Role of polyphenols and polyphenol-rich foods in the modulation of PON1 activity and expression

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ABSTRACT

Paraxonase 1 (PON1) is a HDL-associated enzyme involved in the protection of LDL and HDL-lipoproteins against lipid peroxidation. Several studies documented the capacity of polyphenols to stimulate PON1 transcription activation.

The objective of the present review is to provide the main evidence about the role and the potential mechaninsm of action of polyphenols and polyphenol-rich foods in the modulation of PON1 gene expression and activity.

A total of 76 *in vitro* and in vivo studies were included in the review. Overall, while evidence obtained *in vitro* are limited to quercetin and resveratrol, those deriving from animal models seem more convincing for a wide range of polyphenols but only at pharmacological doses. Evidence from human studies are promising but deserve more substantiation about the role of polyphenol-rich foods in the regulation of PON1 activity and expression.

Research focused on the understanding of the structure-activity relathionship of polyphenols with PON1 and on the mechanisms at the base of PON1-modulation is warranted. Well-designed human intervention studies are encourage to corroborate the findings of polyphenols also at physiological doses.

1. INTRODUCTION

Paraoxonases (PON) are a family of three enzymes named PON1, PON2 and PON3. PON1 and PON3 are predominantly synthesized in the liver and secreted into the plasma where they are associated with HDL. PON2 is not generally present in plasma but widely distributed also in cells and tissues such as liver and kidneys. Both PON2 and PON3 have antioxidant properties but lack of paraoxonase or arylesterase activities compared to PON1. Although all the three enzymes have shown anti-atherogenic activity, PON1 is considered the major protective factor against LDL and HDL oxidation [1]. Studies investigating the role of PON1 in cardiovascular disease have provided evidence that PON1 status is a better predictor of disease than PON2 and PON3. The mechanism by which PON1 protect LDL from oxidation seems to be related to its capacity to hydrolyze oxidized fatty acids derived from phospholipids, cholesterylester and triglycerides hydroperoxides that are potentially atherogenic compounds [2]. In this regard, data from several animal models of atherosclerosis demonstrated the ability of PON1 to retard and reverse atherosclerosis through a reduction of oxidized-LDL, a reduction of macrophages oxidative stress and foam cell formation, an increase in reverse cholesterol transport and an improvement of arterial function. In addition, PON1 is involved in the detoxification of homocysteine (Hcy)-thiolactone, a reactive metabolite that, through a process of N-homocysteinylation, affects the structure and function of proteins and lipoproteins including HDL [3].

Several studies support the hypothesis that a low paraxonase and lactonase activity of PON1 has been associated with an increased oxidative stress and vulnerability to plaque formation, atherosclerosis and cardiovascular diseases [1,4-9]. Moreover, alterations in circulating PON1 levels have been found in a variety of diseases including diabetes mellitus, hepatic and renal diseases, psoriasis and rheumatoid arthritis [10]. It is well know that PON1 activity can be influenced by several factors such as lifestyle and diet.

Very recently, Lou-Bonafante and colleagues critically revised the role of Mediterranean diet, and its components, in the modulation of PON1 activity [11]. The authors suggested that the Mediterranean diet, through the intake of nuts, fruit and vegetable may affect PON1 activity by protecting the enzyme from oxidative stress-induced inactivation and/or by improving its activity.

Regarding the effects of dietary constituents, several *in vivo* studies showed an increase in PON1 activity/expression following vitamin C [12-13], vitamin E [14-16], folate [13], carotenoids [17], mono- and poly- unsaturated fatty acids [18-22], selenium [21,22], and polyphenols supplementation [23-25]. Polyphenols are a heterogeneous family of bioactive compounds widely distributed in the plant kingdom. Chemically, they are characterized by the common presence of at least one aromatic ring in their structure, linked with other phenolic-, hydroxyl-, carbon- or other chemical groups [26]. Polyphenols can be classified into *flavonoids* (i.e. flavonols, flavanones, flavones, isoflavones, anthocyanidins, and flavan-3-ols) and *nonflavonoids* (i.e. condensed and hydrolysable tannins, stilbenes, phenolic acids, hydroxibenzoic and hydroxycinnamic acids and lignans) depending of their chemical structure [26,27]. They can be in the form of oligomers and polymers, or esterified with other chemical compounds (mainly sugars or organic acids), while rarely are present as aglycones (without sugar). Minor *nonflavonoids* include also derivatives of colonic microbiota metabolites such as phenylvaleric, phenyl-lactic, phenylpropionic, phenylmandelic and phenylhydracrylic acid [28]. In the last years, several studies focused on the bioactivity of polyphenols and polyphenol-rich foods. Most of the studies have been performed *in vitro* and in animal models, while limited are those in humans. In particular, observational and intervention studies documented an effect of polyphenols in the prevention/modulation of metabolic syndrome [28], endothelial dysfunction [29], hypertension [30-32] and cardiovascular and coronary diseases [33,,34]. The effects seem related to the antioxidant and anti-inflammatory activity [35,36], to vascular function modulation [33,37] and to lipid/cholesterol regulation [38]. In addition, it has been hypothesized

that polyphenols effects may be mediated also by the regulation of PON1 activity and gene expression. In the present review, we attempt to summarize the main evidence on the potential effects of polyphenols and polyphenol-rich foods on PON1 expression and activity also considering, when available, the contribution of genetic factors and the mechanisms of action. The review will focus on both *in vitro* and *in vivo* studies.

2.OVERVIEW OF *IN VITRO* AND *IN VIVO* STUDIES ON POLYPHENOLS AS MODULATORS OF PON1 EXPRESSION AND ACTIVITY

A systematic search for literature focused on the effect of polyphenols and polyphenol-rich foods in the modulation of PON1 was carried out. The search of the studies was performed based on the preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow diagram (**Figure 1**). PUBMED, ScienceDirect and Scopus databases were searched to identify pertinent articles. The systematic computerized literature search was performed from January 2000 up to November 2016. The exploration used the combination of the following terms: 'polyphenols', 'polyphenol-rich foods', 'flavonoids', 'anthocyanins' and 'paraoxonase 1'. Reference lists of the obtained papers were also searched for additional articles. The selection of the *in vitro* and *in vivo* studies was performed according to the following inclusion and exclusion criteria. *Inclusion criteria*: 1- be performed in cells and/or in animal models and/or in humans; 2-be a study evaluating PON-1 activity and/or expression; 3-be a study evaluating polyphenols and/or polyphenol-rich foods. *Exclusion criteria*: a) evaluating foods not having polyphenols as major bioactive compounds; b) performed *in vitro* but not using cells; c) written not in English; d) performed without a statistical analysis. A total of 406 records were screened and 323 out of them were excluded based on title or abstract or because duplicate papers. Eighthy-three full-text articles were obtained from the databases and from the reference lists of the obtained papers. Based on the full-text, inclusion and exclusion criteria, 7 articles were excluded while 76 papers were analyzed. Five of them combined two or three experimental

models [39-43] for a total of 81 studies. Among them, 11 were *in vitro* studies, 44 were performed on animal models and 26 were intervention studies in humans. The studies included in the review are described in **Tables 1-3** (*provided as supplemental material*) and the following details were included: polyphenol/s or polyphenol rich-food (composition was reported when available) tested, cell model, animal model or subjects selected and their characteristics, study design, type of intervention and main findings.

2.1 In vitro studies

Eleven *in vitro* studies evaluated the role of polyphenols and polyphenol-rich extracts on PON1 expression and activity (*see* Supplementary **Table 1** under "Supplemental data" in the online issue) [39,42-51].

The main polyphenols considered were resveratrol [47-51], used in two studies also as positive control [42,43], and quercetin [39,43,45]. The human hepatoma cell line Huh7 was the main cell line tested, being utilized in 9 out of the 11 *in vitro* studies considered [39,42-45,49,50]. The duration of treatments generally ranged from 24 to 48 h, while the doses of resveratrol ranged from 2 to 25 µM with the exception of one study that used also concentrations of 200 µM [48]. Gouédard *et al.*[44] and Guyot *et al.*[50] reported an increased PON1 gene expression in human hepatocyte primary cultures and in HuH7 hepatoma cell line following 48 h supplementation with 10 µM of resveratrol. Similar results were also observed by Gupta and colleagues following incubation of HepG2 cells for 48 h with 15 µM of resveratrol [51]. Curtin *et al.*[50] found the optimal induction of intracellular and extracellular PON1 activity within 2–20 µM of resveratrol while no effect was observed at doses higher than 20 µM, which in turn resulted cytotoxic leading to a decrease of cell metabolic activity.

Three studies found a dose-dependent in increase of PON1 activity [42,45,46]. Schrader and colleagues documented that Huh7 liver hepatoma cells supplemented with curcumin (1-20 µM for 48 h) increased PON1 activation in a dose-dependent manner for concentrations higher than

10 μM [42]. Khateet *et al.*[46] reported that supplementation with pomegranate juice polyphenols such as punicalagin and gallic acid (from 17.5 to 70 μg gallic acid equivalent/mL for 24 h) increased HuH7 hepatocyte-secreted PON1 arylesterase activity and the effect was dose-dependent. Garige and coworkers showed a progressive up-regulation of PON1 expression and activity following increasing quercetin supplementation (from 0 to 20 μM for 48 h) in HuH7 cell line [45].

On the whole, studies documented a different effect of polyphenols in the modulation of PON1 activity and expression dependent on the compound tested. For example, Gouédard *et al.* [45] reported that supplementation of HuH7 cells for 48 h with flavone, catechin and quercetin (10 μM) and naringenin (50 μM) resulted in a significant increase of PON-1 activity even if the maximum induction was observed only with quercetin. Schader *et al.*[39] studied the effect of different flavonoids (concentration range 1–25 μM for 48 hrs) on induction of PON1 in stably transfected Huh7 liver cells. The authors documented that genistein was the most potent flavonoid with PON1-inducing activity, followed by daidzein, luteolin, isorhamnetin and quercetin. Other flavonoids such as naringenin, cyanidin, malvidin and catechin showed only little or no PON1-inducing activity. Quercetin and resveratrol proved to increase both PON1 mRNA expression and PON1 activity when compared to the related control groups (untreated cells). However, a comparison of the findings from the different studies appear complicated due to the variability in terms of type and dose of compounds tested.

2.2 Animal studies

The effect of polyphenols and polyphenol-rich foods on animal models has been evaluated in 44 studies (*see* Supplementary **Table 2** under "Supplemental data" in the online issue) [25,39,40,42,43,52-90]. Most of them were performed on mice or rats, while two studies on hamsters [62,74]. The main polyphenols tested were quercetin [43,52,54,58,63,68,71,87] and catechin [54,58,71,87], whereas pomegranate juice was the most polyphenol-rich food used

173 [40,53,69,72] followed by vegetable oils [62,77,81]. Several studies were also performed using
174 grapevine derived products in the form of extracts or concentrate [25,67,79], red wine [54] or
175 polyphenols [64]. Numerous studies did not provide information regarding the polyphenols
176 concentration of food [25,53,56,57,69,70,78,85,87]. Other studies provided only an estimation
177 of the polyphenol content evaluated through indirect techniques, for example measuring the total
178 phenolic content by the Folin-Ciocalteau method. This lack of information makes comparison
179 among studies, even those using the same food, particularly complex. All the studies were
180 placebo-controlled. A large variability regarding the duration of the interventions (ranging from
181 2 to 20 weeks), as well as the dose of the tested compounds was observed. In spite of this, 39
182 out of 44 studies found a significant effect of supplementation with polyphenols on PON1
183 expression and/or activity. No effect was found in 5 studies [39,40,56,82]. The lack of effect
184 could be, at least in part, related to the relatively short duration of the intervention in several
185 studies (2-3 weeks) [39,42,56,88] compared to others (12-20 weeks)
186 [40,53,60,64,65,70,74,75,78,80]. For example, El-Beshbishy *et al.* [56] documented that the
187 intake of a polyphenol-rich food (500 mg kg^{-1} day) for 2 weeks did not increase serum PON1
188 activity in rats, but showed to protect LDL from oxidation hypothesizing that this effect was not
189 mediated by PON1 but a direct effect of polyphenols on LDL itself. Schrader *et al.*[39] failed to
190 observe a modulation on plasma PON1 activity in rats fed with genistein for 3 weeks, in spite
191 the same authors found genistein as the most potent inducer of PON1-transactivation at
192 concentrations higher than 5 μM in the cell model. Other than duration of the study, the lack of
193 effects on PON1 mRNA induction, protein and activity levels in the *in vivo* study may be
194 attributed to the concentrations of genistein that were much higher than those found in plasma
195 and liver of rats.

196 Some investigations revealed high variability in PON1 gene expression and activity
197 when using the same compound or food. For instance, 3 studies documented an increase in
198 PON1 expression following quercetin administration, while no effect was observed after

199 catechin [54,71,88] which was suggested to be a poor inducer of PON1 mRNA and PON1
200 transactivation. On the contrary, Hamelet *et al.*[58] found catechin, but not quercetin, able to
201 counteract homocysteine-induced impairment of PON1 gene expression and activity in liver of
202 hyperhomocysteinemic mice. These conflicting results may be at least partially explained by the
203 different animal model used or different absorption and metabolism of polyphenols.
204 Regarding the studies investigating the effect of pomegranate juice intake, a significant
205 increase in PON1 activity was found by Kaplan *et al.*[53] and Rosenblat *et al.*[69] who showed
206 a significant increase in serum PON activity in mice supplemented with pomegranate for 1 and 2
207 months, respectively. Similarly, Betanzos-Cabrera *et al.*[72] documented a significant increase
208 in liver PON1 activity after 4 months of supplementation with pomegranate juice (0.35 mmol/L)
209 used in combination with a high fat diet compared to the high fat diet alone, but not compared to
210 the control group. Conversely, Aviram *et al.*[40] showed no effect on PON1 activity in mice
211 following 14 weeks of supplementation with 6.25 or 12.5 mL pomegranate juice/day
212 (corresponding to 0.175 and 0.350 mmol of total polyphenols, respectively). This variability
213 among studies suggests that many variables (e.g. dose and duration of the studies, animal
214 model) may affect findings from different studies.
215
216 *2.3 Human studies*
217 The effect of polyphenols and polyphenol-rich foods on PON1 gene expression and activity has
218 been investigated in 26 human intervention studies (*see* Supplementary **Table 3** under
219 "Supplemental data" in the online issue) [40,41,43,91-113]. Differently from what observed in
220 *in vitro* and animal studies, the evidence of a modulation of PON1 expression and activity
221 through polyphenols is promising but needs more substantiation. In fact, half of the studies
222 reported an increase in PON1 expression and/or activity, while the other half showed no effect
223 or even a reduction following polyphenol/polyphenol-rich food supplementation. These
224 conflicting findings can be attributed to large differences among studies such as experimental

design, duration of the intervention, type of food and amount used, and last but not least, the study population selected. In this regard, 13 out of 26 studies were performed on healthy volunteers [40,41,43,91,94,96,100,101,104,107,108,110,112], whereas the remaining 13 investigations involved subjects with asymptomatic severe carotid artery stenosis [93], diabetes [95,99,103,105,106], peripheral arterial disease [97], cardiovascular risk [92,102,110,111], hemodialysis [113] and end-stage renal disease [98].

Regarding the study design, 14 studies did not include a control group evaluating the effects at baseline and post-intervention with polyphenols or polyphenol-rich foods [41,42,92,94,95,98-100,103,105,107,108,110,112]. A cross-over design was adopted in 5 studies [96,97,101,104,111], while 7 studies used a parallel design [43,91,93,102,106,109,113]. Twenty-three out of 26 trials investigated the effect of polyphenol-rich foods in the medium-long term, with a duration generally ranging from 2 to 24 weeks [40,43,91-97,99-103,105-113] except for one study that was a 3-year long term intervention [93]. Three studies evaluated also the effect of a single serving of decaffeinated green tea extracts, blackcurrant-based juice and 5 different beverages and wine on PON1 activity [41,104,108]. Compared to the long-term intervention, the acute effect of polyphenol-rich foods failed to positively modulate the activity of PON1. Only the administration of an orale dose of decaffeinated green tea extracts (455 mg equivalent to 4 cups of green tea) showed to increase PON1 activity in a group of end-stage renal disease patients.

Pomegranate and derived products were the most examined food [40,41,93,95,101,103,105,112,113], followed by berries provided mainly in the form of juice [41,104,106,108]. The amount of food varied depending on the type of product and bioactive composition. For example, the amount of pomegranate and berry juice, and red wine ranged from 50 to 250 mL/day while that of virgin and extravirgin olive oil was around 25 mL/day. Eight studies did not provide information about the food matrix composition of polyphenols [91,92,97,100,105-107,109,112], while 10 trials provided an estimation of the total polyphenols

content [40,41,94,99,101,103,104,108,111,113]. Regarding pomegranate, a significant modulation of PON1 activity was observed both in healthy and unhealthy subjects at low doses (50 mL per day). For example, Aviram *et al.*[40] showed that a 2-week consumption of pomegranate juice (PJ; 50 mL/day) significantly increased serum PON1 activity in a group of healthy subjects. The same authors reported an improvement of PON1 activity in a group of patients with asymptomatic severe carotid artery stenosis following 1-3 year of PJ intervention (50 mL/day) [93]. Rosenblat and coworkers found an increase in serum PON1 arylesterase activity after 12 weeks of PJ in a group of diabetic subjects [95]. A 4 and 6-week intervention with PJ (50 mL/day) and pomegrate extract contributed to PON1 stabilization, increased association with HDL, and enhanced catalytic activities in a group of diabetic and overweight individuals [99]. Moreover, Fuhrman and colleagues documented that a 4-week intake of PJ (50 mL/day) increased HDL-rePON1/free rePON1 ratio in diabetic subjects [103]. Only 2 studies utilized PJ at higher doses [41,105]. Rosenblat *et al.*[41] showed that 250 mL/day of PJ per 1 week improved serum PON1 lactonase activity in healthy subjects, while no effect was observed following a single dose of PJ. Finally, a 6-week intervention with PJ (200 mL/day) documented an increase in paraoxonase and aryl esterase PON1 activity in a group of diabetic subjects [105].

Two studies examined the role of pomegranate extract on PON-1 activity [112,113]. Tracy *et al.*[112] reported that a 3-month supplementation with 1g per day of pomegranate capsule extract increased serum PON-1 activity in a group of recurrent stone formers but not in the non-stone former group. Wu and colleagues [113] showed that a daily oral supplementation for 6 months of purified pomegranate extract (1g per day) improved serum PON-1 lactonase activity (but not paraoxonase and arylesterase PON-1 activity) in a group of hemodialysis patients.

The effect of berries, alone or in combination with other foods, on PON1 activity was evaluated in 6 studies [40,91,97,104,106,108]. The results have shown high variability between studies. For example, 1 week of intervention with blackcurrant juice (250 mL/day) increased

serum PON1 lactonase activity in healthy subjects [56]. In a double-blind randomized clinical trial, the intake of 240 mL/day of cranberry juice for 12 weeks increased PON1 activity in type 2 diabetic male patients [106]. Conversely, Kardum *et al.*[108] reported no effect of a 12-week intervention with polyphenol-rich chokeberry juice (100 mL/day) on PON1 activity in a group of healthy subjects. Similar findings were also observed by Huebbe and colleagues, which documented no effect on PON1 activity following a post-prandial consumption of 250 g of blackcurrant-based juice [104].

Three studies specifically evaluated the effects of virgin and extravirgin olive oil, and virgin argan oil [94,97,111]. Chercki *et al.* [94] showed that a 3-week intervention with 25 mL/day of virgin argan and extra virgin olive oil (providing about 3.3 mg/kg and 790 mg/kg of total polyphenols, respectively) increased PON1 activity in a group of healthy subjects. Farràs and coworkers [111] reported that a 3-week intervention with a functional virgin olive oil (25 mL/day providing about 500 ppm of total phenolic compounds) significantly improved PON1 activtiy in a group of hyperlipidemic individuals. On the contrary, Loued *et al.*[107] documented that a 12-week intervention with extra virgin olive oil (25 mL/day) did not affect serum PON1 activity in young and elderly healthy subjects.

A comparison of findings from animal and human studies testing the same food products appears difficult since most of the animal studies used larger doses compared to those in human trials. To give an example, the supplementation with 5-10 mL/day of pomegranate juice in mice weighting ~200 g would correspond to 1.75-3.5 L/day when consumed by a subject of 70 kg. Thus, an appropriate extrapolation of animal dose to human dose and viceversa through normalization to the body surface area should be used.

3. HYPOTHESIZED MECHANISMS OF PON1 REGULATION TROUGH POLYPHENOLS

In **Figure 2** are reported the possible mechanisms of action of polyphenols in the regulation of PON1 expression and activity. One of the most putative pathway of upregulation of PON1 could

be the activation of the AhR. The AhR is a ligand-activated transcription factor belonging to the basic helix-loop-helix/per-aryl hydrocarbon receptor nuclear translocator protein-(ARNT)-single-minded protein (Sim) family of proteins. It is classically activated by synthetic xenobiotics such as dioxins, polycyclic aromatic hydrocarbons but also polyphenols (i.e. resveratrol and quercetin). Upon ligand binding, AhR translocates to the nucleus and forms a heterodimer with the ARNT. The AhR/ARNT heterodimer binds to xenobiotic responsive elements (XREs) within the PON1 promoter (_126 and _106 region) and induces an upregulation as documented in human breast cancer and hepatoma cell line following quercitin supplementation [44,114].

Another plausible pathway could involve the transcription factor sterol regulatory element-binding protein-2 (SREBP-2) via specificity protein 1 (Sp1). SREBPs are a new class of membrane-bound transcription factors that modulate lipid homeostasis. SREBP-2 is the major regulator of cholesterol biosynthetic pathway. Recent studies have reported that quercetin may modulate PON1 gene via SREBP-2 [45,115]. In particular, it has been hypothesized that quercetin can cause PON1 translocation through SREBP-2 from the endoplasmic reticulum to the nucleus, where interacts with sterol responsive elements-like sequence on the PON1 promoter [45]. It has been reported that an interaction between Sp1 and protein kinase C (PKC) could represent a potential mechanism of PON1 transcription in HuH7 liver cells [56]. This process seems activated through a phosphorylation of PKC mediated by polyphenols (i.e. resveratrol and epigallocatechin gallate) in HepG2 cells [116].

SREBP-2 is linked to p44/42 mitogen-activated protein kinase (MAPKs) signaling cascade. MAPKs regulate the synthesis of chemokines, cytokines, adhesion molecules and prostaglandins involved in inflammation. MAPKs seem to play an important role in the regulation of PON1 activity and PON1 protein expression in Huh7 cells [117]. However, the role of polyphenols on MAPK regulation has not been deeply investigated. For example, epigallocatechin gallate has shown to inhibit interleukin-1beta-induced activation of MAPK in

human chondrocytes through the inhibition of c-Jun NH2-terminal kinase (JNK) dependent activity [118]. It is plausible that polyphenols may stimulate PON1 transcription through the activation of JNK or acting as scavenger by inhibiting ROS production and oxidation. In this regard, protocatechuic acid, the main metabolite of cyanidin-3-glucoside, was able to induce the activation of JNK in macrophages which, in turn, determined the increase of nuclear receptor Nrf2, leading to inhibition of the early ROS overproduction [119].

The intracellular signalling cascade of peroxisome proliferator-activated receptors (PPARs) pathway plays a critical role in the regulation of diverse biologic processes within the cardiovascular system, including PON activity. In this regard, recently pomegranate juice polyphenols, gallic acid and ellagic acid were demonstrated to upregulate PON1 expression and PON1 release from hepatocytes through the activation of PKA and PPARγ signaling pathway [46].

Several studies have demonstrated that also inflammation can negatively affect PON1 activity [120]. The inflammatory process is orchestrated by nuclear factor kappa-B (NF-κB), an oxidative stress sensitive transcription factor, predominantly existing in the cytoplasm in an inactive state bound to a member of the IκB family of inhibitory proteins [121]. Phosphorylation of IκB by PKC or IκB kinase (IKK) results in its degradation and dissociation from the NF-κB complex. Once NF-kB is activated, it stimulates the expression of a number of genes including those responsible for the production of citokines and interleukins. The production of citokines and interleukins such as C reactive protein, interleukin-6, interleukin-1 and tumor necrosis factor alpha have shown to reduce PON1 activity and PON1 mRNA levels in murine and human hepatoma cell lines [120]. In particular, polyphenols have been recognized to block the phosphorylation of IκB by inhibiting the activation of NF-kB and of the inflammatory cascade as documented in *in vitro* and in animal models [122,123]. Inhibitors of NF-kB translocation or the transient over-expression of IkB have shown to partially restore PON1 mRNA levels [120].

The specific chemical structure of polyphenols seems to have a role in the modulation of PON1 activity and expression. The presence of hydroxyl groups on the flavonoid rings seems to increase their affinity to re-PON1, while the glucuronidation and sulfatation processes, which mask important hydroxyl groups of the flavonoid molecules decrease their PON1-inducing activity. Flavones and flavonols (i.e. luteolin, quercetin, kaempferol and apigenin), that show different numbers of hydroxyl groups on their rings, interact with higher affinity to re-PON1 than other flavonoids. These compounds present a double bond at their C ring, making it planar due to coupling of the A and B rings' electrons, so the hydroxyl group at position 3 and the oxygen at position 4 on the C ring are on the same plane [124].

However, the rePON1-flavonoid interaction, not only depends on the number and presence of flavonoids hydroxyl groups, but also on the flavonoids substructure. In fact, although apigenin and naringenin (flavone and flavanone, respectively) have the same number of hydroxyl groups at the same positions, apigenin shows a higher affinity to PON1 than naringenin probably due to a 2,4-substituted resorcinol moiety in the A ring [124].

Recently, Atrahymovic and colleagues showed that the isoflavan glabridin could link re-PON1, despite the high hydrophobic subunit, protecting re-PON1 in a dose-dependent (1−100 μM) manner [125]. The authors hypothesized that the mechanism governing the protective effect was not related to the antioxidant action, but rather to a physical interaction with the enzyme. The bind glabridin-re-PON1 affected the enzyme structure and significantly enhanced the ability of the enzyme to remove Ox-LDL associated cholesteryl ester hydroperoxides.

The different chemical structure of polyphenols and the impact of PON1 polymorphisms in the response make it difficult to elucidate the ability of these dietary compounds to modulate PON1 activity and gene expression and the specific mechanisms involved.

4. CONCLUSIONS

379 Several observational studies outlined the importance of PON1 in the prevention of
380 atherogenesis and preservation of HDL from oxidation. The mechanism by which PON1 can
381 preserve HDL from oxidation is not completely elucidated and more research should focus on
382 this aspect. In this context, the present review provides results supporting the role of
383 polyphenols in the modulation of PON1, even if much remains to ascertain. In fact, the studies
384 performed *in vitro* are few and most of the positive effects were observed only for quercetin and
385 resveratrol at doses not comparable to those achievable *in vivo*. The evidence deriving from
386 animal models seem to be more convincing; the majority of studies found a significant effect of
387 polyphenols and polyphenol-rich foods supplementation on both PON1 expression and PON1
388 activity, even if high doses have been generally used. Regarding human trials, it has been shown
389 a positive modulation of PON1 gene expression and activity following the consumption of some
390 polyphenol-rich foods, especially pomegranate juice at the dose of 50 mL/day. However, results
391 deserve further investigations because of some methodological issues. In fact, the population
392 characteristics were different among the studies and, in addition, in most of the trials the
393 experimental designs were not placebo-controlled. This latter represents a limitation, since it is
394 not clearly possible to attribute the effects observed specifically to the polyphenol-rich food
395 treatment.
396 Future studies should be performed to understand the mechanisms by which polyphenols can
397 modulate PON1 activity, and to verify whether the effects can be obtained at physiological
398 doses. This consideration highlights the importance of using reasonable doses of foods and
399 related bioactive compounds in intervention studies as suggested by the Food and Drug
400 Administration in clinical trials for therapeutics [126]. In addition, the adoption of rigorous and
401 well controlled human intervention studies is encouraged. Moreover, since polyphenols are
402 extensively metabolized in the human gut and liver, the contribution of their metabolic products
403 should be considered.
404

5. ACKNOWLEGMENTS

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FIGURE CAPTION 1

Fig. 1 Flow chart highlighting the study selection

FIGURE CAPTION 2

Fig. 2 Polyphenols in the modulation of PON1 actvity and expression: the mechanisms of action