3 rather than PON1 after physical training. The results of the mentioned studies on the effect of regular as well as single physical activity are summarized in Table 1.

Effects of a single physical activity

When performing strenuous exercise, oxygen consumption in humans increases up to 20-fold. Apart from supplying working muscles with energy, excessive oxygen free radicals are

Table 1. Selected studies on the effect of regular and single exercise on PON1 activity

Author	Subjects	Geno/ Pheno-type	Regular exercise		Single exercise		
			PON activity	ARE activity	Timing	PON activity	ARE activity
Humans							
Tomas et al., 2002 [84]	17 subjects after 16-week training	overall	no change		bout of exercise	increase	
		LL, LM and MM	no change		0.5-2 h after exercise	decrease	
		QR	decrease		24 h after exercise	overall: no change	
						untrained QR: decrease	
						trained QR, QQ and untrained QQ: no change	
		QQ	increase				
Evelson et al., 2002 [26]	15 rugby players vs 15 sedentary subjects		no difference	higher			
Senti et al., 2003 [77]	421 physically active vs 917 sedentary subjects		higher				
Richter et al., 2005 [66]	after 12-week endurance exercise program in 32 patients at risk of CAD			no change			
Briviba et al., 2005 [14]	10 half-marathon and 12 marathon runners				within 20 min after exercise	no change	
Brites et al., 2006 [13]	18 trained triathlettes vs 18 sedentary men	overall	no difference	no difference			
		QR	no difference	higher			
		QQ	no difference	no difference			
Roberts et al., 2006 [67]	after 3-week aerobic exercise program in 22 obese men at risk of CAD		no change				
Cabrera de Leon et al., 2007 [16]	physically active vs sedentary (5814 subjects)		higher				
Goldhammer et al., 2007 [30]	after 12-week aerobic exercise program in 37 patients with CAD		increase				
Iborra et al., 2008 [35]	after aerobic exercise program in patients with DM t.2* and healthy controls		no change				
Tsakiris et al., 2009 [85]	10 basketball players					decrease	decrease
Rats							
Pawłowska et al., 1985 [61]	trained					decrease	
Romani et al., 2009 [70]	10 trained for 10-weeks vs 10 untrained rats		no difference	higher	trained rats 30 min after exercise	no change	decrease
					untrained rats 30 min after exercise	no change	no change

* DM t.2 – diabetes mellitus type 2.

generated, which leads to deleterious effects of augmented oxidative stress status [60]. During acute exercise, plasma antioxidant defense mechanisms are mobilized to avoid oxidative tissue damage. The total combined capacity of all individual antioxidants in plasma is reflected by the total peroxyl radical trapping antioxidant capacity of plasma (TRAP). Liu et al. [45] reported that strenuous physical activity significantly increases TRAP. There are many endogenous and exogenous plasma antioxidants acting synergistically to provide protection against free radicals. They may be divided into a group of nonenzymatic antioxidants, which includes antioxidant vitamins (α-tocopherol, ascorbic acid, and β-carotene), glutathione, the major non-protein thiol source in the cell, ubichinone (Q_{10}), α-lipoic acid, uric acid, bilirubin and certain phytochemicals (polyphenols, carotenoids, etc.), and a group of antioxidant enzymes, i.e. superoxide dysmutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). However, the role of each antioxidant in exercise has not yet been fully defined. Excessive free radical production may lead to an imbalance of pro-oxidants and antioxidant defense, which can cause oxidation of LDL particles. Sanchez-Quesada et al. [73] showed that after intense long-lasting aerobic exercise, LDL particles become more susceptible to $CuSO_4$ oxidation. Liu et al. [45] obtained similar results in 11 healthy male marathon runners. The susceptibility of their plasma LDL to *in vitro* oxidation increased after the marathon run. The change was not transient, but persisted over four days. After intense aerobic physical activity there is a higher proportion of negatively charged LDL [74], a subfraction proven to be cytotoxic and causing inflammation in endothelial cells [22].

Strenuous exercise resulting in excessive radical formation and lipid peroxidation leads to a decrease in PON1 activity. PON1 appears to be inactivated by oxidized LDL through interaction between oxidized lipids and PON1's free sulfhydryl groups [4]. Pawłowska et al. [61] showed in a study performed on rats that a single physical exercise diminished paraoxonase activity in the liver and serum. In a study on rats by Romani et al. [70], different changes in arylesterase activity were shown after a single bout of exercise depending on the level of activity. In trained rats, arylesterase activity decreased, while in untrained rats it did not change after a single bout of exercise. Paraoxonase activity did not change in either group. Similarly, Briviba et al. [14] documented no significant changes in paraoxonase assays after a half-marathon and a marathon run in healthy amateur runners. Arylesterase activities in basketball players were significantly decreased after a single bout of physical exercise. However, after one month of α-tocopherol supplementation, the plasma levels of PON1 arylesterase activities remained unchanged after single exercise, suggesting a protective role of α-tocopherol supplementation from free radical production [85]. Tomas et al. [84] observed that a bout of exercise leads to an increase and a subsequent decrease in PON1 activity in the following 2 hours and a recovery at 24 h. There was also an increase in oxidized LDL just after and 2 h after the bout of exercise, originating from augmented oxidative stress. When divided according to *PON1*-192 polymorphism, recovery of the basal PON1 activity levels at 24 h was found in QQ subjects regardless of their training status and in trained R carriers, but in untrained R carriers the activity remained decreased. In this study, the effects of acute exercise varied depending on the training status and the *PON1*-192 polymorphism, but not the *PON1*-55 polymorphism.

The results of current research show that the effects of physical activity on PON1 level are modified by environmental and lifestyle factors as well as *PON1* polymorphism. It seems that it would be contributory to precede with further research in order to establish the effects of physical activity on PON1 according to the enzyme's status, i.e. activity and concentration. If PON1 proves to be an antioxidant enzyme, it would be of great clinical significance to establish which environmental and lifestyle manipulations can affect its activity and whether the preventive and therapeutic management of patients would differ depending on their PON1 genotype.

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