

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

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16 **RUNNING TITLE:** Polyphenols in the modulation of paraxonase-1

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18 **KEYWORDS**

19 Polyphenols; Polyphenol-rich foods; Paraoxonase-1; *in vitro* studies; *in vivo* studies

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27 **ABSTRACT**

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

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

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

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

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## 45 **1.INTRODUCTION**

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

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

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

75 Regarding the effects of dietary constituents, several *in vivo* studies showed an increase in  
76 PON1 activity/expression following vitamin C [12-13], vitamin E [14-16], folate [13],  
77 carotenoids [17], mono- and poly- unsaturated fatty acids [18-22], selenium [21,22], and  
78 polyphenols supplementation [23-25]. Polyphenols are a heterogeneous family of bioactive  
79 compounds widely distributed in the plant kingdom. Chemically, they are characterized by the  
80 common presence of at least one aromatic ring in their structure, linked with other phenolic-,  
81 hydroxyl-, carbon- or other chemical groups [26]. Polyphenols can be classified into *flavonoids*  
82 (i.e. flavonols, flavanones, flavones, isoflavones, anthocyanidins, and flavan-3-ols) and  
83 *nonflavonoids* (i.e. condensed and hydrolysable tannins, stilbenes, phenolic acids,  
84 hydroxibenzoic and hydroxycinnamic acids and lignans) depending of their chemical structure  
85 [26,27]. They can be in the form of oligomers and polymers, or esterified with other chemical  
86 compounds (mainly sugars or organic acids), while rarely are present as aglycones (without  
87 sugar). Minor *nonflavonoids* include also derivatives of colonic microbiota metabolites such as  
88 phenylvaleric, phenyl-lactic, phenylpropionic, phenylmandelic and phenylhydracrylic acid [28].

89 In the last years, several studies focused on the bioactivity of polyphenols and polyphenol-rich  
90 foods. Most of the studies have been performed *in vitro* and in animal models, while limited are  
91 those in humans. In particular, observational and intervention studies documented an effect of  
92 polyphenols in the prevention/modulation of metabolic syndrome [28], endothelial dysfunction  
93 [29], hypertension [30-32] and cardiovascular and coronary diseases [33,,34]. The effects seem  
94 related to the antioxidant and anti-inflammatory activity [35,36], to vascular function  
95 modulation [33,37] and to lipid/cholesterol regulation [38]. In addition, it has been hypothesized

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

## 101 **2.OVERVIEW OF *IN VITRO* AND *IN VIVO* STUDIES ON POLYPHENOLS AS** 102 **MODULATORS OF PON1 EXPRESSION AND ACTIVITY**

103 A **systematic** search for literature focused on the effect of polyphenols and polyphenol-rich  
104 foods in the modulation of PON1 was carried out. **The search of the studies was performed**  
105 **based on the preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA)**  
106 **flow diagram (Figure 1).** PUBMED, ScienceDirect and Scopus databases were searched to  
107 identify pertinent articles. **The systematic computerized literature search was performed from**  
108 **January 2000 up to November 2016.** The exploration used the combination of the following  
109 terms: ‘polyphenols’, ‘**polyphenol-rich foods**’, ‘flavonoids’, ‘anthocyanins’ and ‘paraoxonase  
110 1’. Reference lists of the obtained papers were also searched for additional articles. **The**  
111 **selection of the *in vitro* and *in vivo* studies was performed according to the following inclusion**  
112 **and exclusion criteria. *Inclusion criteria:* 1- be performed in cells and/or in animal models**  
113 **and/or in humans; 2-be a study evaluating PON-1 activity and/or expression; 3-be a study**  
114 **evaluating polyphenols and/or polyphenol-rich foods. *Exclusion criteria:* a) evaluating foods not**  
115 **having polyphenols as major bioactive compounds; b) performed *in vitro* but not using cells; c)**  
116 **written not in English; d) performed without a statistical analysis. A total of 406 records were**  
117 **screened and 323 out of them were excluded based on title or abstract or because duplicate**  
118 **papers. Eighty-three full-text articles were obtained from the databases and from the reference**  
119 **lists of the obtained papers. Based on the full-text, inclusion and exclusion criteria, 7 articles**  
120 **were excluded while 76 papers were analyzed.** Five of them combined two or three experimental

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

127

### 128 2.1 *In vitro* studies

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

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

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

147 10  $\mu\text{M}$  [42]. Khateet *et al.*[46] reported that supplementation with pomegranate juice  
148 polyphenols such as punicalagin and gallic acid (from 17.5 to 70  $\mu\text{g}$  gallic acid equivalent/mL  
149 for 24 h) increased HuH7 hepatocyte-secreted PON1 arylesterase activity and the effect was  
150 dose-dependent. Garige and coworkers showed a progressive up-regulation of PON1 expression  
151 and activity following increasing quercetin supplementation (from 0 to 20  $\mu\text{M}$  for 48 h) in  
152 HuH7 cell line [45].

153 On the whole, studies documented a different effect of polyphenols in the modulation of  
154 PON1 activity and expression dependent on the compound tested. For example, Gouédard *et al.*  
155 [45] reported that supplementation of HuH7 cells for 48 h with flavone, catechin and quercetin  
156 (10  $\mu\text{M}$ ) and naringenin (50  $\mu\text{M}$ ) resulted in a significant increase of PON-1 activity even if the  
157 maximum induction was observed only with quercetin. Schader *et al.*[39] studied the effect of  
158 different flavonoids (concentration range 1–25  $\mu\text{M}$  for 48 hrs) on induction of PON1 in stably  
159 transfected Huh7 liver cells. The authors documented that genistein was the most potent  
160 flavonoid with PON1-inducing activity, followed by daidzein, luteolin, isorhamnetin and  
161 quercetin. Other flavonoids such as naringenin, cyanidin, malvidin and catechin showed only  
162 little or no PON1-inducing activity. Quercetin and resveratrol proved to increase both PON1  
163 mRNA expression and PON1 activity when compared to the related control groups (untreated  
164 cells). However, a comparison of the findings from the different studies appear complicated due  
165 to the variability in terms of type and dose of compounds tested.

166

## 167 2.2 Animal studies

168 The effect of polyphenols and polyphenol-rich foods on animal models has been evaluated in 44  
169 studies (*see* Supplementary **Table 2** under “Supplemental data” in the online issue)  
170 [25,39,40,42,43,52-90]. Most of them were performed on mice or rats, while two studies on  
171 hamsters [62,74]. The main polyphenols tested were quercetin [43,52,54,58,63,68,71,87] and  
172 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-Ciocalteu 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<sup>-1</sup> 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

225 design, duration of the intervention, type of food and amount used, and last but not least, the  
226 study population selected. In this regard, 13 out of 26 studies were performed on healthy  
227 volunteers [40,41,43,91,94,96,100,101,104,107,108,110,112], whereas the remaining 13  
228 investigations involved subjects with asymptomatic severe carotid artery stenosis [93], diabetes  
229 [95,99,103,105,106], peripheral arterial disease [97], cardiovascular risk [92,102,110,111],  
230 hemodialysis [113] and end-stage renal disease [98].

231 Regarding the study design, 14 studies did not include a control group evaluating the effects at  
232 baseline and post-intervention with polyphenols or polyphenol-rich foods [41,42,92,94,95,98-  
233 100,103,105,107,108,110,112]. A cross-over design was adopted in 5 studies  
234 [96,97,101,104,111], while 7 studies used a parallel design [43,91,93,102,106,109,113].

235 Twenty-three out of 26 trials investigated the effect of polyphenol-rich foods in the medium-  
236 long term, with a duration generally ranging from 2 to 24 weeks [40,43,91-97,99-103,105-113]  
237 except for one study that was a 3-year long term intervention [93]. Three studies evaluated also  
238 the effect of a single serving of decaffeinated green tea extracts, blackcurrant-based juice and 5  
239 different beverages and wine on PON1 activity [41,104,108]. Compared to the long-term  
240 intervention, the acute effect of polyphenol-rich foods failed to positively modulate the activity  
241 of PON1. Only the administration of an orale dose of decaffeinated green tea extracts (455 mg  
242 equivalent to 4 cups of green tea) showed to increase PON1 activity in a group of end-stage  
243 renal disease patients.

244 Pomegranate and derived products were the most examined food  
245 [40,41,93,95,101,103,105,112,113], followed by berries provided mainly in the form of juice  
246 [41,104,106,108]. The amount of food varied depending on the type of product and bioactive  
247 composition. For example, the amount of pomegranate and berry juice, and red wine ranged  
248 from 50 to 250 mL/day while that of virgin and extravirgin olive oil was around 25 mL/day.

249 Eight studies did not provide information about the food matrix composition of polyphenols  
250 [91,92,97,100,105-107,109,112], while 10 trials provided an estimation of the total polyphenols

251 content [40,41,94,99,101,103,104,108,111,113]. Regarding pomegranate, a significant  
252 modulation of PON1 activity was observed both in healthy and unhealthy subjects at low doses  
253 (50 mL per day). For example, Aviram *et al.*[40] showed that a 2-week consumption of  
254 pomegranate juice (PJ; 50 mL/day) significantly increased serum PON1 activity in a group of  
255 healthy subjects. The same authors reported an improvement of PON1 activity in a group of  
256 patients with asymptomatic severe carotid artery stenosis following 1-3 year of PJ intervention  
257 (50 mL/day) [93]. Rosenblat and coworkers found an increase in serum PON1 arylesterase  
258 activity after 12 weeks of PJ in a group of diabetic subjects [95]. A 4 and 6-week intervention  
259 with PJ (50 mL/day) and pomegrate extract contributed to PON1 stabilization, increased  
260 association with HDL, and enhanced catalytic activities in a group of diabetic and overweight  
261 individuals [99]. Moreover, Fuhrman and colleagues documented that a 4-week intake of PJ (50  
262 mL/day) increased HDL-rePON1/free rePON1 ratio in diabetic subjects [103]. Only 2 studies  
263 utilized PJ at higher doses [41,105]. Rosenblat *et al.*[41] showed that 250 mL/day of PJ per 1  
264 week improved serum PON1 lactonase activity in healthy subjects, while no effect was observed  
265 following a single dose of PJ. Finally, a 6-week intervention with PJ (200 mL/day) documented  
266 an increase in paraoxonase and aryl esterase PON1 activity in a group of diabetic subjects [105].

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

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

277 serum PON1 lactonase activity in healthy subjects [56]. In a double-blind randomized clinical  
278 trial, the intake of 240 mL/day of cranberry juice for 12 weeks increased PON1 activity in type  
279 2 diabetic male patients [106]. Conversely, Kardum *et al.*[108] reported no effect of a 12-week  
280 intervention with polyphenol-rich chokeberry juice (100 mL/day) on PON1 activity in a group  
281 of healthy subjects. Similar findings were also observed by Huebbe and colleagues, which  
282 documented no effect on PON1 activity following a post-prandial consumption of 250 g of  
283 blackcurrant-based juice [104].

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

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

299

### 300 **3. HYPOTHESIZED MECHANISMS OF PON1 REGULATION THROUGH POLYPHENOLS**

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

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

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

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

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

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

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

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

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

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

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

377

#### 378 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



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410

411 **6. REFERENCES**

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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 activity and expression: the mechanisms of action