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Review

Polyphenols and mitochondria: An update on their increasingly emerging ROS-scavenging independent actions

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ABSTRACT

Polyphenols, ubiquitously present in fruits and vegetables, have been traditionally viewed as antioxidant molecules. Such contention emerged, mainly from their well established *in vitro* ability to scavenge free radicals and other reactive oxygen species (ROS). During the last decade, however, increasing evidence has emerged supporting the ability of certain polyphenols to also exert numerous ROS-scavenging independent actions. Although the latter can comprise the whole cell, particular attention has been placed on the ability of polyphenols to act, whether favorably or not, on a myriad of mitochondrial processes. Thus, some particular polyphenols are now recognized as molecules capable of modulating pathways that define mitochondrial biogenesis (*i.e.*, inducing sirtuins), mitochondrial membrane potential (*i.e.*, mitochondrial permeability transition pore opening and uncoupling effects), mitochondrial electron transport chain and ATP synthesis (*i.e.*, modulating complexes I to V activity), intra-mitochondrial oxidative status (*i.e.*, inhibiting/inducing ROS formation/removal enzymes), and ultimately mitochondrially-triggered cell death (*i.e.*, modulating intrinsic-apoptosis). The present review describes recent evidence on the ability of some polyphenols to modulate each of the formerly mentioned pathways, and discusses on how, by acting on such mitochondrial processes, polyphenols may afford protection against those mitochondrial damaging events that appear to be key in the cellular toxicity induced by various xenobiotics as well as that seen during the development of several ROS-related diseases.

Introduction

The early recognition of the apparent cause-consequence relationship that exists between the oxidation of certain macromolecules (*i.e.*, lipids, proteins and nucleic acids), induced by free radicals and other reactive oxygen species (ROS), and the alterations of their biological functions has given place to a number of hypotheses which link the occurrence of oxidative damage with the initiation and/or progression of various non-transmissible chronic diseases (NTCD)[1] [1,2]. In coherence with the former hypotheses, a large number of studies, conducted mostly in cultured cells and in animal models, demonstrated the effectiveness of a diversity of ROS-scavenging molecules to prevent the elevation of those markers of oxidative and functional damage that are believed to be key in the genesis of such diseases [1–4]. Based on such type of studies, and prompted by epidemiological data which inversely correlated the relative risk of developing cardiovascular and/or tumoral diseases with the consumption of foods rich in ascorbic acid, α-tocopherol and/or β-carotene [5], several cohort studies and randomized clinical trials were conducted to address the potential health-protecting effects of such antioxidant vitamins. Unfortunately, however, the results of most trials revealed very disappointing outcomes in terms of clinical effectiveness [6]. Moreover, in some studies, the use of supplements containing large doses of α-tocopherol and/or β-carotene was associated with a significant increment in the (for all-causes) mortality of the supplemented individuals [7], precluding since the use of such type of antioxidant supplements as a means to lower the relative risk of developing cardiovascular diseases and diverse forms of cancer.

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[1] *Abbreviations used:* MPTP, mitochondrial permeability transition pore; ETC, electron transport chain; NTCD, non-transmissible chronic diseases; NOX, NADPH oxidase; NO, nitric oxide; XO, xanthine oxidase; MAO, monoamine oxidase; Keap1, Kelch ECH-associating protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response elements; GSH, glutathione; PGC-1α, proliferator-activated receptor coactivator-1α; TFAM, transcription factor A, mitochondrial; SIRT1, silent mating type information regulation 2 homolog 1; MAC, mitochondrial apoptosis-induced channel; EGCG, epigallocatechin-3-gallate; AIF, apoptosis initiating factor; MPP^+, 1-methyl-4-phenyl pyridinium ion; PD, Parkinson's disease; AD, Alzheimer's disease; UCP-2, uncoupling protein-2; ΔΨ, transmembrane potential; ΔpH, pH gradient; OGD, oxygen glucose-deprivation; OxPhos, oxidative phosphorylation.

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As reported for the antioxidant vitamins, inverse correlations have also been established between the consumption of foods rich in polyphenols and the relative risk of developing various NTCD [3,8,9]. In view of the well-recognized ability of polyphenols to act as ROS-scavengers [10,11], such correlations have been often interpreted as an indirect support for those hypotheses that mechanistically link the occurrence of ROS-induced oxidative damage with the development of NTCD. However, as discussed below, on the basis of the often large differences that exist between the concentrations of polyphenols required to exert a ROS-scavenging action *in vitro* and those likely to be attained after their dietary consumption, the actual possibility that polyphenols act *in vivo* through a ROS-scavenging mechanism has been increasingly questioned [12]. Nonetheless, besides ROS-scavenging, polyphenols can exert an intracellular antioxidant action *via* a number of other mechanisms [4], which generally require lower *in vitro* concentrations and do not involve the stoichiometric oxidative consumption of the polyphenol molecules. Such mechanisms primarily comprise the favorable modulation of the expression and/or activity of ROS-removing [13], ROS-producing [14] and/or endogenous-antioxidant synthetizing enzymes [15]. Although these antioxidant mechanisms could concur at different levels, affecting various subcellular compartments, the reach of their modulation by polyphenols at the mitochondrial level has been recently recognized as a particularly important one [9,16,17]. The latter relates to the fact that mitochondria represent not only the major site of superoxide-generation within cells but also a major target for the oxidative action of ROS [16]. In addition to the formerly mentioned antioxidant-related mechanisms, during the last decade compelling evidence has emerged revealing the potential of certain polyphenols to modulate a diversity of other processes which pertain to the structural integrity and the metabolic functionality of the mitochondria. For instance, these compounds are now being recognized for their ability to modulate the capacity of mitochondria to undergo biogenesis (*i.e.*, inducing sirtuins) [18,19]; to control its membrane potential (*i.e.*, mitochondrial permeability transition pore (MPTP) opening and uncoupling effects) [20–25] and its electron transport chain and ATP synthesis (*i.e.*, through complexes I to V activity) [26–31]; and ultimately, to trigger cell death (*i.e.*, inhibiting/inducing intrinsic-apoptosis). The present review summarizes some of the most relevant findings concerning the potential of polyphenols to modulate each of the above-referred processes. Additionally, since some of the latter are believed to be altered in the pathogenesis of several diseases (*e.g.*, inflammatory, degenerative and tumoral) [32], and known to be directly affected by certain xenobiotics (*e.g.*, MPP^+, rotenone, statins and non-steroidal anti-inflammatory drugs) [30,31,33,34], the potential of polyphenols to exert a health-protecting effect against such type of diseases and xenobiotic induced-toxicities is also discussed. Due to the often low bioavailability and high biotransformation undergone by most polyphenols, a concentration sufficient to exert an *in vivo* modulatory effect at the mitochondrial level can be difficult to be attained. Thus, in this review, a brief discussion on some of the major current approaches to develop mitochondria-targeted polyphenols is also included.

Polyphenols: Biologically active molecules

Polyphenols are molecules whose structure contains one or more benzene rings to which at least two hydroxyl groups are attached. The term also applies, however, to some simple phenols where only one free hydroxyl group is attached to a single benzene ring. Dietary polyphenols are found in nature primarily as secondary metabolites of edible plants. Due to the large number of foods containing polyphenols and the broad scope of bioactivities attributed to these compounds (see below), polyphenols remain amongst the most intensively investigated molecules by nutrition and food chemistry researchers. Currently, over five hundred different polyphenols have been described in regularly consumed foods. The systematic study of dietary polyphenols has generated valuable information on their consumption and bioavailability and led to the development of databases on polyphenolic food composition. From a structural point of view, polyphenols can be classified under the form of two major distinguishable subgroups, namely flavonoids and non-flavonoids. In turn, each subgroup comprises six different subclasses [9]. Fig. 1 depicts the basic structural formulae of each subclass and exemplifies those polyphenols that thereafter are most cited in the text. Regarding their bioactivity, although dietary polyphenols have been considered for over five decades primarily as antioxidant molecules, they are now increasingly recognized as pleiotropic molecules, capable of exerting a diverse range of bioactivities (Fig. 2). Among the most studied bioactivities is the ability of selected polyphenols to exert anti-inflammatory, anti-atherogenic, vasodilating, anti-platelet aggregation, anti-nutritional (interfering metal absorption and carbohydrate and lipid digestion/absorption) and antimicrobial actions. Also many polyphenols are recognized for their ability to exert anti-mutagenic and anti-carcinogenic actions (by affecting cell regulation, differentiation, proliferation and apoptosis). Finally, anti-allergic, antiviral, immunomodulating, lipid profile-normalizing, estrogenic, neurosedating, insulin-potentiating and anti-angiogenic actions of some polyphenols have also been well described (Fig. 2).

Excepting for the ROS-scavenging action featured by all polyphenols, each of the before-mentioned bioactivities are quite limited to a more selected number of molecules, and for each of such compounds, what often underlies their selectivity of action appears to be their structural particularity. The latter has warranted the search for structural elements (pharmacophore) defining the health-related beneficial effects of these compounds. Nonetheless, since oxidative stress is likely to be part of the mechanisms involved in the pathogenesis of most known NTCD, whether as a primary cause or as a worsening factor, it is not surprising to see many reports where, beyond their structural differences and particularities, a large number of polyphenols appear to be able to protect against diseases which, from a physiopathological point of view, can greatly differ each other (such as cancer, cardiovascular and neurodegenerative disorders).

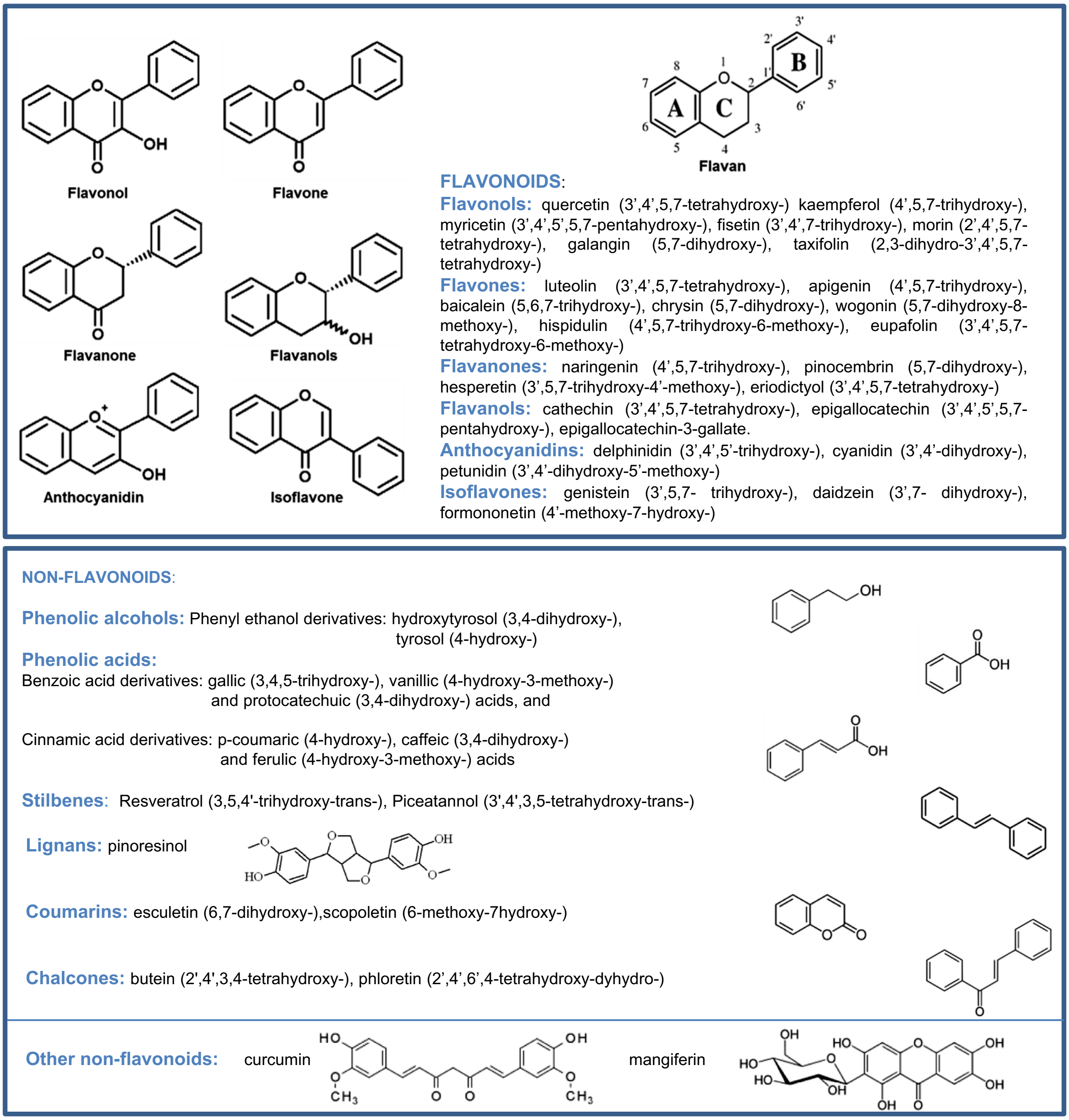
Antioxidant actions of polyphenols

In general terms, polyphenols can act as antioxidants *via* two major modes of action. The first one, referred to in Fig. 2 as actions exerted at the "ROS-removing level", comprises a direct ROS-scavenging mechanism, and a favorable modulation of the activity of ROS-removing and endogenous antioxidant-synthesizing enzymes. The second mode of antioxidant action, referred to as "ROS formation level", comprises a direct inhibitory action of polyphenols on the metal-dependent formation of free radicals (*i.e.*, superoxide and hydroxyl), and on ROS-producing enzymes (Fig. 2). A brief description on these two modes of antioxidant action is presented below.

Actions promoted at the ROS-removing level

ROS-scavenging mechanism

As mentioned before, vast evidence indicates that polyphenols can exert a direct scavenging action against free radicals or ROS in general [10,11]. Through this mechanism, polyphenols can sacrificially react with free radicals such as hydroxyl ($HO^•$), superoxide



FLAVONOIDS:

Flavonols: quercetin (3’,4’,5,7-tetrahydroxy-) kaempferol (4’,5,7-trihydroxy-), myricetin (3’,4’,5’,5,7-pentahydroxy-), fisetin (3’,4’,7-trihydroxy-), morin (2’,4’,5,7-tetrahydroxy-), galangin (5,7-dihydroxy-), taxifolin (2,3-dihydro-3’,4’,5,7-tetrahydroxy-)

Flavones: luteolin (3’,4’,5,7-tetrahydroxy-), apigenin (4’,5,7-trihydroxy-), baicalein (5,6,7-trihydroxy-), chrysin (5,7-dihydroxy-), wogonin (5,7-dihydroxy-8-methoxy-), hispidulin (4’,5,7-trihydroxy-6-methoxy-), eupafolin (3’,4’,5,7-tetrahydroxy-6-methoxy-)

Flavanones: naringenin (4’,5,7-trihydroxy-), pinocembrin (5,7-dihydroxy-), hesperetin (3’,5,7-trihydroxy-4’-methoxy-), eriodictyol (3’,4’,5,7-tetrahydroxy-)

Flavanols: cathechin (3’,4’,5,7-tetrahydroxy-), epigallocatechin (3’,4’,5’,5,7-pentahydroxy-), epigallocatechin-3-gallate.

Anthocyanidins: delphinidin (3’,4’,5’-trihydroxy-), cyanidin (3’,4’-dihydroxy-), petunidin (3’,4’-dihydroxy-5’-methoxy-)

Isoflavones: genistein (3’,5,7- trihydroxy-), daidzein (3’,7- dihydroxy-), formononetin (4’-methoxy-7-hydroxy-)

NON-FLAVONOIDS:

Phenolic alcohols: Phenyl ethanol derivatives: hydroxytyrosol (3,4-dihydroxy-), tyrosol (4-hydroxy-)

Phenolic acids:
Benzoic acid derivatives: gallic (3,4,5-trihydroxy-), vanillic (4-hydroxy-3-methoxy-) and protocatechuic (3,4-dihydroxy-) acids, and

Cinnamic acid derivatives: p-coumaric (4-hydroxy-), caffeic (3,4-dihydroxy-) and ferulic (4-hydroxy-3-methoxy-) acids

Stilbenes: Resveratrol (3,5,4'-trihydroxy-trans-), Piceatannol (3',4',3,5-tetrahydroxy-trans-)

Lignans: pinoresinol

Coumarins: esculetin (6,7-dihydroxy-),scopoletin (6-methoxy-7hydroxy-)

Chalcones: butein (2',4',3,4-tetrahydroxy-), phloretin (2’,4’,6’,4-tetrahydroxy-dyhydro-)

Other non-flavonoids: curcumin mangiferin

Fig. 1. Basic structural formulae of major sub-classes of flavonoids and non-flavonoids. Examples of those polyphenols that are most cited in the review are given. In each case, the substitutions bound to the basic structure of the subclass to which the polyphenol belongs are described within the accompanying bracket.

$(O_2^{\cdot -})$, nitric oxide (NO$^{\cdot}$), alkoxyl and peroxyl radicals, and with non-radicals such as peroxynitrite (ONOO$^-$) and hypochlorite (ClO$^-$) [10]. The ROS scavenging-mediated antioxidant activity of polyphenols is primarily attributed to the presence of those benzene ring-bound hydroxyl groups that are capable of donating either one hydrogen atom or a single electron to the ROS, stabilizing the reactive species [10,11]. As a consequence, a phenoxyl radical of the polyphenol is generated, and after reacting with a second radical, a stable quinone structure is formed [11]. It should be noted however, that *in vitro* some polyphenols (such as quercetin, myricetin, gallic acid and epigallocatechin gallate) can also act as pro-oxidants, depending on their concentration and their hydroxyl substituent pattern. For a thorough review on the ROS-scavenging properties and structure–activity relationships of flavonoids see [11]. Regarding the possible relevance of the ROS-scavenging properties of polyphenols as a mechanism capable of contributing significantly to the *in vivo* antioxidant actions of these compounds, most *in vitro* studies indicate that the concentrations (IC$_{50}$) of polyphenols required to efficiently scavenge ROS are generally within the 10–100 μM range. Such concentrations are one to two orders of magnitude greater than those reported to be reached in plasma after the ingestion of polyphenol-rich foods [9,35]. It should be noted, however, that a direct ROS scavenging action of polyphenols could be more relevant in those anatomical sites that are more directly exposed to polyphenols, such as the mucosa of the gastrointestinal tract [30,36], and eventually the skin after their deliberately direct application onto this tissue. Another condition where there is a reasonable probability that a natural polyphenol could act *in vivo* as antioxidant directly through a ROS-scavenging mechanism, is that featured by those polyphenols (*i.e.*, quercetin and epigallocatechin-3-gallate) which have been shown to enter and concentrate in a substantial manner within mitochondria.

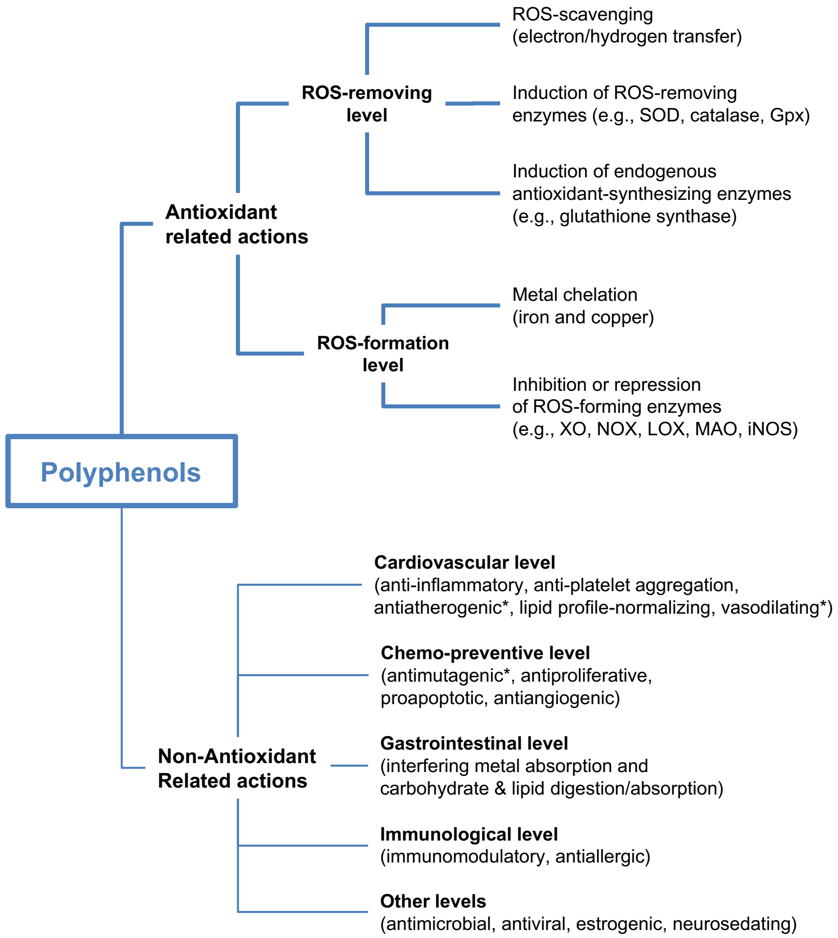


Fig. 2. Diagrammatic classification of the major known biological actions of natural polyphenols. For those actions defined as non-antioxidant related, the classification was made on the basis of the currently available evidence that points out to the overall participation of mechanisms that would not necessarily involve antioxidant actions. Nonetheless, in some cases (marked by an asterisk), the action attributed to the polyphenols would involve the participation of ROS-related as well as ROS-independent mechanisms.

Induction of ROS-removing and endogenous antioxidant-synthesizing enzymes

The major part of the antioxidant cell's defense is provided by effective enzymatic systems, among which ROS-removing enzymes like superoxide dismutase (SOD; dismutating superoxide), catalase (reducing hydrogen peroxide) and glutathione peroxidase (reducing hydrogen peroxide and lipid hydroperoxides), and endogenous-regenerating enzymes such as glutathione reductase (reducing oxidized glutathione) and thioredoxin reductase (reducing oxidized thioredoxin) are included. An increasingly recognized mechanism by which polyphenols could exert an antioxidant action *in vivo*, refers to the ability that some of these compounds have to up-regulate, via activation of the Keap1/Nrf2/ARE (Kelch ECH-associating protein 1/NF-E2-related factor 2/antioxidant response elements) signaling pathway, the expression of the above referred enzymes [13]. In addition, some polyphenols are able to induce, via the Nrf2 pathway, certain phase I and phase II enzymes which participate in the detoxification of (potentially pro-oxidant) xenobiotics [13]. The increment in the activity of all these enzymes follows an NRf2-mediated enhancement of the expression of their corresponding coding genes. Most compounds that increase Nrf2-dependent transcription are believed to act promoting a pro-oxidant action in Keap1, presumably by reacting with cysteine sulfhydryl groups, whether as electrophiles or as thio-oxidants, releasing and activating Nrf2. In addition to the above-referred ROS-removing enzymes, cells contain a number of endogenously-synthesized antioxidant molecules such as glutathione (GSH), dehydrolipoic acid and ubiquinol. Among these, the ability of polyphenols such as quercetin, kaempferol or apigenin to increase -*via* the Keap1/Nrf2/ARE pathway – the transcription of the gene coding for the glutamate cysteine ligase (or glutathione synthase, rate limiting enzyme in the synthesis of GSH), elevating the intracellular levels of this thiol, has been documented [15].

Compared to the classical direct ROS-scavenging antioxidant mechanism (generally associated with actions exerted within the 10–100 μM range), the Keap1/Nrf2/ARE indirect mechanism of action would be more efficient since it requires lower concentrations of polyphenols (generally seen within the 0.5–5 μM range) and does not depend on their direct stoichiometric consumption. Also, this system relies on an enzymatic ROS-removing catalytic mechanism which allows amplifying the initial antioxidant activity of polyphenols beyond the time-frame of their immediate occurrence in the ROS-containing milieu.

Actions promoted at the ROS-formation level

Metal-chelation

In addition to the major known enzymatic sources of superoxide generation (*e.g.*, the membrane-bound NOX, the cytosolic xanthine oxidase (XO), and the mitochondrial complexes I, II and III), the former as well as hydroxyl radicals can also be generated *via* reactions catalyzed by redox-active transition metals such as copper and iron. Within mitochondria, free iron can occur under conditions leading to an elevated production of superoxide. For instance, during the mitochondrial dysfunction featured by dopaminergic neurons in Parkinson's disease (PD), or that induced by MPP^+ or rotenone, neurotoxins employed to produce experimental models of PD. An intra-mitochondrial release of iron mediated by superoxide affects in particular the [4Fe–4S] cluster-containing enzymes aconitase (one cluster) and NADH-ubiquinone dehydrogenase (eight clusters). Some flavonoids (e.g., baicalein, quercetin, myricetin) as well as certain non-flavonoids (e.g., gallic, 2,3-dydroxybenzoic and protocatechuic acids) have been shown to be particularly active able to chelate iron and copper ions rendering them inactive to participate in free radical generating reactions. The SAR studies of polyphenols related to iron binding have been generally established for catechol and pyrogallol-containing compounds. In the case of flavonoids in particular, despite the existence of large differences in the metal-chelating capacity of different congeners, some molecular aspects have been proposed to be common and important towards their metal-chelating properties [37]. It should be noted however, that the metal-chelating property of a given polyphenol will become relevant in terms of its antioxidant potential, only if besides sequestering a given metal, the latter indeed becomes redox-inactive. Otherwise, the flavonoid–metal complex formed could catalyze the formation of free radicals, acting thus as a pro-oxidant.

Inhibition of ROS-producing enzymes

A number of polyphenols are also recognized for their ability to reduce ROS levels by directly inhibiting the major ROS-forming enzymes. An example of the latter is the well-established ability of some flavonoids (*e.g.*, apigenin, luteolin, kaempferol) to act as competitive inhibitors of XO, implicated in the oxidative injury to tissues that follows post-ischemic reperfusion conditions. Some flavonoids (such as apocynin and epicatechin) are known to inhibit NOX activity, possibly by interfering with the assembly or inhibiting the expression of its various subunits. Another ROS producing enzyme susceptible to inhibitory modulation by certain polyphenols (*e.g.*, cyanidin, cyanidin-3-glucoside, curcumin) is the mitochondrial bound monoamine oxidase (MAO). Although the inhibition of MAO is of interest for its potential to influence the development of ROS-related neurodegenerative diseases like Parkinson's and Alzheimer's, the antioxidant-related health benefits associated to MAO-inhibiting polyphenols are likely to emerge not only from such activity, but also from a direct ROS-scavenging action.

Particularly important, as a free radical-generating source, are the ETC-complexes I and III, as these enzymatic complexes are generally considered the two main producers of superoxide anions within mitochondria. Recent evidence indicates that, by acting at the ETC-complexes level, certain polyphenols would be able to potentially modulate the rate of mitochondrial superoxide production [14,30,31]. This aspect is dealt with in the subsequent Section "Electron transport chain".

Effects of polyphenols on mitochondrial processes

Mitochondrial biogenesis

Given the significant contribution of the mitochondria to cellular ATP synthesis, the high energetic demand of most cells requires these organelles to be highly dynamic, being biogenesis a particularly important process. Although mitochondrial biogenesis is regulated by a large number of coactivators and transcription factors, the proliferator-activated receptor coactivator-1α (PGC-1α) plays a role as a master regulator of such process [38], up-regulating the activity of nuclear respiratory factor 1 (NRF-1) and transcription factor A, mitochondrial (TFAM); the two latter are key for mitochondrial DNA (mtDNA) replication and transcription [38]. PGC-1α activity is highly regulated by post-translational phosphorylation and acetylation. Reversible acetylation of PGC-1α significantly modifies its transcriptional activity, and the latter seems to be primarily controlled, both *in vitro* and *in vivo*, by the silent mating type information regulation 2 homolog 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase [38]. Several polyphenols have been shown to activate SIRT1 *in vitro*, and thus are currently being thoroughly investigated as potential inducers of mitochondrial biogenesis through the deacetylation-mediated activation of PGC-1α [18] (Fig. 3). For instance, Baur et al. [39] and Lagouge et al. [40] showed that resveratrol (given as 0.04% of the diet) is effective in inducing a SIRT1-mediated deacetylation of PGC-1α, activating its transcriptional activity in mice liver and muscle, respectively. Interestingly, the latter effect of this stilbene resulted not only in an increased number of mitochondria in the studied tissues but also in an increased rate of survival [39] and improved motor function [40] of mice fed a high calorie/fat diet. More recently, the SIRT1/PGC-1α-dependent effect of resveratrol on mitochondrial biogenesis was also reported *in vitro* at 10 μM in endothelial cultured cells [41], and *in vivo* in aorta of type 2 diabetic mice (given at a 20 mg/kg dose) 41, and in cardiac tissue of double transgenic rats harboring human renin and angiotensinogen genes (given at a 800 mg/kg dose) [42].

Another polyphenol that has been well shown to effectively induce mitochondrial biogenesis is quercetin. Studies conducted by Davis et al. [43] reported on the ability of this flavonol (7 day treatment of mice with 12.5 or 25 mg/kg of weight) to induce the expression and activation of SIRT1 and PGC-1α and to increase the content of mtDNA and cytochrome c in both skeletal muscle and brain. As a possible functional significance of such increment, the authors showed a concomitant increase in the maximal physical endurance capacity of the treated animals [43]. The possible influence of quercetin on both mitochondrial biogenesis and exercise endurance has also been studied in untrained subjects [19] orally administered the flavonoid (given as 1000 mg/day for 2 weeks). In the latter study, quercetin administration increased the relative number of copies of muscle mtDNA and mRNA for mitochondrial biogenesis markers (though not to a significant extent) and induced a small (yet significant) increase in the physical performance of the untrained adult males. The result of the latter study seems to be in agreement with a former report by MacRae and Mefferd [44] where the administration of an antioxidant-rich beverage containing 300 mg of quercetin to trained cyclists, significantly increased their physical performance after 6 weeks of consumption (no effect was seen in controls given a quercetin-free antioxidant-rich beverage). Unfortunately, in this early study no mitochondrial biogenesis markers were reported.

A third polyphenol that has been extensively linked to mitochondrial biogenesis is hydroxytyrosol, a phenolic alcohol present in olives (and in extra-virgin olive oils). In studies conducted *in vitro*, Liu et al. reported on the ability of hydroxytyrosol (1–10 μM) to activate PGC-1α through SIRT1 deacetylation and to induce mitochondrial biogenesis in 3T3-L1 murine adipocytes and in ARPE-19 human retinal pigment epithelial cells [45]. More recently, the same group reported that the administration of hydroxytyrosol to rats (25 mg/kg daily during 8 weeks) stimulated the activity of PGC-1α and the expression of several markers of mitochondrial biogenesis in skeletal muscle of both treadmill-exercised and sedentary animals, and increased their exercise endurance capacity [46]. Interestingly, the authors noted that, in contrast to the mitochondrial biogenesis-inducing effect of moderate exercise, excessive exercise was associated with a decrease in PGC-1α levels. Such effect was totally prevented by the administration of hydroxytyrosol to the animals [46].

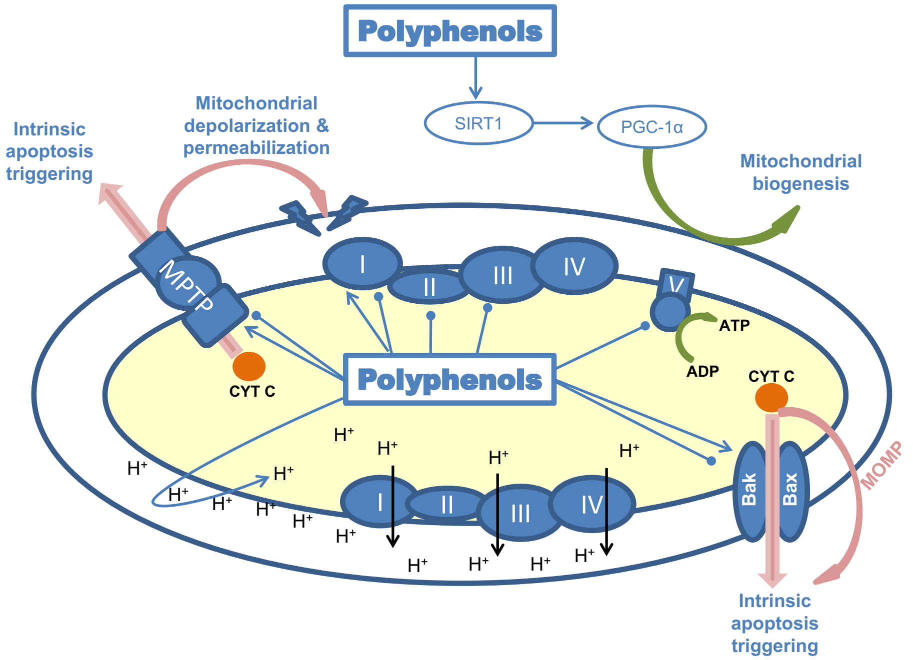


Fig. 3. Schematic representation of the main mitochondrial actions of polyphenols. Certain polyphenols, both flavonoids and non-flavonoids, are able to induce mitochondrial biogenesis by a SIRT1-activated PGC-1α-mediated mechanism, to induce or inhibit the triggering of intrinsic apoptosis by modulating the Bcl-2 family members (like Bak and Bax), to modulate (whether inhibiting or inducing) the opening of the MPTP, to uncouple oxidative phosphorylation by acting as weak protonophores, and to inhibit complex V (affecting only the ATPase activity or both the ATP synthase and ATPase activities). In addition, some polyphenols (particularly flavonoids) are able to inhibit complexes I, II or III of the ETC, although recent evidence indicates that certain flavonoids could also interact with the ETC complexes by acting as ubiquinone/ubiquinol-like molecules. This scheme does not include those actions for which a ROS-scavenging action of polyphenols appears to be the primarily underlying mechanism.

In addition to resveratrol, quercetin and hydroxytyrosol, several other polyphenols have been shown to induce mitochondrial biogenesis. For instance, isoflavones (like daidzein, genistein and formononetin) in rabbit renal proximal tubular cells [47], flavones (like baicalein, its 7-*O*-glucuronide baicalin, and wogonin) in L6 skeletal muscle cells [48] and the flavan-3-ol epigallocatechin-3-gallate in skin fibroblasts from subjects with Down's syndrome [49], all are able to activate the SIRT1/PGC-1α pathway. Interestingly, in contrast to the above-mentioned SIRT1-inducing effect of genistein [47], this isoflavone has also been reported to be able to reduce SIRT1 mRNA and protein expression, and to induce SIRT1 nuclear exclusion into the cytosol of the human prostate cancer cell lines LNCaP and PC-3 [50]. Unfortunately, PGC-1α protein levels or acetylation degree were not measured in such study. These two different SIRT1-mediated actions of genistein could relate to the possible existence of differences in the basal levels and activity of SIRT-1 or in the mechanisms that modulate its expression in normal compared to the under study cancer cells.

A greater consistency of SIRT-1-inducing effects of polyphenols is seen when the results of *in vivo* studies are compared. In a study conducted in humans, Taub et al. [51] reported that the administration of an epicatechin-rich cocoa (total epicatechin was 100 mg/day during 3 months) to patients with type 2 diabetes and heart failure stimulates mitochondrial biogenesis in (biopsies from) skeletal muscle, as expressed by an increment in SIRT1-dependent activation of PGC-1α and as an elevation in mitofilin, porin and mitochondrial complexes I and V protein levels. Also addressing the effect of a mixture of polyphenols, but this time in rats, Rehman et al. [52] reported an increment in mtDNA and in mRNA and protein levels of PGC-1α, complex IV and TFAM, and a decreased acetylation of PGC-1α in kidneys of cyclosporine A-treated rats given a polyphenol-rich green tea extract (incorporated as a 0.1% of diet with 47.2% of the polyphenols represented by EGCG). At high doses, cyclosporine A inhibits mitochondrial respiration and MPTP opening, and decreases ATP production *in vitro* and *in vivo*. Attention has also been placed on the long-term effects of curcumin, a polyphenol derived from turmeric, incorporated to the diet, on various markers of mitochondrial biogenesis. For instance, curcumin (5-month dietary supplementation at 0.05%) has been shown to up-regulate PGC-1α protein expression in the brain of senescence-accelerated mouse-prone 8 (SAMP8), a fast-aging mice strain, improving MMP and ATP levels and restoring mitochondrial fusion [53]. In agreement with the latter, dietary curcumin (3-month dietary supplementation at 0.2%) has also been shown to increase the expression of TFAM and PGC-1α in association with a greater expression of mitochondrial respiratory complexes, especially of complex IV, and an increase in ATP levels in the brain of apolipoprotein (APO) E3-targeted gene replacement mice. Comparatively, curcumin, although ineffective to increase the above-referred mitochondrial biogenesis markers, was able to significantly increase ATP levels in the higher Alzheimer-risk APOE4-mice [54]. These latter effects of curcumin are interesting since they are seen *in vivo* despite its known low bioavailability. For a recent review on the neuroprotective effects of polyphenols on brain ageing and Alzheimer's disease please see [17].

The above description shows that polyphenols belonging to a broad range of subclasses (namely, stilbenes, flavonols, phenolic alcohols, isoflavones, flavones and flava-3-ols), all are able to induce mitochondrial biogenesis. Most of such data arises from studies conducted *in vitro*, and although some have been conducted *in vivo*, the studies available are still insufficient to conclude on the actual potential that (some) polyphenols may have to induce mitochondrial biogenesis in humans. When applicable, future research should extend the relationship between a polyphenol-induced increase in parameters related to mitochondrial number and an increment of those parameters that account for the expected improvement of the corresponding tissue or organ functionality. Since so far no systematic structure–activity-relationship studies addressing the possible existence of "pharmacophores" for a mitochondrial biogenesis-inducing action of polyphenols have been reported, at this point in time it is not possible to affirm how structurally-selective this action would be. However, since a number of polyphenols that largely differ in their chemical structure have already been shown to be effective, the possibility should not be ruled out that the biogenesis-inducing ability of the so far positively tested molecules could involve at least part of the structural determinants that define the ROS-scavenging property of all the tested polyphenols.

Apoptosis

Mitochondria host the main regulators of intrinsic apoptosis, a process triggered by diverse toxic stimuli, among which increased ROS formation and extensive DNA damage are of particular relevance [55]. Intrinsic apoptosis is initiated by the release of cytochrome c into the cytosol, and occurs as a consequence of an extensive mitochondrial membrane permeabilization induced by the formation of either the mitochondrial permeability transition pore (MPTP), or the mitochondrial apoptosis-induced channel (MAC) [56]. Once in the cytosol, cytochrome c binds an adaptor protein named apoptotic protease activation factor (Apaf-1), allowing the ATP-dependent recruitment of procaspase-9 molecules. The formation of such multiprotein complex (apoptosome) allows the proteolytic self-activation of caspase 9, which in turn is responsible for the down-stream activation of the effector caspases 3 and 7 that ultimately lead to apoptosis [55]. Cellular consequences of the latter caspases activation include cell shrinkage, chromatin and nucleosomal condensation and DNA fragmentation, all of which ultimately cause cell death [55]. Physiologically, intrinsic apoptosis is controlled by several pro- and anti-apoptotic factors of the Bcl-2 protein family and by members of the inhibitors of apoptosis family (IAPs) [55]. As already mentioned in the introduction section, several polyphenols are able to trigger, and in some cases to inhibit, intrinsic apoptosis (Fig. 3). Some of those polyphenols which are able to induce intrinsic apoptosis have been studied as putative cancer-chemopreventing agents. An interesting example is quercetin, for which relatively low micromolar concentrations (5–10 μM) were reported to activate the mitochondrial apoptosis in MOLT-4 human leukemia cells and to inhibit mitochondrial activity and cellular proliferation [57]. The same report also showed that the cytotoxic effect of quercetin was synergistically potentiated by similar concentrations of ellagic acid, a distinctive ellagitannin polyphenol of muscadine grapes, suggesting an enhanced anti-carcinogenic potential for this polyphenol combination [57]. Other authors have reported on the ability of quercetin to induce typical parameters of intrinsic apoptosis in LNCaP human prostate carcinoma cells (50–200 μM) [58], MDA-MB-231 human breast cancer cells (150–300 μM) [59], U2-OS/MTX300 methotrexate-resistant human osteosarcoma cells (20–100 μM) [60], KB human epidermoid carcinoma cells and their multi-drug resistant variant KBv200 cells (IC_{50} of 19 μM) [61] and in HeLa human cervical cancer cells (50–150 μM) [62]. Of note, although in all these cell lines quercetin was shown to induce the release of cytochrome c into the cytosol and the activation of caspase-3, and in most studies the polyphenol upregulated the proapoptotic Bax protein and downregulated the antiapoptotic Bcl-2 protein, in the case of the study conducted in KB and KBv200 cells, intrinsic apoptosis induced by quercetin was shown to be Bcl-2 and Bax independent [61]. Based on the latter study, it would seem that quercetin could induce mitochondrial apoptosis through at least two different mechanisms, a classical Bcl-2/Bax dependent and an alternative Bcl-2/Bax independent; although what actions of quercetin would be underlying the latter mechanism remains to be established [61].

Another polyphenol that has been broadly studied as a potential inducer of mitochondrial apoptosis is epigallocatechin-3-gallate (EGCG). In an early study by Kazi et al. [63] EGCG was shown to induce apoptosis in LNCaP and PC-3 human prostate cancer cells through the release of cytochrome c to the cytosol and the activation of caspase 9. The authors postulated that such action of EGCG (which was shown only at a 50 μM concentration) would relate to its ability to downregulate the levels of the antiapoptotic protein hyperphosphorylated-Bcl-X_L, which prevents the release of cytochrome c and is highly expressed in prostate cancer cells [63]. In the same line, Qanungo et al. [64] showed that EGCG (50–200 μM) is also able to induce mitochondrial-dependent apoptosis in several human pancreatic cancer cell lines (though some of the tested lines were non-responsive). The latter authors report also that EGCG would induce the oligomerization of the proapoptotic Bax (one of the main components of MAC) and the depolarization of the mitochondrial membrane, and the downregulation of XIAP, a member of the IAP family. Using concentrations within the 10–100 μM range, EGCG was shown to exert mitochondrial-dependent apoptotic effects in MKN45 gastric cancer cells [65], in BEL-7402 and SMMC7721 hepatocarcinoma cells [66], and in JJ012 human chondrosarcoma cells [67]. When tested in Hep2 human laryngeal epidermoid carcinoma cells, Lee et al. [68] showed that EGCG (25–200 μM) is able to induce mitochondrially-dependent apoptosis thorough a caspase-independent mechanism, postulating that the effect of EGCG would be mediated by the release of apoptosis initiating factor (AIF) and endonuclease G into the cytosol, and their subsequent translocation into the nucleus where they induce chromatin condensation and large-scale DNA fragmentation [68]. Most of the referred reports give insights indicating that the mitochondrial apoptosis-inducing effect of EGCG would be mediated by the initial regulation of either p53 or the MAPK cascade [67].

In addition to quercetin and EGCG, several other polyphenols (tested in concentrations within the 5–100 μM range) such as curcumin [69], theaflavins [70], the polymeric catechins found in tea infusions, and baicalin, baicalein and wogonin [71], the main polyphenols of *Scutellaria baicalensis*, have been reported to induce mitochondrial apoptosis by regulating the activity and/or expression of various members of the Bcl-2 family. It is important to mention that, whenever assessed, the latter studies reported the absence of a pro-apoptotic effect of polyphenols on non-tumoral cell lines, supporting the contention that some of such polyphenols would selectively have a potential to be used as putative chemopreventive molecules.

While under conditions of abnormal cellular proliferation, a pro-apoptotic action of polyphenols can be considered a desirable effect, under other conditions, such as those where apoptosis is itself a mechanism of tissue damage, an antiapoptotic action becomes a protecting goal. Thus, despite the numerous reports on the ability of different polyphenols to induce intrinsic apoptosis, a comparable number of studies have addressed the potential of particular polyphenols to protect against chemically-induced or ischemia-triggered mitochondrial apoptosis. For instance, recently quercetin has been reported to protect *in vivo* rat cardiac tissue (when given i.p. at 10 mg/kg) [72] and *corpus cavernosum* tissue (when given s.c. at 20 mg/kg) [73] against the apoptosis-triggering effect of ischemia/reperfusion, and to protect *in vitro* (at 10 μM) against the apoptosis induced by glutamate in HT22 mouse hippocampal neuronal cells [74]. Similarly, studies using EGCG have reported on the ability of such polyphenol (added in a 5–50 μM range) to protect against several insults that induce mitochondrially-triggered apoptosis in HLEB-3 human lens epithelial cells [75] and in human dental pulp cells [76]. Several other polyphenols have been reported to protect, either *in vitro* or *in vivo*, against intrinsic apoptosis-inducing conditions. For instance, studies conducted in rats show that curcumin (given i.p. at a 30 mg/kg dose) prevents neuronal apoptosis triggered by ischemia/reperfusion [77], and that, by preventing mitochondrial apoptosis, the hydroxycinnamic acid derivative chlorogenic acid (given orally at 20 mg/kg) is able to protect against acetaminophen-induced liver toxicity [78]. Additionally, Anandhan et al. [79], assessing the effect of theaflavin against the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurodegeneration in C57BL/6 mice, found that the administration of this polyphenol (at a 10 mg/kg dose) attenuated apoptotic markers such as caspase-3, 8, 9 in *substantia nigra* and tended to normalize the accompanying behavioral alterations. On the other hand, working *in vitro*, and using concentrations as low as 0.1–1 μM, Campos-Esparza et al. [80] showed that morin and mangiferin are able to protect primary rat brain neurons against the glutamate-induced excitotoxicity and intrinsic apoptosis, and Bournival et al. [81] reported that (at 0.1 μM) resveratrol and quercetin are also effective to protect rat PC12 neuronal cells against the apoptosis induced by MPP^+. The latter study [81] showed that the antiapoptotic effect was mediated through the modulation of the Bax and Bcl-2 protein expression. Although using higher concentrations of polyphenols (50 and 100 μM), Du and Lou [82] found that catechin and proanthocyanidin B4 are able to protect rat cardiomyocytes against doxorubicin-induced mitochondrial apoptosis via a modulation of Bcl-2 protein. It should be pointed out, however, that some polyphenols (among which EGCG, resveratrol and luteolin are included), besides modulating caspase-dependent programmed cell death pathways in neuronal apoptosis, may also exert an antiapoptotic action by inhibiting AIF release from mitochondria, providing a new caspase-independent mechanism of action for polyphenols.

Although most of the referred studies point out to the modulation of several pro- and antiapoptotic proteins (such as the Bcl-2 family members and the AIF) as putative targets of the antiapoptotic action of polyphenols, it should be mentioned that most of the experimental conditions used to induce apoptosis obligatory imply triggering events that lead to an increased mitochondrial oxidative stress. Thus, based on the so far available evidence, it would be difficult to assert that the protective effects of polyphenols against the proapoptotic insults/conditions are indeed independent of an antioxidant mechanism; particularly, in those studies in which an early ROS production precedes apoptosis, a direct ROS-scavenging and/or a metal-chelating action, or a direct inhibition of free radical-forming enzymes (*e.g.*, XO, NOX, complex I) by the antiapoptotic polyphenols cannot be ruled out. Similarly, a modulating effect of the antiapoptotic polyphenols on the expression of ROS-removing or endogenous antioxidant-synthesizing enzymes could be assumed to occur under experimental conditions where apoptosis, or its inhibition, manifest within a time frame which is compatible with the operation of such antioxidant mechanisms.

Electron transport chain

Within mitochondria, oxidative phosphorylation (OxPhos) is the major source of ATP. OxPhos couples the electron transporting chain (ETC) with the ATP synthase activity in a process which involves redox reactions and transmembrane proton translocations. The ETC comprises four enzymatic complexes, namely NADH:ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II), ubiquinol:cytochrome c oxidoreductase (complex III) and cytochrome c oxidase (complex IV) [16]. The electron transferring process is initiated by the donation of two electrons from NADH or succinate to complexes I and II, respectively, allowing the subsequent reduction of ubiquinone to ubiquinol. The latter molecule reduces complex III, allowing cytochrome c reduction and thereby the subsequent reduction of complex IV. Along the ETC functioning, protons are pumped into the intermembrane space by complexes I, III and IV, building a proton gradient across the mitochondrial inner membrane which is essential for the operation of the ATP synthase. However, in addition to its proton gradient building function, the ETC continuously generates superoxide anions. The latter occurs under physiological conditions as a result of the interaction between oxygen and one-electron reduced intermediate molecules that are transiently formed mainly at the complex I and III levels. Conditions leading to a diminished activity of any of these three complexes, as it occurs in certain diseases [32] or as a result of exposure of mitochondria to known ETC inhibitors (such as rotenone for complex I or Antimycin A for complex III) or to drugs recently described to inhibit complex I, such as the non-steroidal anti-inflammatory drugs (NSAIDs) [30,31], have been clearly associated with a substantial increment in mitochondrial superoxide production [30,31,33].

In view of the known consequences associated with an alteration in the activity of the ETC, studying the potential of polyphenols to interact with some of its complexes has been warranted. In the present section we review some of the main studies addressing the potential ability of certain polyphenols to affect the activity of ETC complexes in a direct manner, whether inhibiting them or not, shortly after their addition to isolated mitochondria (Fig. 3). Focusing on such type of studies is necessary to distinguish the former type of actions (direct) from those (indirect) which most likely require longer times (and possibly the participation of extra-mitochondrial factors), such as the modulation of the activity of ETC complexes that could take place *via* changes induced in their genomic and or proteomic expression.

The systematic assessment of the potential of polyphenols to directly affect the activity of ETC complexes I and II, includes early works by the laboratory of Pardini conducted in beef heart isolated mitochondria [26,27]. For instance, in a SAR study by Hodnick et al. [26] in which the effect of 15 different polyphenols (of which only one was a non-flavonoid) on complex I activity was assessed (manometrically as NADH-dependent oxygen consumption), only four of them exhibited IC_{50} lower than 50 μM (estimated from data presented as nmol of flavonoid/mg of mitochondrial protein). The most active were myricetin and luteolin, which exhibited IC_{50} of between 10 and 15 μM, and fisetin and the chalcone butein, with an IC_{50} near 5 μM. In a subsequent study, the same research group addressed the effect of six other flavonoids, finding that robinetin, rhamnetin, eupatorin and baicalein inhibited complex I activity with IC_{50} lower than 50 μM [27]. According to their results, these authors have proposed that the absence of a hydroxyl substituent in the C-5 position increases the potency of flavonoids to inhibit mitochondrial NADH-oxidase [26,27]. Also, they observed that rhamnetin, with a 3,5,3′,4′-tetrahydroxy-7-methoxyflavonol configuration, is 3.5-fold more potent inhibitor than quercetin, with a 3,5,7,3′,4′-pentahydroxy configuration, suggesting that a methoxyl configuration on the A-ring increases the inhibitory activity when compared to a hydroxyl group at the same position. In contrast, they found that converting a hydroxyl into a methoxyl group in the B ring decreases the inhibitory activity. On the other hand, the importance of the C-2, C-3 double bond was assessed by comparing the higher potency of those flavonoids whose structure includes such feature (fisetin and quercetin) with that of their corresponding 2,3-dihydroflavonols (fustin and taxifolin), suggesting that the planarity of the chromone (pyrone) structure enhances the NADH-oxidase inhibitory activity. Similarly, the importance of the C-4 keto group was shown by the low activity of the anthocyanidins and catechins. When a series of 3,5,7-trihydroxyflavones differing in the number and position of the B ring hydroxyl groups was compared, myricetin, containing a pyrogallol configuration, was the most potent inhibitor, followed by quercetin, containing a catechol configuration, and by morin with a meta-hydroxyl group. Kaempferol, which contains a mono-hydroxyl group, and galangin, containing no B ring hydroxyls were totally devoid of inhibitory activity. Regarding the ability of polyphenols to inhibit complex II (assessed manometrically as succinate-dependent oxygen consumption), out of the 21 molecules tested in the two formerly referred studies [26,27], only myricetin, luteolin, fisetin and butein were reported to have IC_{50} values lower than 50 μM.

Regarding a possible inhibitory effect of polyphenols on complex III, Zini et al. [83] reported that resveratrol was able to inhibit this complex in mitochondria isolated from rat brain, with an IC_{50} of 5.49 nM. Although such extremely low value suggests that resveratrol could exert such action *in vivo*, the maximal inhibitory effect reported by the authors was only near 20% of the basal complex III activity. On the other hand, Dabaghi-Barbosa et al. [84] showed that the flavone hispidulin (added to rat liver mitochondria at concentrations ranging from 50 to 200 μM) inhibits state 3 respiration. Although complex III activity was not directly assessed, based on measurements of the activity of complexes I, II, I-III and II-III, the latter authors concluded that the inhibition of respiration induced by hispidulin be mediated through the inhibition of complex III. In a similar manner, Herrerías et al. [85] showed that eupafolin (also a flavone), added at concentrations between 50 and 200 μM, was able to inhibit respiration, exerting its effect primarily at the complex III level, but also affecting complexes I and II. More recently, a work from the same laboratory showed that the formerly referred inhibitory effects of hispidulin and eupafolin on complex III are also seen with the hydroxyl-free basic flavone structure, implying that such core would suffice to induce the inhibition of this complex [29].

Other researchers have addressed the potential effect of polyphenols on the functioning of the whole ETC by assessing mitochondrial respiration rather than a specific complex activity. For instance, Santos et al. [20], by means of a Clark-type oxygen electrode, showed that quercetin and various derivatives (3′-*O*-methyl-quercetin; 3,5,7,3′,4′-penta-*O*-methyl-quercetin; 3,7,3′,4′-tetra-*O*-methyl-quercetin) were able to inhibit state 3 respiration, finding that a concentration of 25 μM was needed to induce the minimal measurable effect. The most active inhibitors were quercetin and 3′-*O*-methyl-quercetin, while the tetra-*O*-methyl- and the penta-*O*-methyl-quercetin derivatives showed the lowest activity, suggesting that multiple methylation of the hydroxyl groups in quercetin reduces its mitochondrial respiration inhibitory activity. An inhibitory effect of quercetin on mitochondrial respiration was also observed by Dorta et al. [22], who reported that, when added at concentrations of 25 and 50 μM, quercetin (but not taxifolin, catechin or galangin) caused a substantial inhibition of state 3 respiration of mitochondria energized by either succinate, a complex II substrate, or glutamate + malate, complex I substrates.

Additional studies on complex I activity were conducted by Filomeni et al. [86], who by using different substrates and inhibitors of the ETC reported that the ability of kaempferol, which lacks the C-3′ hydroxyl group present in quercetin, to inhibit (50 μM) the respiration of mitochondria isolated from mouse liver would involve an inhibition of complex I activity. Similarly, Lagoa et al. [14] reported that, in addition to kaempferol, quercetin and apigenin are able to inhibit complex I activity when added directly to rat brain mitochondria in a 10–100 μM range. Of note, the inhibition induced by these flavonoids was competitively reversed by the co-addition of increasing concentrations of coenzyme Q1, suggesting that the flavonoids might affect the binding of this coenzyme to complex I. Based on the latter, the authors point out that coenzyme Q deficient mitochondria should be more prone to inhibition of complex I activity by these flavonoids, due to the observed competition between coenzyme Q and the flavonoid. Lagoa et al. [14] also suggested that the complex I-inhibitory ability of the active flavonoids may be of relevance in the context of prevention of certain neurodegenerative and cardiovascular diseases, particularly in those cases where deficiencies in complex I function are well documented. It is not clear however, how an inhibitory action of flavonoids on complex I could be of any benefit since it would only contribute to further lowering the activity of a complex which is already diminished, and that is believed to be mechanistically key in the mitochondrial dysfunction that characterizes such conditions. On the other hand, in their study Lagoa et al. [14] reported that in addition to inhibiting complex I, quercetin and kaempferol are also able to diminish the basal, the rotenone-induced and the antimycin A-induced mitochondrial production of H_2O_2. Again, it is not clear how, contrary to the increase in superoxide production expected to arise from their inhibiting complex I, these flavonoids actually lower H_2O_2 production. Since the IC_{50} (between 1 and 2 μM) for the inhibition of H_2O_2 production by quercetin and kaempferol is, as reported in the work of Lagoa et al. [14], at least one order of magnitude lower than that reported in the same study for these flavonoids to act as complex I inhibitors, at this point in time, it would seem that the overall effect that these flavonoids would exert on the mitochondrial ETC functioning be that of lowering ROS production (basal and inhibitor-induced), and that such effect would be most likely mediated by a direct ROS-scavenging mechanism.

While all the above-discussed studies focused primarily on describing the ability of some polyphenols to inhibit the ETC complexes, other researchers have focused on their potential to protect against the consequences associated with the diminished activity of the ETC complexes that either follows the exposure of mitochondria to certain xenobiotics known to inhibit complexes I or III, or appears to be key in the mitochondrial dysfunction associated with some neurodegenerative diseases. For instance, during the last few years evidence has accumulated showing that oxidative stress and mitochondrial dysfunction would be key and early events in the development of the gastrointestinal (GI) toxicity induced by NSAIDs. Aiming at exploring the potential that polyphenols may have to protect against such deleterious effects of NSAIDs, our laboratory found that APPE, a standardized apple peel polyphenol extract (given orally at 175 mg/kg, of which 58% were quercetin glycosides), was highly effective in protecting *in vivo* against the functional (loss of GI barrier function), macroscopic (gastric ulceration and bleeding), microscopic (GI mucosal necrosis) and inflammatory (neutrophil infiltration) damage induced by indomethacin [36]. *In vitro* studies conducted in Caco-2 cells demonstrated that the latter agent induced an early inhibition of mitochondrial complex I that was followed by an increment in mitochondrial superoxide production, a subsequent drop in ATP, and an elevation in xanthine oxidase activity and in various extra-mitochondrial oxidative stress parameters [30]. Interestingly, while the extra-mitochondrial oxidative effects of indomethacin were significantly prevented by allopurinol (a xanthine oxidase inhibitor) and by tempol (a SOD mimetic), only APPE (added at 0.1 μg/ml) was found to be able to also fully protect against the inhibition of complex I and the drop in ATP [30]. Such findings revealed that the mitochondrial and cellular protection induced by the polyphenols present in APPE, rather than being limited to a ROS-scavenging action, critically depended on their ability to prevent the indomethacin-induced inhibition of complex I. More recently, the inhibition of complex I induced by indomethacin was extended to four other NSAIDs (diclofenac, piroxicam, ibuprofen and aspirin) and demonstrated to be totally prevented by 10 μM quercetin, whether co-added to Caco-2 cells or to mitochondria isolated from rat duodenal epithelium [31]. Since adding increasing concentrations of ubiquinone to NSAIDs-incubated mitochondria totally reversed complex I inhibition, we have proposed that the protection afforded by coenzyme Q and quercetin would involve displacing the NSAIDs molecules from the ubiquinone binding site. Furthermore, we have demonstrated that in the absence of indomethacin and ubiquinone, quercetin concentration dependently (5–10 μM) allowed complex I functioning and the NADH-dependent transfer of electrons from such complex to complex III [31]. The ubiquinone/ubiquinol-like behavior of quercetin suggested by the latter results is coherent with the presence of a complex I-susceptible to be reduced *o*-quinone structure in the B ring of the flavonoid, which may be partially formed during the dissolution of quercetin in an alkaline media that briefly precedes its final dilution in neutral media. In fact, we have observed that when quercetin is directly dissolved in ethanol, a condition under which no quinone is expected to be formed, the flavonoid fails to serve as a complex I electron acceptor (unpublished data). Elucidating the chemical identity of the quercetin-derived species that would actually interact with complexes I and III is necessary and can be of major importance to define the potential that quercetin and other flavonoids might have to favorably modulate the activity of some of the ETC complexes. Carrasco-Pozo et al. [87] reported that, relative to quercetin, much higher concentrations of resveratrol (15-fold) and rutin (50-fold) were required to prevent the drop in ATP and the alteration in mitochondrial membrane potential induced by indomethacin in Caco-2 cells; a result which suggests that only quercetin would be able to effectively protect against the inhibition of complex I induced by such NSAID.

In research aimed at understanding the pathophysiology of Parkinson's disease and at evaluating the potential of different molecules as drug candidates, different cell and animal models have been used chemically inducing lesions by means of neurotoxins like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or its active metabolite MPP^+, by 6-hydroxydopamine and by rotenone. All such agents share the ability to inhibit complex I, and thereby represent a good experimental approach to the study of PD, a pathological condition typically featuring a diminished complex I activity [32]. Regarding the effect of polyphenols on the latter models of PD, quercetin, added *in vitro* at concentrations of 0.1 μM [81,88] or 40 μM [89], has been shown to offer protection against the dopaminergic neuronal death induced by MPP^+ or by its precursor in neuronal cultures; a neuroprotective effect of quercetin (given at 100 mg/kg) was also seen in laboratory animals exposed to MPP^+ [90], and in rotenone-exposed rats (given at 50 mg/kg of quercetin) [34]. Nonetheless, the neuroprotection against the complex I-inhibiting toxins is not limited to quercetin, but has been also shown for other polyphenols such as resveratrol [88], catechin, chrysin, puerarin, naringenin, genistein [89], EGCG [91], certain curcuminoids [17] and various anthocyanins [92]. In these type of studies, evaluating the extent to which the neuroprotective effect that each polyphenol exerts against the cytotoxicity induced by the latter agents is linked to an early and direct protection against the inhibition of complex I induced by the neurotoxins used is of major importance. Regarding the latter, worth to note is the recent work by Karuppagounder et al. [34] in which quercetin was shown to effectively protect against the neurotoxicity induced by rotenone in a PD animal model. The authors observed that the neuroprotection afforded by quercetin (seen at both cellular and functional levels) was substantiated by a significant reversal (by up-regulation) of the rotenone-induced decrease in mitochondrial complex I activity.

Oxidative stress, as known, represents a key triggering event (and also a major expression) of the downstream consequences associated with complex I inhibition in the pathogenesis of PD [32]. Therefore, at this point in time, it is not possible to distinguish the extent to which the neuroprotection exerted by the polyphenols relates to the ability that some of these compounds may have to either prevent or reverse complex I inhibition (clearly for the case of quercetin), from the less selective ability that all tested polyphenols share in terms of being able to act as neuro-protectants through a direct ROS-scavenging mechanism.

Mitochondrial membrane potential

According to the chemi-osmotic theory of OxPhos coupling, a proton-impermeable inner mitochondrial membrane is a condition *sine qua non* for the energy transduction between the ETC and the synthesis of ATP at the level of complex V. Additionally, since intrinsic apoptosis is triggered upon the release of some intramitochondrial factors into the cytosol, the maintenance of proper inner and outer mitochondrial membrane structure is vital for cell survival. Despite the latter, two important processes exist under physiological conditions that are able to induce membrane permeabilization and disruption. The first one, which primarily dissipates the membrane potential, is the transport of protons from the intermembrane space into the matrix through uncoupling proteins (particularly uncoupling protein-2; UCP-2), and the second one, which involves a major permeabilization of both inner and outer mitochondrial membranes, is the membrane permeability transition induced by the opening of the multiprotein complex MPTP [56]. In addition to such mechanisms, some xenobiotics are known to induce either proton-permeabilization of the inner membrane (*e.g.*, dinitrophenol, CCCP and FCCP) or MPTP opening, while others are known to be able to inhibit the latter (*e.g.*, sanglifehrin A and cyclosporine A). As with all the mitochondrial processes mentioned in the previous sections, some polyphenols have also been reported to be able to induce mitochondrial uncoupling (*i.e.*, favoring the direct translocation of protons from the intermembrane space to the matrix) and/or to modulate MPTP (Fig. 3). In the former case, the most studied subgroup of polyphenols corresponds to the flavonoids. For instance, in a study using cytochrome c oxidase-containing vesicles capable of generating a transmembrane potential ($\Delta\Psi$) and a pH gradient (ΔpH), the flavones chrysin and 7-hydroxyflavone and the flavanone pinocembrin were found to be able to dissipate both $\Delta\Psi$ and ΔpH in a concentration-dependent manner, with an uncoupling efficiency (UE_{50}) of 90, 110 and 110 μM, respectively [21]. The same study also showed that other flavonoids, such as 5-hydroxyflavone, apigenin and luteolin (flavones), quercetin and morin (flavonols), and naringenin and eriodictyol (flavanones) were almost ineffective (when tested at concentrations lower than 150 μM) in dissipating both gradients. Similarly, in a study using isolated rat liver mitochondria, when tested at 25–50 μM, the flavonol galangin, but not quercetin, taxifolin (a 2,3-dihydroflavonol), or catechin, was able to reduce the $\Delta\Psi_m$ and stimulate state 4 respiration, two characteristic effects of uncouplers of oxidative phosphorylation [22]. In a study by Trumbeckaite et al. [23], quercetin and its glycosylated derivatives hyperoside and rutin (3-*O*-galactoside and 3-*O*-rutinoside, respectively) were reported to be effective in stimulating state 2 respiration in rat heart mitochondria, while quercitrin, the 3-*O*-rhamnoside derivative of quercetin, was almost ineffective. Of note, the uncoupling effect (UE_{100}) of quercetin, hyperoside and rutin was seen at extremely low concentrations (3.6, 32.7 and 72.7 nM, respectively). Interestingly, the apparent oxidative phosphorylation uncoupling condition of quercetin (and that of rutin and hyperoside) is not only in contrast with the former findings by Dorta et al. [22] and by van Dijk et al. [21], but also indicate that such effect was seen at a concentration two-to-three orders of magnitude lower than those reported to be attained in human plasma after the consumption of quercetin-rich foods [35]. A more recent study conducted in rat heart mitochondria showed that dehydrosilybin, but not silybin (both flavonolignans added at 2–25 μM), caused a marked increase in state 2 respiration and a decrease in $\Delta\Psi_m$, both effects being consistent with dehydrosilybin causing the uncoupling of mitochondrial respiration [93]. In all the above-mentioned studies, the uncoupling effect of the flavonoids has been attributed to their weak-acidic and overall high lipophilic nature, which is consistent with their putative ability to be protonated in the low-pH external side of the inner mitochondrial membrane, to pass through the lipid layer and to be de-protonated in the high-pH mitochondrial matrix milieu, thus dissipating the proton gradient across the inner mitochondrial membrane. However, a more recent study assessing the uncoupling effect of the isoflavone genistein proposes that such effect, seen at a 1 μM concentration, might be mediated by an up-regulation of the UCP-2 gene expression induced through the activation of the estrogen receptor β [94]. Beyond what exactly are the mechanisms by which the referred polyphenols induce oxidative phosphorylation uncoupling, several investigators coincide in that such effect would be associated with a diminished rate of ROS formation through the ETC (reviewed by Modrianský and Gabrielová [24]). Thus, the uncoupling effect of some polyphenols might be viewed as another ROS scavenging-independent mechanism by which such compounds might exert their antioxidant activity. As proposed by Modrianský and Gabrielová, most superoxide is formed *in vivo* under the "resting" state of mitochondrial respiration (*i.e.*, under non-phosphorylating conditions where the electron flow across the ETC is slow) and therefore, increasing mitochondrial respiration (by uncoupling) would shorten the lifetime of the ETC-related radicals responsible for oxygen monovalent reduction and lead to a lower oxygen tension in the mitochondrial matrix, reducing the formation of superoxide by electron leaks from the ETC.

As mentioned before, several polyphenols have also been described as potential modulators of the opening of the MPTP. For instance, Santos et al. [20] reported that quercetin and pinocembrin, and their methylated derivatives 3,5,7,3′,4′-penta-*O*-methyl quercetin and 7-*O*-methyl pinocembrin, added to rat liver isolated mitochondria at a 25 μM concentration, were all able to effectively inhibit the opening of the MPTP (measured as mitochondrial swelling) induced by either inorganic phosphate or mefenamic acid. Similarly, the group of Panickar and Anderson reported that a water-soluble cinnamon polyphenol extract [95] and the flavonol myricetin [25], added in the range of 0.01–0.1 mg/ml and 100 pM–10 nM, respectively, were able to inhibit cell swelling and mitochondrial dysfunction induced by oxygen glucose-deprivation (OGD) in C6 rat glial cells. In both studies, the authors postulate that the protection afforded would involve the observed inhibition of the opening of the MPTP induced by OGD. Noteworthy, in the latter study [25], the effect of myricetin was seen at concentrations as low as 1 nM. Similarly, a study conducted in rats subjected to ischemia/reperfusion (I/R; a condition known to induce MPTP opening) showed that the continuous infusion of theaflavin (20 μM) protected the cardiac tissue from damage and postulated that such effect would be mediated by the inhibition of the I/R-induced opening of the MPTP [96]. Such postulate was based solely on the fact that the co-administration of atractyloside (a known MPTP opener) to the animals completely abolished the cardio-protection induced by theaflavin. Unfortunately, no *in vivo* evidence is available linking opposing effects of polyphenols and the atractyloside at the MPTP functioning level.

Also addressing the potential of quercetin to modulate the opening of MPTP, De Marchi et al. [97] showed that depending on the experimental conditions (*i.e.*, patch clamping or cell swelling assays, or presence or absence of iron or copper in the media) and on the flavonoid concentration, quercetin could act both, as an inhibitor (5 μM) or as an inducer (40 μM) of MPTP opening. Assessing MPTP opening by means of cyclosporine A-sensitive mitochondrial calcium release, Ortega and García [98] reported that quercetin acts as an MPTP inducer but only when added at concentrations equal to or greater than 30 μM. Differing from both former studies, Panickar and Anderson [25], studying MPTP-related cell swelling induced by OGD, found that quercetin (25 μM) was effective in preventing the swelling but had no effect on membrane depolarization, suggesting that the protective effect of this polyphenol would not involve an inhibition of the MPTP opening. In addition to quercetin, genistein (10 μM) [99], taxifolin (10 μM) and catechin (25 μM) [22] have also been reported to significantly induce the opening of the MPTP. For all the above-referred polyphenols, a potential pro-oxidant mechanism, in which these compounds would undergo oxidation into quinone-derivatives, is believed to underlie their MPTP-inducing activity [22,97,99]. In such scenario, the oxidized metabolites could react with sulfhydryl groups present in the MPTP complex leading to the thio-arylation of such groups and the opening of the pore [22,97,99]. It should be mentioned, however, that quercetin (already at 5 μM) has also been shown postulated to be able to induce MPTP by inhibiting the adenine nucleotide translocase (ANT), a putative component/regulator of the pore [98]. The possibility of conducting more in deep studies aimed at assessing the specific effect that some polyphenols might have in inducing or inhibiting particular proteins responsible for the MPTP formation and/or opening is still limited by the fact that the precise structure of the MPTP has not been established yet [56].

ATP synthesis

Intra-mitochondrial ATP synthesis is mainly driven by complex V, a ubiquitous multimeric enzyme found in all living organisms (*i.e.*, bacteria and eukaryotic cells). In animal cells, complex V is located in the inner mitochondrial membrane, where it employs the $\Delta\Psi_m$ built up by the ETC to drive the synthesis of ATP from ADP and inorganic phosphate (P_i). Structurally, complex V resembles a rotating motor and contains two main parts, an inner mitochondrial membrane-embedded F_0 base and a globular rotating F_1 headpiece (located at the mitochondrial matrix), both of which are linked by a common rotor shaft which couples proton flow to ATP synthesis [100]. Each part of the complex V is formed by several subunits; for instance, F_0 comprises three distinct subunits (a, b and c) present in a stoichiometric ratio of ab_2c_n (where n ranges between 8 and 15), whereas F_1 exhibits five different subunits (α, β, γ, δ and ε), in a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ [100] Under physiological conditions, the driving force of the proton gradient passing through the F_0 subunit drives F_1 rotation clockwise (as seen from the membrane) allowing ATP synthesis. The F_1 subunit can also rotate anti-clockwise, functioning under such condition as an ATPase.

Given its relevance in ATP synthesis, and therefore in the survival of bacteria and animal cells, inhibition of the ATP synthase activity of complex V represents a potential target primarily for the action of antimicrobial and chemotherapeutic molecules [28]. Apart from the long-known complex V inhibitors (*e.g.*, oligomycin, efrapeptin, aurovertin B and azide), several polyphenols have been reported to inhibit complex V activity (Fig. 3). For instance, in an early work conducted in beef heart mitochondria by Lang and Racker [101], the flavonols quercetin, myricetin, fisetin and morin were all shown to be able to inhibit ATPase activity of complex V, but not its ATP synthase activity, at concentrations near to 0,1 μM. In another study in which a large number of different subclasses of polyphenols where addressed in terms of their ability to inhibit the rat brain mitochondria ATPase activity of complex V, Zheng and Ramirez [102] showed that piceatannol and resveratrol (stilbenes), EGCG and epicatechin-3-gallate (flavan-3-ols), genistein, daidzein and biochanin A (isoflavones), quercetin, kaempferol and morin (flavonols), apigenin (flavone), phloretin (chalcone), and curcumin are all effective, although with different potencies (IC_{50}) ranging from 8 to 100 μM. However, only piceatannol, EGCG and resveratrol showed an IC_{50} value lower than 20 μM (8, 17 and 19 μM, respectively). In contrast, the same study reported that catechin, epicatechin and epigallocatechin (flavan-3-ols which lack a gallate ester bound to them), caffeic acid, gallic acid and salicylic acid (one-benzene based phenolic acids), and the glycosylated compounds genistin (genistein glycoside), quercitrin (quercetin glycoside) and phloridzin (phloretin glycoside) had little or no inhibitory effect over complex V. Interestingly, in their study Zheng and Ramirez [102] demonstrated that, unlike the exclusively ATPase-inhibitory effect of quercetin on (beef heart mitochondria) complex V reported earlier by Lang and Racker [101], resveratrol is also able to inhibit the (rat brain mitochondria) ATP synthase activity of such complex, doing it with an IC_{50} of 28 μM.

Other studies have aimed at further understanding the possible mechanism by which resveratrol, piceatannol and quercetin are able to inhibit complex V activity. For instance, using isolated F_1-ATPase from bovine heart mitochondria, Gledhill et al. [100] determined that these three polyphenols are able to bind, *via* H-bonds and hydrophobic interactions, to a common site in the inside surface of F_1, near to an annulus made from loops in the three α- and three β-subunits. More recently, working with *Escherichia coli*-isolated F_1, Dadi et al. [103] showed that similar to what was shown for resveratrol [102], piceatannol (IC_{50} of 14 μM) is also able to inhibit both ATP synthesis and hydrolysis. Results from such study, coupled with those from Lang and Racker [101], Zheng and Ramirez [102] and Gledhill et al. [100], would imply that while resveratrol and piceatannol interfere with both senses of F_1 rotation (clockwise and anti-clockwise), quercetin, being unable to inhibit the ATP synthase activity [101], would only impede the anti-clockwise movement. However, the exact mechanism by which the binding of the three molecules to a common pocket in F_1 causes such different effects still remains to be determined [28,100]. Interestingly, the study by Dadi et al. [103] also reported that resveratrol and piceatannol (which inhibit both activities of complex V), but not quercetin or two of its glycosides, quercitrin and quercetin-3-β-D glucoside (which only inhibit the ATPase activity), are able to inhibit the growth of *E. coli* in glucose-limited or in succinate-rich media, suggesting that the inhibition of ATP synthase would be responsible, at least in part, for the cytotoxic effects of the two former stilbenes. Additionally, Sekiya et al. [104] showed that piceatannol (IC_{50} near 20 μM) was able to interfere with the rotation of the F_1 part of *E. coli* complex V, and that it stays bound throughout the rotational catalytic cycle of the ATPase activity. Finally, it is worth mentioning that a recent study assessing the potential of four theaflavins (theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin-3,3′-digallate) to inhibit complex V showed that all such compounds are able to inhibit the ATPase activity, and that such inhibition would most likely involve the F_1 portion of complex V [105]. Relative to theaflavin, which showed an IC_{50} of 60 μM, the three other oligomers were more potent, showing IC_{50} values of 20, 15 and 10 μM, respectively. Results from Li et al. [105] reveal that the inhibition of complex V correlated well with the size of the polyphenols, and these authors have suggested that the latter could probably be related to the fact that the ATP synthase is a rotary motor and many of its inhibitors appear to be mechanical disruptors.

Experimental evidence on the effect of polyphenols on complex V activity indicate that while only few of these compounds are able to inhibit both ATPase and ATP synthase activities, most other tested polyphenols are able to inhibit (to different extents) only the former activity. According to available evidence, however, it would seem that none of the so far tested natural polyphenols be able to specifically inhibit the ATP synthase activity without affecting the ATPase one; thus, the relative inability of most compounds to inhibit the synthase activity might reflect the existence of significant differences in terms of the accessibility of certain polyphenols to their putative binding pockets involved in each catalytic cycle, and/or differences in terms of the possibility that such polyphenols establish potentially modulatory interactions with those binding site-related aminoacidic residues which are more relevant towards defining the sense of F_1 rotation. Probably reflecting the latter, several studies addressing SAR for polyphenols as inhibitors of the ATPase activity have been published. For instance, the early study by Lang and Racker [101] indicated that the C-3 and C-3′ hydroxyl groups in the flavonoid structure would be determinant of a good ATPase inhibitory effect, since flavonoids that do not possess both of such features, like apigenin or morin, are far worst inhibitors than those molecules that do present such hydroxylation sites, like quercetin, myricetin and fisetin. In the same line, the work conducted by Zheng and Ramirez [102] supports the contention that ATPase inhibition would require polyphenols to bear at least two phenolic rings, since simple phenolic acids (caffeic, gallic and salicylic acids) are not able to interfere with ATP hydrolysis. The same study suggests that the C-5 and C-7 hydroxyl groups in the flavonoid structure would be relevant for inhibition, since all molecules bearing such substituents were effective ATPase inhibitors, and that the addition of a galloyl molecule to flavan-3-ols would also be important for their inhibitory effect, since it converted inactive epicatechin and epigallocatechin into the active inhibitory esters epicatechin-3-gallate and EGCG. More recently, in a study conducted to investigate the potential of several modified polyphenols to inhibit *E. coli* ATPase, Ahmad et al. [106] observed that the hydroxylation pattern of simple hydroxyphenols and more complex imino-diphenolic compounds affects the inhibitory potency of such molecules over complex V; those molecules with more distant hydroxyl groups were found to be better inhibitors than those whose hydroxyl groups were more proximal each other. Noteworthy, the same study also showed that the addition of a NO_2 group with electron withdrawing power to the polyphenol structure results in stronger inhibition, a result that could arise from stronger hydrogen bonding between the phenolic hydroxyl groups and the polyphenol binding pocket residues [106]. Although further SAR studies are needed to better elucidate the structural determinants of the interaction between the ATPase-inhibiting polyphenols and complex V, it would seem that future exploration of this issue would be facilitated by the fact that the structure of complex V coupled with the above-mentioned three polyphenolic inhibitors has already been defined through crystallization techniques [100].

Up to date, no study has addressed the issue of whether complex V activity would depend on or be modulated by ROS. Thus, based on the recently emerging evidence forth a direct molecule-complex V protein interaction between those polyphenols that have been shown to be active inhibitors, it seems reasonable to postulate that a ROS-dependent action would definitively not be involved in the inhibitory activity of such active compounds.

Mitochondria-targeted polyphenols

The former discussion accounts for the potential ability that certain polyphenols would have to modulate a variety of biological processes which either take place or directly affect the mitochondria (*i.e.*, mitochondrial superoxide production, mitochondrial biogenesis, intrinsic apoptosis, ETC, OxPhos coupling, MPTP and ATP synthesis). However, since most evidence arises from *in vitro* studies, a fundamental point is whether the concentrations described *in vitro* can be indeed attained within mitochondria *in vivo*. Towards that end, research conducted during the last decade has intensively focused on certain chemical modifications of antioxidant molecules that are aimed at taking advantage of the fact that, relative to the intermembrane space, the mitochondrial matrix exhibits a ΔpH and a ΔΨ which favor the accumulation within the latter compartment of any molecule carrying a positively charged moiety or structure. Rhodamine and the lipophilic triphenylphosphonium (TPP) derivatives represent two widely used positive-charge carrying moieties. Linkage of phosphonium cations to classical antioxidants has led to development of various mitochondria-targeted molecules, among which the ubiquinone derivative Mito-Q was early described [107] and shown to efficiently concentrate within mitochondria and to offer significant *in vitro* and *in vivo* protection against various forms of ROS-related damage. In addition, some TPP-derivatives have been designed to lower superoxide (Mito-SOD and Mito-TEMPOL), H_2O_2 (Mito-peroxidase) or prevent lipid peroxidation (Mito-E) (reviewed by Murphy and Smith [108]). Another type of synthetic cationic mitochondria-targeted antioxidants are the plastoquinonyl-TPP derivatives (SkQ1), developed by the group of Skulachev [109], for which also increasing evidence has emerged on their ability to experimentally retard the development or reduce the damage seen in a number of age- and ROS-related pathologies. Currently, SkQ1 are also actively investigated in some clinical trials [109]. The same TPP-linking approach has been used to build mitochondria-targeted polyphenols, where resveratrol [110] and quercetin [112] are so far the only two studied molecules. In the case of resveratrol, the TPP derivative was reported to accumulate within energized mitochondria from rat liver (although the extent of such accumulation was not reported) and appears to be cytotoxic for fast-growing but not for slower-growing cells when supplied in the 1–5 μM range [110]. In a more recent work, the same group reported that when tested in such low micromolar range, resveratrol derivatives, bearing the *O*-linked TPP group at either position C-3 or position C-4′, are pro-oxidant for cultured cells, while resveratrol was found not to exert such effect. The cytotoxicity of these mitochondriotropic derivatives was found to be primary necrotic, ROS-mediated, potentiated by SOD and susceptible to be prevented by the addition of a membrane-permeant catalase. Interestingly, the two resveratrol derivatives also induced ROS-independent mitochondrial depolarization. The cytotoxicity effectiveness of the TPP-linked compounds was increased upon methylation of the remaining hydroxyls, suggesting that such groups would not be involved in ROS generation and depolarization. In the same line, studies conducted on two TPP quercetin-derivatives, the 3-*O*-TPP and the 7-*O*-TPP ones, indicate that, when tested at a 3–5 μM concentration range, both compounds are toxic for fast-growing cells, also probably due to a common pro-oxidant action [112,113]. Nonetheless, despite the latter similarities, the two former quercetin derivatives have been shown to exert different effects at the level of mitochondrial membrane potential and MPTP. For instance, while the 3-*O*-TPP quercetin (20 μM) was reported to induce the opening of the MPTP and to act as a mild uncoupler of the OxPhos, causing mitochondrial depolarization and increasing oxygen consumption [114], the 7-*O*-TPP derivative (30 μM) showed no ability to either stimulate oxygen consumption or to induce MPTP [113]. Of note, the former quercetin-derivative (at 50 μM) has also been shown to inhibit mitochondrial ATPase activity in permeabilized mitochondria (in a similar manner to that previously referred for quercetin [101]), indicating that the TPP-modification of the C-3 hydroxyl group would not hinder the flavonoid's ability to interact with complex V [112] It should be mentioned that quercetin, in concentrations at which the TPP derivatives were effective (1–20 μM), was almost totally inactive in inducing MPTP opening, OxPhos uncoupling or cell death [112–114]. Recently, Durante et al. [115] reported that while the 3-*O*-TPP quercetin derivative was effective in protecting rat aorta rings against glyceryl trinitrate-induced tolerance and endothelial dysfunction, its tetra-acetylated form turned out to cause endothelial dysfunction *per se*. Although the latter study may merit further *in vivo* investigation, its results also suggest that particular attention should be placed at the possibility that blockade of the phenolic hydroxyls of these TPP-compounds, whether by early chemical modifications or by their subsequent biotransformation, may have a biological impact different from that sought *in vivo* or attained *in vitro* with their parent TPP-polyphenolic derivatives.

Cancer cells are constitutively under oxidative stress and an intensification of such stress may lead to their selective removal. Thus, the above-reported ability of TPP-linked resveratrol and quercetin molecules to increase ROS, inducing a greater cytotoxicity in fast growing cells, prompts the possibility that these compounds may represent a new class of chemotherapeutic agents whose mechanisms of action and *in vivo* activity also warrant further investigation [111,113]. The latter, however, raises some questions on the potential that these polyphenol derivatives may have to protect against the oxidative and functional damage that follows exposure of mitochondria, cells or experimental animals to chemicals such as rotenone, MPP^+ or NSAIDs, or to ischemia/reperfusion conditions, where the parent polyphenols have already been shown to exert antioxidant and cytoprotective actions. The cytotoxicity induced by rotenone, MPP^+ or NSAIDs is known to directly involve their ability to inhibit complex I of the ETC [30,31,33] and to that extent, the damage induced by such agents should be susceptible to reversal by ubiquinone or ubiquinone-like acting molecules. Since James et al. [116] reported that MitoQ is unable to behave as a substrate for complexes I and III, the potential usefulness of the latter modified molecule would be limited to preventing those forms of damage which do not directly depend on the normal functioning of the ETC. MitoQ was shown to be reduced by complex II as well as by α-glycerophosphate dehydrogenase [116], allowing the reduced form of MitoQ to behave as a ROS-scavenging antioxidant [107]. The other TPP-derived antioxidant molecules Mito-E, Mito-SOD and Mito-peroxidase have also been shown to serve as effective ROS-scavengers [108]. On the face of the previously described (Section "Effects of polyphenols on mitochondrial processes") capacity of a large number of selected polyphenols to modulate certain mitochondrial functions, future studies on their TPP-derivatives should address the antioxidant *versus* pro-oxidant actions of such derivatives, as well as their potential to either directly interact with particular components of the OxPhos system (from complexes I to V), or to activate SIRT1/PGC-1α, inducing mitochondrial biogenesis.

Conclusions

Mitochondria distinguish from any other intracellular organelles, since they concentrate the cell's largest ability to synthesize ATP, host the main regulators of the intrinsic programmed-cell death, represent the major intracellular source of superoxide (and its derived ROS), and are also probably the most critical target of such species. Since alterations in some mitochondrial functions, such as in the ETC activity or in ATP synthesis, could lead to an increased production of ROS and/or to a decreased ROS-removal capacity, most of the research conducted on the potential of polyphenols to modulate mitochondrial processes has focused on their ability to act as antioxidants. Less explored, however, has remained the ability that certain polyphenols have to exert a modulatory action on other mitochondrial processes, not necessarily ROS-related, such as biogenesis, membrane potential maintenance, electron transport chain, ATP synthesis and cell death triggering. As reviewed above, polyphenols belonging to the flavonol, flavone, isoflavone, flavan-3-ol, stilbene, phenolic alcohol and phenolic acid subclasses are now recognized to be able to activate SIRT1/PGC-1α in different cell types increasing thereby mitochondrial biogenesis which, in some cases, has been shown to translate into an improved physical endurance (seen preliminarily in experimental animals and humans). On the other hand, some polyphenols have been shown to exhibit an *in vitro* ability to modulate (whether preventing or inducing) MPTP opening and/or to uncouple the OxPhos system. While the uncoupling action relates to the potential of certain polyphenols (belonging to the flavone, flavanone, flavonol and isoflavone subclasses) to favor a mild futile cycling within the ETC, thereby increasing energy expenditure (a condition which might be desirable for weight control), preventing the MPTP opening could relate to the cytoprotective effects shown by some of such compounds (belonging to the flavonol, flavanone and flavan-3-ol subclasses) against ischemia/reperfusion and various MPTP-inducing chemicals. Prevention of MPTP opening could also underlie the protective effect that some flavonols, flavan-3-ols, stilbenes and phenolic acids have against pro-apoptotic chemicals (such as acetaminophen, methotrexate or the neurotoxins glutamate and MPP^{+}). Nonetheless, the down-regulation of the pro-apoptotic Bcl-2 family members, displayed by polyphenols like curcumin, quercetin, EGCG, baicalein and theaflavin, represents another potential antiapoptotic mechanism by which these compounds could cytoprotect. Conversely, some polyphenols, among which members of the flavonol, flavan-3-ol and flavone subclasses are included, are particularly able to induce MPTP opening, triggering thereby the intrinsic apoptosis pathway. The latter action may underlie their putative chemopreventive potential. Another mitochondrial action, also restricted to few polyphenols (but comprising various subclasses) is the ability of certain flavonols, isoflavones and flavan-3-ols to inhibit the ATPase activity of complex V, and that of the stilbenes resveratrol and piceatannol to inhibit both the ATPase and ATP synthase activities. Although the actual biological significance and reach of inhibiting ATPase activity is not yet evident, the inhibition of ATP synthesis at the level of complex V has been associated with the *in vitro* anti-microbial and anti-tumoral activities of the ATP synthase-inhibiting stilbenes. One of the most earlier studied effects of polyphenols on mitochondria refers to their ability to modulate *in vitro* the activity of some of the ETC complexes. Most studies addressing the latter have revealed that, in ubiquinone-added or -containing isolated mitochondria, several polyphenols would act as inhibitors of complexes I, II (showed particularly by some members of the flavone and flavonol subclasses) or III (restricted to few flavones and stilbenes). To establish the actual biological significance of the reported inhibitions (generally seen at medium to high μM concentrations), the latter studies await further investigations addressing the concomitant consequences of inhibiting the normal functioning of the ETC (*i.e.*, increment in mitochondrial superoxide, drop in ATP and loss of mitochondrial membrane potential). In contrast to what can be expected from the ETC-inhibiting polyphenols, some of the latter compounds have proven to be effective in protecting cultured cells and experimental animals against the oxidative, cellular, and occasionally functional damage induced by their exposure to known inhibitors of complex I (such as rotenone or MPP^{+}). The latter type of studies are valuable as they suggest the therapeutic potential that some particular polyphenols may have in pathological conditions which feature a diminished complex I activity as an ethiogenically relevant event, but need to be further complemented with studies that include the assessment of the *in vivo* effects of such polyphenols at the complex I level. More recently emerged evidence indicates that, in a rotenone-induced animal model of PD, quercetin as a paradigm, afforded neuroprotection (both cellular and functional) that was substantiated by a significant reversal (by up-regulation) of the rotenone-induced decrease in mitochondrial complex I activity. Therefore, the potential effects of some flavonoids on the ETC complexes would not be limited to those direct actions seen *in vitro*, but would also seem to extend to *in vivo* gene modulation. Also broadening the potential that certain polyphenols may have to favorably modulate the ETC activity is the recent evidence revealing their ability to protect against complex I inhibition induced by NSAIDs, and against its oxidative and cellular consequences. Notably, in such protection quercetin has emerged as a polyphenol capable of either preventing or reversing the direct inhibition of complex I induced by such drugs by acting as a coenzyme Q-like molecule. The latter result, although still limited to isolated mitochondria, once confirmed in more complex models would open the possibility for the potential use of quercetin, and probably that of some other flavonoids, to contribute in the treatment of those conditions where an increment in the mitochondrial availability of coenzyme Q could be convenient. The latter includes physiological conditions like aging, pathological conditions such as PD and AD, some inherited complex I-diminished conditions, and certain toxicological conditions which arise from the use of NSAIDs and statins (where the synthesis of ubiquinone has been reported to be significantly reduced).

The information summarized in the present review clearly evidences that mitochondria represents an important intracellular target of polyphenols. Until recently, major emphasis had been placed on the antioxidant actions of these compounds, which clearly would not be limited to their traditional ROS-scavenging property. Currently, in turn, it is evident that besides acting as antioxidants, the opportunities of action of polyphenols extend to their direct modulation of a myriad of mitochondrial events that could affect the whole cell, ranging from energy generation to cell death control. However, excepting for those actions of polyphenols that appear to be independent of their interacting with other molecules (such as their uncoupling effect) and those that seem to involve their direct interaction with a particular macromolecule (such as with complex I and complex V), a direct ROS-scavenging activity cannot be precluded from contributing to most other modulating effects of polyphenols, such as those occurring at the level of mitochondrial biogenesis, intrinsic apoptosis triggering and MPTP control. Thus, future studies aimed at discriminating the extent to which a cytoprotective effect of a given polyphenol is a consequence of its direct modulatory action on the formerly-referred mitochondrial processes or whether it also critically arises from a ROS-scavenging action, are warranted.

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