Review

Dietary polyphenols: Good, bad, or indifferent for your health?

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Received 19 June 2006; received in revised form 16 August 2006; accepted 4 October 2006

Available online 13 October 2006

Time for primary review 27 days

Abstract

Flavonoids and other polyphenolic compounds have powerful antioxidant effects in vitro in many test systems, but can act as pro-oxidants in some others. Whether pro-oxidant, antioxidant, or any of the many other biological effects potentially exerted by flavonoids account for or contribute to the health benefits of diets rich in plant-derived foods and beverages is uncertain. Phenolic compounds may help to protect the gastrointestinal tract against damage by reactive species present in foods or generated within the stomach and intestines. The overall health benefit of flavonoids is uncertain, and consumption of large quantities of them in fortified foods or supplements should not yet be encouraged.

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Keywords: Atherosclerosis; Cell culture/isolation; Redox signalling

1. Introduction

The traditional “trio” of antioxidants (ascorbate, β-carotene, α-tocopherol) has had a bad press recently, with human intervention trials giving mostly-negative results, and some meta-analyses and other studies suggesting that these agents not only fail to protect against disease, but also that some of them may accelerate development of cancers or cardiovascular disease in certain subjects [1–6]. Does this mean that the concept that free radicals and other reactive species contribute to the development of age-related diseases such as cancer is incorrect? Possibly, but I do not believe so (reviewed in [7]). Instead, I think it to be more likely that the antioxidant administrations simply failed to protect against oxidative damage (discussed in detail in [2,7–9]). For example, high doses of α-tocopherol are poorly-effective at decreasing levels of lipid peroxidation in humans, as measured by reliable biomarkers such as F2-isoprostanes [7,10,11]. Whether other tocopherols, or tocotrienols, work better remains to be determined. α-Tocopherol acts much more effectively as an antioxidant in mice than it does in humans, and its ability to protect against atherosclerosis and neurodegeneration in mice is correspondingly greater [12–16].

2. Fruits and vegetables

Among the few things uncontested by nutritionists are that increased consumption of grains, fruits, and vegetables, decreased saturated fat intake, a moderate degree of exercise and a judicious consumption of red wine (or other alcoholic beverages) seem associated with a lower risk of developing cardiovascular disease, some forms of cancer, and perhaps Alzheimer’s disease [15,17–23]. However, foods and beverages derived from plants are chemically complex, and protective effects could arise from many components or mixtures of components present, including fibre, immunostimulatory agents, inducers of antioxidant or xenobiotic-metabolizing enzymes, monounsaturated fatty acids, agents that modulate cholesterol synthesis, B-vitamins, folic acid (which may minimize homocysteine levels), agents modulating nitric oxide production, cyclooxygenase inhibitors and...
even the humble ethanol molecule itself [2,17–28]. Are fruits and vegetables beneficial because of these other components? Or is it that fruits and vegetables act (in whole or in part) by antioxidant actions, but that the active antioxidants are not vitamin C, α-tocopherol or β-carotene? Some studies suggested yes to the latter question [29–31]. For example, Verhagen et al. [29] found that urinary excretion of 8-hydroxy-2′-deoxyguanosine (8OHdG), a putative biomarker of oxidative damage to DNA and DNA precursors [32,33], was decreased by feeding human volunteers Brussels sprouts, but not by giving them α-tocopherol, ascorbate, or β-carotene [34].

Several other authors have shown that consumption of antioxidant-rich foods decreases levels of oxidative damage in vivo in humans (reviewed in [35]). Others have found little effect (e.g. [36]), and some registered increases in biomarkers of oxidative protein damage, such as 2-aminoadipic and γ-glutamyl semialdehydes [37]. One must be cautious in all such studies to rule out confounding effects of refeeding fasted individuals, as opposed to the effects of antioxidants in the food, on biomarkers of oxidative damage. Thus Vissers et al. [38] showed that olive oil administration to human volunteers decreased the propensity of low-density lipoproteins (LDL) subsequently isolated from their blood to undergo oxidation in vitro, but feeding oil without antioxidants had the same effect. In 2000, we reported [39] that dark soy sauce has powerful antioxidant abilities in vitro. Recently, we attempted to see if dark soy sauce decreased oxidative damage in vivo in human volunteers, and indeed it was able to decrease levels of F2-isoprostanes [40]. We administered the soy sauce with rice, using a placebo colouring on the same amount of rice as a control. The rice meal (devoid of antioxidants) also had effects on F2-isoprostanes and urinary 8OHdG excretion [40], although the soy sauce meal did better than the placebo in lowering F2-isoprostane levels. Similarly, Richelle et al. [41] and Lee et al. [42] suggested that fasting may raise plasma F2-isoprostane levels. At the moment, the balance of evidence does suggest that antioxidant effects contribute to the benefits of a high intake of fruits and vegetables (reviewed in [35,43]) but the extent of their contribution is uncertain. More work needs to be done on the effect of diet on oxidative damage, using suitable controls.

3. Pro-oxidant effects

Some authors have claimed that “antioxidants” can stimulate oxidative damage in vivo, especially ascorbate, alleged in several studies to increase oxidative DNA damage (reviewed in [44]). Indeed, it was suggested that mega-doses of ascorbate might kill cancer cells in vivo by oxidizing to produce H2O2 [45]. We found small and transient increases in oxidative DNA damage in human volunteers fed mixtures of ascorbate, β-carotene and α-tocopherol, but there was wide variation between experiments [8]. Overall, the available data that ascorbate, β-carotene or α-tocopherol are pro-oxidant in vivo are (in my view) equivocal and inconclusive. Nor is the evidence that they are antioxidants a great deal better.

4. Enter the flavonoids

Flavonoids and other polyphenols have powerful antioxidant activities in vitro, being able to scavenge a wide range of reactive species, including hydroxyl radicals, peroxyl radicals hypochlorous acid and (sometimes) superoxide radical, O2− (reviewed in [46]). Flavonoids can also inhibit biomolecular damage by peroxynitrite in vitro [47–49], although they are less good at doing this in the presence of physiological levels of HCO3/CO2 [49,50]. Peroxynitrite reacts fast with CO2/HCO3− to form reactive products that flavonoids appear to scavenge less well. Many flavonoids chelate transition metal ions such as iron and copper, decreasing their ability to promote reactive species formation [46,51,52].

Two observations drew attention to the potential biological importance of flavonoids. First, phenolics in red wine were shown to be able to inhibit the oxidation of LDL in vitro and this was suggested as an explanation of the “French paradox” [19,53]. Second, the Zutphen study, an epidemiological study in the Netherlands, suggested an inverse correlation between the incidence of coronary heart disease and stroke and the dietary intake of flavonoids, especially quercetin [54]. Since then, several other epidemiological studies have confirmed similar associations, although a few have not, and there is little evidence that flavonoids protect against cancer [55]. Some suggestions of protection against neurodegenerative disease have been made [22,23,43,56–58], although it is unclear to what extent flavonoids can enter the human brain [59].

Thus could flavonoids be major contributors to the disease-protective effects of fruits and vegetables? If so, is this due to antioxidant effects? Many polyphenols are absorbed, although rarely completely, and most of the remainder are broken down in the colon to generate high levels of monophenols [60,61]. Are the amounts of polyphenols absorbed sufficient to exert significant antioxidant effects? Several studies administering flavonoid-rich foods and beverages and measuring biomarkers of oxidative damage suggest yes, but others no (discussed in [35,62]). “Feeding effects” alluded to earlier could account for some of the apparent positive effects. Are significant antioxidant effects likely in vivo? Plasma levels of unconjugated flavonoids rarely exceed 1 μM and the metabolites tend to have lower antioxidant activity because radical-scavenging −OH groups are blocked by methylation, sulphation, or glucuronidation [60,61]. Since plasma total antioxidant capacities (TAC) are often in the range of 1 mM or more (reviewed in [7]), it seems difficult to imagine how an additional 1 μM polyphenol could exert a powerful antioxidant effect in vivo. Some studies have shown effects of flavonoid-rich foods in raising plasma TAC in humans. But one must be cautious here; many such foods can increase plasma uric acid levels, and urate is detected by
several TAC assays [62–64]. Since elevated urate may be a risk factor for some diseases, the alleged “antioxidant benefit” may not be what it seems [62]. Finally, flavonoids and other phenols are complex molecules and have multiple potential actions other than antioxidant ones, including inhibiting telomerase, gluta-
mate dehydrogenase, cyclooxygenase, lipoxygenase, xanthine oxidase, matrix metalloproteinases, angiotensin-converting enzyme, proteasome, cytochrome P450 and sulfotransferase enzyme activities, affecting signal transduction pathways and interacting with sirtuins [25,35,43,56,57,65–73]. Flavonoids may also interact with cellular drug transport systems, compete with glucose for transmembrane transport, interfere with regulation of the cell cycle, inhibit protein glycation, modulate paraoxonase, myeloperoxidase and thyroid peroxidase activities, increase endothelial nitric oxide production and affect platelet function [74–82]. Again, it is uncertain whether some of these effects occur in vivo, given the low concentration of bioavailable polyphenols.

5. Do polyphenols work pre-absorption?

It has been proposed [83] that antioxidant and other protective effects of flavonoids and other phenolic compounds could occur before absorption, i.e. within the stomach, intestines and colon (Fig. 1). This could account for the suggested ability of flavonoid-rich foods to protect against gastric, and possibly colonic, cancer, although again it must not be assumed that any protective effect of flavonoid-rich foods is attributable to antioxidant actions of the flavonoids, or to flavonoids at all, rather than to other components in the foods. However, ingestion of green tea was reported to rapidly decrease prostaglandin E2 concentrations in human rectal mucosa, consistent with inhibition of cyclooxygenase activity, a potential anti-cancer mecha-
nism [25]. The levels of individual flavonoids in faecal water are fairly low (μM or less), but monophenols (many derived from polyphenol breakdown) are present at much higher concentrations [84]. By contrast to the colon, poly-
phenols are likely to be present in the stomach and intestines at high (≥mM) concentrations after consumption of polyphenol-rich foods and beverages.

Why should antioxidant protective effects of polyphenols be important to the stomach and intestines? The gastrointestinal (GI) tract is constantly exposed to reactive species. Some are released by the GI tract itself, e.g. superoxide and H₂O₂ production by NADPH oxidases and “dual oxidases” in epithelial cells [85–87]. Some reactive species are present in food and beverages, and yet others are generated by chemical reactions of dietary components within the stomach [83,88]. Sources of reactive species include H₂O₂ in beverages [89], the mixtures of ascorbate and Fe²⁺ in the stomach (dietary iron, dietary ascorbate,

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**Fig. 1.** Dietary antioxidants and the gastrointestinal (GI) tract. *Except when supplements are taken. This figure refers to normal dietary intake. +, There is considerable intersubject variability in the efficiency of GI uptake of vitamin E. †Much H₂O₂ may be removed in the oral cavity by catalase and peroxidases in saliva, and by H₂O₂ diffusion into the oral and oesophageal epithelium followed by its rapid catabolism. Adapted from [7] with permission from Oxford University Press. RNS Reactive nitrogen species, OH⁻ hydroxyl radical, RO⁻ alkoxyl radical, RO₂⁻ peroxyl radical, RS reactive species, LOX lipoxygenase, COX-2 cyclooxygenase-2.
and ascorbate normally present in gastric juice [90]), and ingested haem proteins, which can promote oxidation of dietary lipids [88]. Other reactive species that can be present in foods include lipid peroxides, cytotoxic aldehydes, and isoprostanones [91–95]. Nitrite is present at high levels in saliva and in foods [96]. It is converted to HNO₂ by gastric acid, and HNO₂ can then form nitrosating and DNA-deaminating species [97]. Activation of immune cells naturally present in the GI tract by diet-derived bacteria and toxins can also increase ROS production [98].

Flavonoids and other phenolic compounds might exert direct protective effects in the gastrointestinal tract, by scavenging reactive species and/or preventing their formation. For example, polyphenols can inhibit haem protein-induced peroxidation in the stomach [88,99] and decrease DNA base deamination or nitrosamine formation by HNO₂-derived reactive nitrogen species [97,100]. Phenols might also increase levels of toxin-metabolizing or antioxidant defence enzymes in the GI tract, and chelate transition metal ions [83]. Dietary iron is usually not completely absorbed, especially among subjects on Western diets. Unabsorbed dietary iron enters the faeces, where it could represent a pro-oxidant challenge to the colon and rectum [101]. Indeed, diets rich in fat and low in fibre may aggravate this pro-oxidant effect [102]. Phenolic compounds, by chelating iron, may help to alleviate pro-oxidant actions of colonic iron.

6. Flavonoids as pro-oxidant xenobiotics

Why do hot beverages often contain high levels of H₂O₂? Simply because the polyphenols within them oxidize readily at high temperatures [89,103–105]. Polyphenols can also oxidize readily in cell culture media, and several claims of the cytotoxic effects of flavonoids on malignant, and other, cells in culture may have been led astray by this artefact. Flavonoids oxidize especially readily in Dulbecco’s Modified Eagle’s medium (DMEM), but also do so in most other cell culture media, at a slower rate [106,107]. Oxidation generates H₂O₂, quinones and semiquinones that can contribute to (and sometimes entirely account for) cytotoxicity [106–110]. For example, the apparent toxicity of green tea to PCI² cells appeared entirely due to oxidation products generated in the culture medium [109]. This is not to say that all the observed cellular effects of flavonoids are artefacts; indeed, they may exert different effects on different cell types, those on vascular endothelial cells and other cells of the vascular system perhaps being especially important physiologically [43,82]. However, most work with cultured cells has failed to separate real effects from artefacts and may need to be repeated under conditions that slow or prevent phenol oxidation.

The mechanism of polyphenol oxidation in cell culture media is unclear. Metal ions may be involved (since DMEM is rich in iron ions, added to it as ferric nitrate), but it is not simply an iron-catalysed oxidation of polyphenols [110]. Ascorbate also oxidizes in DMEM to make H₂O₂, but mixtures of ascorbate and flavonoids generate less H₂O₂ than would be expected from the rate of its generation by either compound alone [110].

Since there are transition metal ions in the GI tract, it is possible that polyphenols could oxidize there as well. This might even be good for you, generating a pro-oxidant challenge that raises levels of xenobiotic-metabolizing and antioxidant defence enzymes in the GI tract.

7. A caution about supplements

Flavonoid-rich foods appear good for us, although to what extent (if any) the flavonoids contribute to this benefit is uncertain. Other possible protective components in foods were listed in Section 1. So should we consume flavonoid supplements or the flavonoid-enriched foods (e.g. cocoa, chocolate) now coming onto the market in some countries? I would be cautious until we know more [2,77,111]. To me, dietary polyphenols are typical xenobiotics, metabolized as such and rapidly removed from the circulation. They may be beneficial in the gut in the correct amounts. But too much may not be good and thus, I suggest that one should be content with eating a good diet for now.

References

Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do you get?


