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Review

# Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies?

Barry Halliwell *

*Department of Biochemistry, National University of Singapore, University Hall, Lee Kong Chian Wing, UHL #05-02G, 21 Lower Kent Ridge Road, Singapore 119077, Singapore*

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**Abstract**

Diets rich in polyphenols are epidemiologically associated with lower risk of developing some age-related diseases in humans. This apparent disease-protective effect of polyphenols is often attributed to their powerful antioxidant activities, as established *in vitro*. However, polyphenols can also exert pro-oxidant activities under certain experimental conditions. Neither pro-oxidant nor anti-oxidant activities have yet been clearly established to occur *in vivo* in humans, nor are they likely given the limited levels of polyphenols that are achievable *in vivo* after consumption of foods and beverages rich in them. Other actions of polyphenols may be more important *in vivo*. Many studies of the biological effects of polyphenols in cell culture have been affected by their ability to oxidise in culture media, and awareness of this problem can avoid erroneous claims.


*Keywords:* Polyphenols; Cell culture; Antioxidant; Pro-oxidant; Epigallocatechin gallate; Hydrogen peroxide; Ascorbate; Dulbecco's modified Eagle's medium; Green tea; Red wine

Aerobic organisms produce a wide range of oxygen radicals and other reactive oxygen species (ROS)[1], both for useful purposes (*e.g.* defence, redox signalling) and by "accidents of chemistry" (reviewed in [1]). These ROS are metabolised by a series of antioxidant defences, some synthesised *in vivo* and other diet-derived [1]. The purpose of the "antioxidant defence network" [2] is not to remove all ROS, but to control their levels so as to allow useful functions whilst minimising oxidative damage (Fig. 1) [1–4]. But how important are the diet-derived antioxidants such as vitamins C and E to humans? In general, increased intakes of these vitamins do not decrease levels of oxidative damage very much (if at all) in well-nourished humans who are already consuming the recommended dietary allowances [1,5–7]. Indeed, it has been suggested that the main biological function of α-tocopherol in humans is not as an antioxidant [8].

## Polyphenols as antioxidants

Foods and beverages rich in flavonoids and other polyphenols have been associated with decreased risk of age-related diseases in several (but not all) epidemiological studies [9–15]. Flavonoids have powerful antioxidant activities *in vitro*, being able to scavenge [16–23] a wide range of reactive oxygen, nitrogen, and chlorine species, such as superoxide $O_2^{\cdot -}$, hydroxyl radical $OH^{\cdot}$, peroxyl radicals $RO_2^{\cdot}$, hypochlorous acid (HOCl), and peroxynitrous acid (ONOOH). Flavonoids can also chelate metal ions, often decreasing the pro-oxidant activity of metal ions [20,22]. They can inhibit the ability of myeloperoxidase to oxidise low-density lipoproteins (LDL), a potential anti-atherosclerotic effect [24]. Because considerable evidence indicates that increased oxidative damage is associated with, and may contribute to the development of, all major age-related

* Corresponding author. Fax: +65 6775 2207.
*E-mail address:* bchbh@nus.edu.sg

[1] *Abbreviations used:* ROS, reactive oxygen species; LDL, low-density lipoproteins; DMEM, Dulbecco's Modified Eagle's Medium.

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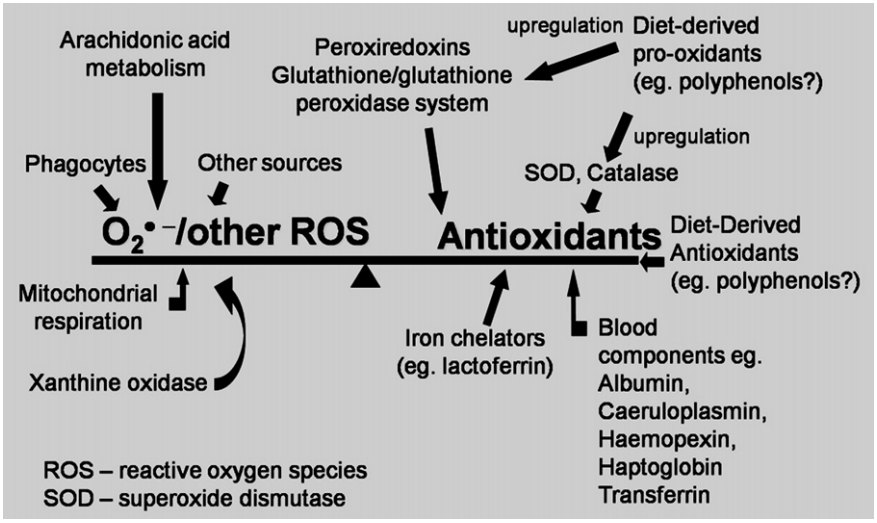


Fig. 1. Balance of antioxidants and reactive species *in vivo*.

diseases [1–3], many have attributed the apparent disease-protective effects of flavonoids to their antioxidant ability (*e.g.* reviewed in [20]).

## Polyphenols as pro-oxidants

Polyphenols oxidise readily in beverages [25–27] such as green tea. They can also oxidise in cell culture media (see below) and even in the oral cavity; holding or chewing green tea in the mouth generates substantial levels of $H_2O_2$ [28]. Often, these pro-oxidant effects involve interactions of polyphenols with transition metal ions [1,29–35]. Oxidation of polyphenols produces $O_2^{\cdot -}$, $H_2O_2$ and a complex mixture of semiquinones and quinones, all of which are potentially cytotoxic [26,31,36,37]. It has been argued that polyphenols may exert antioxidant and other cytoprotective effects in the gastrointestinal tract because of the high levels that can be present [38–40]. However, since there are often unabsorbed transition metal ions (especially iron [41,42]) in the gastrointestinal tract, pro-oxidant effects could conceivably occur there as well. Indeed, such effects have been demonstrated in the gastrointestinal tracts of certain insects consuming high levels of phenols [43,44].

However, in practice pro-oxidant effects can also be beneficial, since, by imposing a mild degree of oxidative stress, the levels of antioxidant defences and xenobiotic-metabolising enzymes might be raised, leading to overall cytoprotection [45], as illustrated in Fig. 1.

## Are polyphenols pro-oxidants or antioxidants *in vivo* in humans?

No data are available on whether polyphenols are antioxidant or pro-oxidant *in vivo* in the human stomach, intestines, and colon, where they can be present at significant levels [38,39,46,47]. As for effects after absorption into the body, multiple well-designed human studies have been done using reliable biomarkers of oxidative damage in plasma ($F_2$-isoprostanes) and urine ($F_2$-isoprostanes, isoprostane metabolites, 8-hydroxy-2′-deoxyguanosine [8OHdG]), essentially testing for systemic antioxidant or pro-oxidant activity. The results have been reviewed in detail elsewhere [40] and are quite variable, but overall no evidence for systemic pro-oxidant effects of polyphenols has emerged. A few studies report that administration of high doses of epigallocatechin gallate to animals leads to the formation of cysteine conjugates detectable in the urine, indicative of some degree of oxidation *in vivo* [36]. However, these effects may not be important at lower doses and may not be relevant to humans [36].

Similarly, only limited and variable evidence for antioxidant effects of flavonoids in humans has been obtained (reviewed in [40]). This is not, to the author, very surprising; although flavonoids can be absorbed through the gastrointestinal tract, maximal plasma concentrations achieved are low, usually not more that 1 μmol/L, in part because of rapid metabolism by human tissues [47–49]. Many of the products of metabolism, such as methylated and glucuronidated forms, have decreased antioxidant (or pro-oxidant) abilities because of the blocking of the phenolic hydroxyl groups involved in such activities [23,48]. Therefore, plasma flavonoid concentrations *in vivo* seem insufficient to exert systemic antioxidant effects.

Another point to consider in interpreting the published human studies is that several groups have studied flavonoid-rich foods (*e.g.* pomegranate [50] or chocolate/cocoa [51,52]) or beverages (*e.g.* green tea) rather than pure flavonoids, and such foods contain other constituents that might be able to modulate oxidative damage. But are such foods and beverages effective as antioxidants *in vivo*? Again, the data are mixed. Some studies showed antioxidant effects (*e.g.* [37,48,51–53]), others no effects (*e.g.* [54–57]) and yet others some indication of mild pro-oxidant effects (*e.g.* [58]). One must be careful in studies with foodstuffs, since