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Invited Review

(Poly)phenols and the regulation of NADPH oxidases

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ABSTRACT

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs) are enzymes that generate superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), and that are widely distributed in mammalian tissues. Many bioactives, especially plant (poly)phenols are being studied for their capacity to regulate NOXs. The modulation of these enzymes are of central relevance to maintain redox homeostasis and regulate cell signaling.

In in vitro and *ex vivo* assays, and in experimental animal models, different (poly)phenols are able to modulate NOX-dependent generation of $O_2^{\bullet-}$ and H_2O_2. Mechanistically, most of the known effects of (poly)phenols and of their metabolites on NOX1, NOX2, and NOX4, include the modulation of: i) the expression of the different constituent subunits, and/or ii) posttranslational modifications involved in the assembly and translocation of the protein complexes. Very limited evidence is available on a direct action of (poly)phenols on NOX active site (electron-transferring protein). Moreover, it is suggested that the regulation by (poly)phenols of systemic events, e.g. inflammation, is frequently associated with their capacity to regulate NOX activation. Although of physiological significance, more studies are needed to understand the specific targets/mechanisms of NOX regulation by (poly)phenols, and the (poly)phenol chemical structures and moieties directly involved in the observed effects. It should be kept in mind the difficulties of NOX's studies associated with the complexity of NOXs biochemistry and the methodological limitations of $O_2^{\bullet-}$ and H_2O_2 the determinations. Studies relating human ingestion of specific (poly)phenols, with NOX activity and disease conditions, are guaranteed to better understand the health importance of (poly)phenol consumption and the involvement of NOXs as biological targets.

1. Introduction

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, or NADPH-oxidases (NOXs) are enzymes that have attracted extensive research because of their ability to generate superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), and of their widespread distribution in mammalian tissues. The modulation of these enzymes can be of relevance to maintain redox homeostasis and regulate cell signaling. Many plant bioactives consumed as part of human diets are studied for their capacity to mitigate different pathophysiological conditions, largely as negative regulators of NOXs. This review summarizes and discusses current information associating the consumption of a class of bioactives, i.e. (poly)phenols, with the regulation of NOXs and their potential health effects. Besides studies in humans and experimental animal, we include select *in vitro* studies when results address mechanistic concepts of physiological relevance. Overall, the gathered information intends to give support to the possibility that NOX regulation could occur because of the ingestion of (poly)phenols.

2. Bioactives and plant (poly)phenols

Bioactives is a term that intends to group substances that the animals could need to optimize its function and sustain health. In humans, a deficiency of bioactives is not linked to known disease states. Bioactives can be ingested as components of foods, as dietary supplements or as part of pharmacological formulas. Other commonly used terms equivalent to 'bioactive' are food factor, nutraceutical, or, in many cases phytonutrient [1].

Based on the above definition thousands of compounds can be considered bioactives, including those from different origin, mechanism of action, and organ/tissue specificity. This heterogeneity results in myriads of potential biological actions. To narrow down this immense matrix, this review is focused on plant (poly)phenols, i.e., polyphenols

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Abbreviations:

LPS	lipopolysaccharide
NOX	NADPH-oxidase
PBMC	peripheral blood mononuclear cell
RFM	ring fission metabolites
SRPM	structurally related polyphenol metabolites
TNFα	tumor necrosis factor alpha

(molecules with multiple phenolic rings) and phenolics (molecules with a single phenolic ring). Plant (poly)phenols are secondary metabolites mostly involved in plant structure and responses to environmental stimuli. Thousands of chemical structures are synthetized by plants and a few hundred are part of plants consumed in human diets [2]. The chemical structures of representative plant (poly)phenols are depicted in Supplemental Material.

Within dietary (poly)phenols, we focus on those compounds that would be of physiological relevance because: i) have been reported to exert significant effects in defined organs/systems; and ii) are molecules which absorption, distribution, metabolism, and excretion (ADME) are known. Additionally, we select data from studies using purified (poly) phenols or their metabolites, and/or plant extracts well characterized in terms of (poly)phenol composition.

3. Oxidants, antioxidants, redox homeostasis and oxidative stress

Extensive research during the last decades has allowed to establish the relevance of redox reactions in biological systems. Thus, the constant consumption of oxygen in aerobic organisms, leads to the production of chemical species that result from the partial reduction of oxygen, i.e., $O_2\bullet^-$, H_2O_2 and hydroxyl radical, which are generically termed oxidants. These species can interact chemically with biological targets, e.g. proteins, lipids, nucleic acids, and carbohydrates, causing their oxidation and potential loss of their biological function. In the case of proteins, their reversible oxidation can lead to redox events, including modulation of redox signaling [3].

Antioxidant defenses protect the body from the undesired oxidation of cellular components, and from oxidant-mediated changes in cellular functions. Antioxidant strategies include enzymes able to metabolize oxidants, e.g. superoxide dismutase, catalase, and peroxidases, enzymes that repair the damage, and non-enzymatic antioxidant defenses [4]. The non-enzymatic defenses can act by: i) directly reacting with oxidants, through redox reactions, and then decreasing or cancelling oxidant reactivity; ii) directly sequestering metal transition ions involved in catalyzing oxidant production; iii) indirectly inhibiting oxidant production through their interaction with oxidant-producing enzymes; or iv) modulating pathways that lead to increased oxidant production, e.g. NF-κB pathway [5].

The maintenance of redox homeostasis is normally associated with reversible changes in oxidant production, and/or with increases in antioxidant defenses. When the capacity of the biological system to respond to the oxidant insult is overwhelmed, an irreversible damage can occur with undesirable consequences. This condition is defined as oxidative stress, and more specifically, oxidative distress [6].

4. (Poly)phenols, and (poly)phenol metabolites as biological antioxidants

(Poly)phenols can act as direct or indirect antioxidants. During decades, and still in many laboratories, the 'antioxidant capacity' of these bioactives has been studied as a mechanism to explain their health benefits [7]. The faulty interpretation of these studies is that (poly) phenols exert beneficial health effects because of their direct reaction with oxidants, i.e., direct antioxidant effects. This assumption neglects the fact that a chemical reaction between an oxidant and a (poly)phenol needs to occur at a rate that is physiologically significant for a biological system. Although thermodynamically feasible, this direct antioxidant reaction will be physiologically relevant only when a large-enough amount of the (poly)phenol is available at the proper place to generate a physiological change [8,9]. In mammals, most of the direct antioxidant actions of (poly)phenols would be only relevant in the upper digestive tract given that, once in the intestine ingested (poly)phenols are extensively metabolized (Fig. 1) [10]. Such metabolism is basically mediated by: i) phase II enzymes in enterocytes, generating structurally related (poly)phenol metabolites (SRPM); or ii) by the resident microbiota which enzymatically generates both, ring fission metabolites (RFM) and small phenolic acids. Moreover, these microbiota metabolites can be subject to additional biotransformation by phase II enzymes (Fig. 1). In brief, either the ingested (poly)phenols or these metabolites can be responsible for the indirect antioxidant actions of (poly)phenols.

In this scenario, indirect antioxidant actions can explain the observed systemic physiological effects of many (poly)phenols. For example, the actions of (poly)phenols regulating NOXs, would explain many of their beneficial health effects. The lower concentrations of (poly)phenols (and their metabolites) necessary to exert such indirect antioxidant actions are compatible with those found in cells and tissues [8,9]. In summary, the regulation of NOXs by (poly)phenols is a relevant example of their indirect antioxidant effects that are of potential relevance for human physiology.

5. NADPH-oxidase (NOX) activity

NOX are oxidoreductases that catalyse the one-electron reduction of O_2 to $O_2\bullet^-$ and the oxidation of NADPH (Fig. 2). The NOX active site (electron-transferring protein) is composed by a C-terminal cytoplasmic region that incorporates the NADPH, and N-terminal transmembrane segments containing two heme-groups that release $O_2\bullet^-$ [11].

The first NOX activity studied was that present in polymorphonuclear phagocytes, which is involved in cell bactericidal actions [12,13]. Subsequent investigations demonstrated the ubiquitous presence of NOXs in non-phagocytic cells. The NOX family known so far is constituted by seven isoforms: NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1 and DUOX2 [14]. These enzymes have been shown to participate in numerous physiological and pathological processes, beyond their well-known contribution to the immune response [15].

A way of organizing NOX isoforms is according to their activation mechanisms:

i) NOX1, NOX2, and NOX3 are activated through the assembly of several subunits in a protein complex, and its translocation to the cell membrane. Essentially, each isoform has a catalytic transmembrane subunit (NOX1, NOX2 -also called gp91phox-, or NOX3), a stabilizer membrane subunit (p22phox), and organizer/activator cytosolic subunits, e.g. NOXO1 and NOXA1 for NOX1; p47phox and p67phox for NOX2; NOXO1, NOXA1, and p67phox for NOX3. The activation of these isoforms requires the phosphorylation of the organizer subunits, and NOX2 in leucocytes requires an extra subunit, p40phox. Finally, an additional subunit, G-protein Rac, binds to the NOX complex to complete the activation of these isoforms to be able to produce $O_2\bullet^-$ [14].

ii) NOX4 is a constitutive isoform with no requirement of cytosolic subunits, being its activity solely determined by the expression of the catalytic transmembrane proteins NOX4 and p22phox [16]. To note, NOX4 releases H_2O_2 because its special topology facilitates the dismutation of the $O_2\bullet^-$ generated in the active site [17].

iii) NOX5, DUOX1 and DUOX2 are activated by calcium and protein phosphorylation [18,19], with no requirement of cytosolic

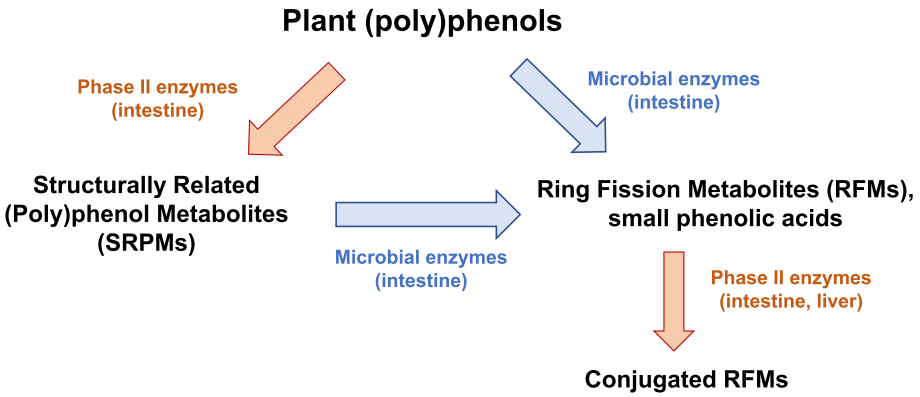

Fig. 1. Metabolism of plant (poly)phenols ingested by humans and rodents. Depending on their chemical structures, (poly)phenols can be metabolized by: i) intestinal phase II enzymes to produce metabolites that maintain most of the original structure: Structurally Related (Poly)phenol metabolites (SRPMs), e.g. glucuronidated, sulfated, and methylated conjugates of the ingested (poly)phenols; ii) microbial enzymes that are able to breakdown the benzene rings to produce Ring Fission Metabolites (RFMs), e.g. small phenolic acids and other small molecules (γ-valerolactones, C6–C3 phenylpropanoic acids, C6–C2 phenylacetic acids, C6–C1 benzoic acids, hydroxyhippuric acid, etc.). These RFMs can be substrates for intestinal phase II enzymes producing their conjugated derivatives resulting in a diverse and complex mixture of compounds, including from larger (e.g. flavonoid conjugates) to smaller molecules (e.g. hydroxyhippuric acid).

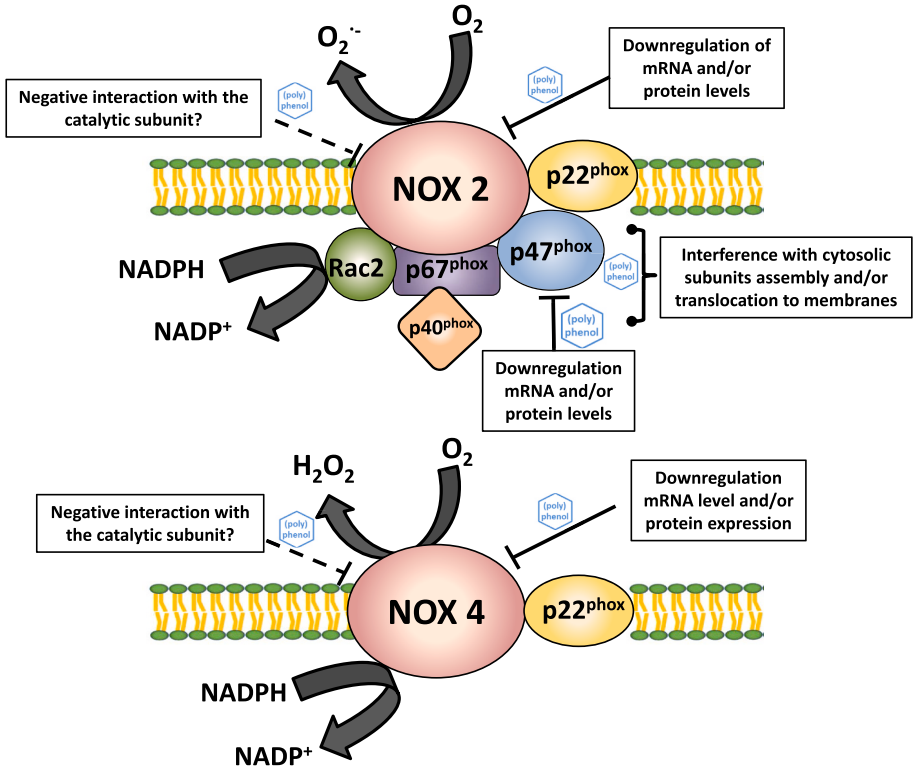

Fig. 2. Schematic structures of NOX2 and NOX4 and potential sites for their regulation by (poly)phenols. For NOX1 regulation, interactions are similar to those on NOX2 (considering differences in the respective subunits).

subunits. NOX5 produces exclusively $O_2\bullet^-$, and DUOX1 and DUOX2 release both $O_2\bullet^-$ and H_2O_2 [14].

NOX1, NOX2 and NOX4 are the most studied isoforms in physiological and pathological conditions given that they are the most widely distributed NOXs in mammalian cells and tissues. Intracellularly, NOX1 and NOX2 are located at the cell membrane, whereas NOX4 has been detected in several alternative locations, as the nuclear membrane, endoplasmic reticulum, and mitochondria [20,21].

Other isoforms have received less attention because of their highly specific localization, e.g. NOX3 is restricted to the inner ear and some fetal tissues [22–24], and DUOX1/2 to the thyroid and lungs. NOX5, although present in human tissues, has been less studied because its absence in rodents [25] limits experimental studies.

6. NADPH-oxidase (NOX) regulation

The assessment of NOXs activity is not simple, essentially because of their ubiquitous and highly reactive substrates and products. In addition, the identification of NOX regulators faces other challenges: i) the diversity of isoforms and the complexity of the enzyme structure; and ii) the experimental strategies used for measuring NOX products.

As explained above, in terms of enzyme structure and activation, NOX1 and NOX2 are substantially different from NOX4. The regulation

(activation or inhibition) of NOX1 and NOX2 can occur at several levels including: i) the expression of transmembrane and/or cytosolic subunits; ii) disruption of the assembly/translocation process and of post-translational modifications; and iii) a direct action on the protein complex (enzyme already assembled), i.e. the regulator directly acting on the protein structure and/or on the active center. In the case of NOX4, its regulation does not involve an assembly process, being only dependent on the amount of protein and/or on a direct action of the regulator on the enzyme [26].

In terms of methodologies, most studies evaluating NOXs activities use chemical probes that react with $O_2\bullet^-$ and/or H_2O_2 generating colorimetric, fluorometric, or chemiluminescent products [27]. These probes do not always present specificity for $O_2\bullet^-$ and H_2O_2, and any assayed compound can compete with the probes for their reactions with $O_2\bullet^-$ or H_2O_2. In both situations, there will be a misestimation of NOX activity that would provide unreliable information about the regulation of the enzyme. Such methodological limitations can be minimized. For example, in *in vitro* conditions, controls with superoxide dismutase and/or catalase can be included to specifically remove $O_2\bullet^-$ and H_2O_2. Additionally, working with the assayed compounds at concentrations below the range of their oxidant scavenging capacity will prevent the artifactual estimation of NOX activity [28].

From a practical point of view, measuring decreases in $O_2\bullet^-$ and H_2O_2 generation will not allow to establish how NOX activity is modified. To evaluate such mechanisms of activation/inactivation it is necessary the concurrent determination of other parameters including protein expression, activation of subunits, translocation/assembly ratio, and posttranslational modifications.

In addition, other open questions in the knowledge of NOX physiology include: i) if inhibiting NOXs is beneficial or damaging in a specific physiological condition; and ii) if the different NOXs are regulated differentially in terms of their time course of activation/inhibition, the inhibition/activation degree, and the overall coordination between inhibition/activation of the different isoforms expressed in a cell type.

7. NOX regulation by (poly)phenols

In this section we will summarize current evidence on how (poly)phenols can act on NOXs modifying their expression, and/or their capacity to generate $O_2\bullet^-$ or H_2O_2. These oxidants, when are present in excess, cause changes in redox homeostasis and oxidative damage to biomolecules, events that are closely associated with inflammation. Therefore, oxidants and inflammation are cause and consequence of multiple pathologies including for example, cardiovascular, kidney and intestinal diseases, diabetes and obesity, and neurodegeneration. Thus, by decreasing excessive production of oxidants, (poly)phenols would be mitigating pathological conditions. However, it also has to be considered that negative regulation of NOXs can have undesirable side effects, e.g. the inhibition of intestinal NOX1 was associated with increased susceptibility to intestinal infection [29].

Additionally, extreme care should be taken when interpreting results addressing the regulation of NOX by (poly)phenols outside studies in animals or humans. It must be considered that being (poly)phenols extremely reactive compounds, their presence can give rise to experimental artifacts when assayed in cell cultures and in in vitro enzyme activity determinations.

7.1. (Poly)phenols and NOX1

The regulation of NOX1 involves several steps including protein expression and enzyme assembly (Fig. 2). We will mainly focus on the effect of (poly)phenols on intestinal NOX1 given the abundance of NOX1 in this tissue, particularly in the colon.

Caco-2 (human intestinal epithelial) cells are used to mimic the intestinal epithelium exposed to food and xenobiotic substances. Treatment of Caco-2 cells, differentiated into mature enterocytes, with tumor necrosis factor-alpha (TNFα), lipopolysaccharide (LPS), bile acids, cholesterol and cholesterol oxidation products induce NOX1 gene, and/or protein expression, and/or oxidant production associated to NOX1 activity [30–36]. Several (poly)phenols attenuate NOX1 upregulation and the associated increase in oxidant production. Such effects were observed for select wine (poly)phenols, e.g. (−)-epicatechin and caffeic acid [31], anthocyanins (cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, peonidin-3-O-glucoside) and for their RFM, protocatechuic acid [32]. Also for flavanols, e.g. (−)-epicatechin [33] and epigallocatechin-3-gallate [34]; ellagic acid [35]; and curcumin [36]. In a cell model of colorectal cancer, (−)-epicatechin gallate and (−)-epigallocatechin gallate dimers not only decreased epidermal growth factor-induced NOX1 transcription, not affecting mRNA stability, but decreased the enzyme activity [37]. Molecular modeling supports that the interaction of both dimers with NOX1 flavin adenine dinucleotide (FAD)-binding pockets through hydrogen bonds and hydrophobic interactions, can be mediating NOX1 inhibition [37].

Increases in NOX1 mRNA and NOX1 protein expression triggered by LPS were suppressed in Caco-2 cells pretreated with *trans*-resveratrol [38]. In Caco-2 cell monolayers, the bile acid deoxycholic acid (DCA) causes a rapid increase in oxidant production, probably associated to NOX1 activation, although NOX1 expression is not affected [39]. NOX1 activation was decreased by (−)-epicatechin [39] and by a series of procyanidins [40,41].

In mice, long-term consumption of a high fat diet causes an increased expression of the NOX1 catalytic subunit and supplementation with both (−)-epicatechin [42], and an anthocyanin (cyanidin and delphinidin)-rich extract [32,36] mitigates the upregulation of NOX1 in the colon and/or the ileum.

Regarding the cytosolic subunits NOXO1 and NOXA1, there are no reports on their regulation by (poly)phenols in the intestine. One report showed that cyanidin-3-glucoside, at a supra-physiological concentration (50 μM), mitigated NOXA1 overexpression induced by TNFα in vascular smooth muscle cells [43]. By contrast, other report found that (−)-epicatechin administration did not affect NOXO1 expression in kidneys from N(ω)-nitro-L-arginine methyl ester (L-NAME)-hypertensive rats [44].

In summary, NOX1 activity and expression can be modulated by different (poly)phenols. This is particularly relevant for intestinal health, given that its epithelium is exposed to large amounts of both, non-metabolized (poly)phenols and their phase II- and microbiota-generated metabolites. In addition, the potential associations among (poly)phenols presence, NOX1 and intestinal barrier permeability, makes the modulation of this enzyme of high relevance for both, intestinal physiology and pathological conditions, e.g. colorectal cancer. However, this idea is mainly based on *in vitro* and rodent studies, with no experimental evidence reported in humans.

7.2. (Poly)phenols and NOX2

Given that NOX2 was the first NOX isoform described. and being abundant in phagocytic cells, early studies on the effects of (poly)phenols on NOX2 were mostly carried out during the phagocyte respiratory burst by measuring either $O_2\bullet^-$ production or O_2 consumption [45]. In experiments exposing phagocytic cells to *in vitro* stimuli, some (poly)phenols, i.e. quercetin, morin, and rutin, diminish $O_2\bullet^-$ production. However, given that (poly)phenols were tested at high concentrations (10–500 μM), their $O_2\bullet^-$ scavenging capacity complicates the interpretation of the results [46,47]. Findings that, in human polymorphonuclear leukocytes, quercetin (100 μM) inhibited the respiratory burst when evaluated as O_2 consumption, suggest an effect on NOX2 activity beyond the potential $O_2\bullet^-$ scavenging action. Authors proposed that quercetin exerts a generalized effect on the cell membrane [48], as was also proposed for (−)-epicatechin and procyanidins [49].

In terms of the molecular mechanisms involved in the regulation of phagocytic NOX2 by (poly)phenols (Fig. 2), it was described that: i)

luteolin decreases gp91phox mRNA levels and p47phox translocation to the membrane in monocytes differentiated into macrophages [50]; ii) eupalin decreases p47phox membrane translocation in human fibroblast [51]; and iii) morin decreases gp91phox and p47phox protein levels, and p47phox phosphorylation in microglial cells [52].

The effects of (poly)phenols on NOX2 were also studied in non-phagocytic cells. We will focus on endothelial cells to describe potential mechanisms of action for circulating (poly)phenols that can also be operative in other cell types. Using human umbilical vein endothelial cells (HUVEC) incubated in the presence of either (−)-epicatechin or its phase-II metabolites, 3'- and 4'-O-methyl epicatechin, the SRPM but not the non-metabolized compound, inhibited NOX2. Such inhibition was mechanistically paralleled with that afforded by apocynin [53,54]. These pioneer studies suggested that a specific chemical structure of SRPMs, i.e. the methoxy-group, is relevant for their capacity to inhibit NOX2 activity as measured by NADPH consumption.

In addition, further studies on the molecular mechanisms involved in the regulation of non-phagocytic NOX2 by (poly)phenols showed that puerarin decreases p47phox expression in vascular smooth muscle cells [55]; and that quercetin decreases gp91phox mRNA and protein levels [56].

In experimental animals, the effects of (poly)phenols on NOX2 have been mostly studied in rodent models in which variations in the expression of NOX2 subunits were triggered by unhealthy challenges. For example, in the heart and blood vessels of hypertensive rodents it was observed that: i) puerarin [57], vaccarin [58], and nobiletin [59] decreased the expression of gp91phox; and ii) curcumin [60], (−)-epicatechin [61,62] and naringin [63] decreased p47phox expression. Similar actions decreasing NOX2 subunits (gp91phox and p47phox) expression were observed for other (poly)phenols in different tissues and conditions, e.g. nobiletin in liver from high fat diet-fed rats [64] and (−)-epicatechin in kidney from high fructose diet-fed rats [65] and LPS-treated rats [66].

Results from clinical studies in healthy populations do not show clear *in vivo* effects of (poly)phenols or (poly)phenol-rich foods on phagocytic NOX2. Most published studies were carried out supplementing healthy volunteers with (poly)phenol-enriched supplements or pure (poly)phenols, isolating phagocytic cells from blood, and measuring the respiratory burst stimulated *in vitro*. Consumption of a blueberry extract by healthy individuals reduced NOX2 activity in peripheral blood mononuclear cell (PBMCs), 1–6 h after consumption [67]. The administration of soluble mate tea during 8 d decreased protein levels of p47phox in non-stimulated immune cells [68]. By contrast, studies reported that quercetin [69–71] or red wine consumption [72,73] do not modify the respiratory burst in phagocytes from healthy volunteers. A cyanidin- and delphinidin-rich extract provided together with a high-fat meal do not modify NOX2 (gp91phox) expression in PBMCs [74]. In individuals under hemodialysis, supplementation with a concentrated red grape juice (and vitamin E) reduced PBMCs NOX2 activity [75].

Acute administration of dark chocolate to smokers [76], and to patients with peripheral arterial diseases [77], decreased the serum level of a soluble derived peptide from NOX2, i.e. sNOX2-dp. However, the determination of sNOX2-dp as indicative of NOX2 activation is not widely used, which reduces the significance of these results.

In summary, the presented studies suggest the regulation of NOX2 subunits expression, and/or of the assembly of the NOX2 complex, as the main mechanisms involved in NOX2 regulation by (poly)phenols in both, phagocytic and non-phagocytic cells.

7.3. (Poly)phenols and NOX4

NOX4 is NOX isoform present in different mammalian cells. Its activity only depends on gene expression and/or protein levels of the different subunits, since NOX4 does not need an assembly process for activation (Fig. 2). We will focus our analysis on the effects of (poly)phenols on NOX4 in kidneys due to its abundance in this tissue.

In renal cells, different (poly)phenols prevented the increase in NOX4 protein levels triggered by exposure to high glucose concentrations: i) (−)-epicatechin and one of its RFM, 2,3-dihydroxybenzoic acid in proximal tubular cells [78]; ii) morin in rat glomerular mesangial cells [79]; and iii) naringin in rat podocytes [80]. However, in the last two studies supraphysiological concentrations (10–80 μM) of polyphenols were used. In terms of the effects of RFM, it was observed that hydroxyhippuric acid, benzoic acid-4-sulfate, isovanillic acid-3-sulfate, and vanillic acid- 4-sulfate, but not the non-metabolized anthocyanidins decreased the levels of NOX4 mRNA in human aortic endothelial cells (HAEC) treated with palmitate [81].

In animal models, regulation of renal NOX4 was reported for: i) puerarin, punicalagin, and naringenin in diabetic nephropathy in mice [80,82,83]; ii) puerarin, and icariin in fibrosis induced by unilateral ureteral obstruction in mice [84,85]; iii) nobiletin, galangin, and genistein in models of two-kidney, one-clip hypertension in rats [86–88]; iv) (−)-epicatechin in hypertensive rats [44,89] and rats subjected to acute endotoxemia [66]; and v) procyanidin B2 in adriamycin-induced nephrotic syndrome in mice.

To the best of our knowledge, no clinical studies are reported on the effects of (poly)phenols on renal NOX4. On the other hand, the reduction of NOX4 mRNA levels in PBMCs was reported in humans consuming an extract rich in cyanidin and delphinidin [74].

In summary, the analyzed' studies suggest the capacity of select (poly)phenols to down-regulate the expression of NOX4 subunits resulting in a decreased generation of $O_2\bullet^-$ and H_2O_2.

8. Conclusions

- Either in *in vitro* and *ex vivo* assays, or in experimental animal models, treatments with different (poly)phenols are associated with a decreased NOX-dependent generation of $O_2\bullet^-$ and/or H_2O_2.
- Most of the observed effects of (poly)phenols and their metabolites on NOXs appear related to the modulation of the expression of the different constituent subunits, and/or to posttranslational modifications needed for the assembly, translocation and consequent activation of the protein complex. Very limited evidence is available for a direct interaction of (poly)phenols and their metabolites with NOX proteins which could lead to enzyme regulation.
- The relationship among NOXs, (poly)phenols and health, has to be framed within the capacity of (poly)phenols to regulate cell signaling and maintain redox homeostasis. This associated with their capacity to mitigate disease and pathologies, e.g. those associated with the chronic inflammation that underlies, among others, cardiovascular, kidney and intestinal diseases, diabetes and obesity, and neurodegeneration. It should be considered that not always the inhibition of NOXs is positive for health, given the known physiological events regulated by NOXs, e.g. bactericidal actions of NOXs present in phagocytic cells, enhancing the activation of signaling cascades.
- The association between (poly)phenols and NOXs, although of physiological significance, still lacks many answers due to the complexity of NOXs biochemistry, including: i) the number of structurally and functionally different NOX isoforms; ii) the tissue-specific NOXs expression patterns; iii) NOXs cellular localization; and iv) the different mechanisms of NOXs regulation among the NOX family members.
- The high number and the diversity of (poly)phenols that affect NOXs expression and function, suggest that (poly)phenols can regulate NOXs by modulating systemic events, like inflammation or calcium homeostasis that are the underlying cause of NOXs upregulation.
- The use and development of appropriate methodologies to evaluate NOX activity is crucial to establish the importance of NOX regulation by (poly)phenols on their health-promoting actions.
- Further studies are needed to better define the underlined mechanisms of action, including both, the specific molecular targets for

(poly)phenol actions, and the chemical structures or moieties involved in the observed effects on NOXs.

Declaration of competing interest

All authors declare that there are no conflicts of interest to be disclosed.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2023.102927.

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