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The antioxidant paradox: less paradoxical now?

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The term 'antioxidant paradox' is often used to refer to the observation that oxygen radicals and other reactive oxygen species are involved in several human diseases, but giving large doses of dietary antioxidant supplements to human subjects has, in most studies, demonstrated little or no preventative or therapeutic effect. Why should this be? First, the role of reactive oxygen species in the origin and/or progression of most human diseases is unclear, although they are probably important in cancer, neurodegenerative diseases and perhaps some others. Second, the endogenous antioxidant defences in the human body are complex, interlocking and carefully regulated. The body's 'total antioxidant capacity' seems unresponsive to high doses of dietary antioxidants, so that the amount of oxidative damage to key biomolecules is rarely changed. Indeed, manipulation of endogenous antioxidant levels (e.g. by supplying weak pro-oxidants) may be a more useful approach to treatment and prevention of diseases in which reactive oxygen species are important than is consumption of large doses of dietary antioxidants.

Introduction

Antioxidants are widely used in the food industry as preservatives for food and beverages and for food packaging, and increasingly they are being added to foods and beverages to increase sales because of their perceived health benefits [1, 2]; the concept that 'antioxidant is good, more antioxidant is better' seems to be embedded in the minds of many members of the public (discussed in [1, 3, 4]). Antioxidant supplementation, in foods or in tablets, is based on the belief that oxygen radicals and other 'reactive oxygen species (ROS)' play a role in many human diseases by causing 'oxidative damage', and that decreasing oxidative damage will delay or prevent disease development. There are many discussions of how to define such terms as 'antioxidant', 'reactive species' and 'oxidative damage' (for recent reviews discussing this in detail, please see [4–9]), but I will not dwell on this here. One point worth emphasizing is that the term 'ROS' does not refer to some monolithic damaging entity; each species of ROS has its special chemical properties and reaction rates. For example, hydroxyl radical ($OH^{\bullet}$) is indiscriminately reactive with almost all biomolecules, whereas superoxide ($O_2^{\bullet-}$) and nitric oxide ($NO^{\bullet}$) radicals are much more selective in what they react with [5, 9–11]. Thus, frequently seen phrases in the literature, such as 'mediated by ROS' or 'ROS are involved', actually convey little useful mechanistic information. It follows that there is no single universal antioxidant; each reacts in a different way with various ROS to generate end-products of variable reactivity [5, 12]. The chemical reactivity of such end-products must be considered when predicting how antioxidants might behave *in vivo* or in food systems.

For example, many polyphenols, such as the flavonoids, have considerable antioxidant activity *in vitro*, as measured by a range of assays [e.g. ferric reducing antioxidant power, oxygen radical absorbance capacity, 1,1-diphenyl-2-picrylhydrazyl and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate)] that claim to measure 'total antioxidant activity'. In fact, the chemistry behind each assay is different and so the results of each assay on the same molecule(s) are different [5, 13, 14]. Despite their impressive *in vitro* antioxidant power, there are few, if any, compelling data that polyphenols exert antioxidant effects *in vivo* (discussed in [4, 5, 15, 16]). Polyphenols are also unstable, easily undergoing oxidation to generate H_2O_2, quinones and semiquinones, among other products. For example, polyphenols readily oxidize in several commonly used cell

culture media. Ironically, as a result many published studies of the 'antioxidant effects' of polyphenols on cells in culture are often studies of effects of the pro-oxidants generated during their oxidation in the cell culture media [17–20]. However, pro-oxidants can be good, exerting a mild stressful challenge that triggers a rapid response, leading to increased levels of endogenous antioxidant defence systems, such as reduced glutathione [4, 5, 20–22]. Polyphenols and other 'antioxidants' have been tested in the laboratory on many small animals (nematodes, rotifers etc.), plants or yeasts to see whether they influence lifespan. Sometimes they do, because ROS are intimately involved in the ageing process; some ROS appear to be good when you age but too many are bad, although the story is complex [5, 23–28]. It is not unlikely that many of the antioxidants tested were oxidizing in the growth media and generating some degree of mild pro-oxidant stress; perhaps that is why they led to lifespan extension in several studies (discussed in [4, 23]). The oxidation of ascorbate and polyphenols in foods and beverages is also a significant problem in the food industry [29–31].

The antioxidant paradox

The term 'antioxidant paradox' is often used to refer to the observation that oxygen radicals and other ROS are implicated in several human diseases, but giving large doses of dietary antioxidants to human subjects has, in most studies, had little or no preventative or therapeutic effect [32].

What accounts for the antioxidant paradox? Let us begin by listing the key beliefs that led to the view that antioxidants would be beneficial.

1 Reactive oxygen species are formed *in vivo* and cause oxidative damage.
2 Oxidative damage contributes to human disease.
3 Diminishing oxidative damage by administering antioxidants will therefore decrease disease incidence.

Let us now examine these concepts one by one.

Reactive oxygen species are formed in vivo *and cause oxidative damage (true)*

Many types of ROS (including the highly reactive hydroxyl radical, $OH^{\bullet}$) are formed *in vivo* and cause damage to biomolecules ('oxidative damage'; reviewed in [5]). Such damage occurs constantly *in vivo*, and cells must repair it (DNA, proteins and RNA to a limited extent) or replace the damaged molecules (lipids, proteins to a large extent and RNA to some extent) [3, 33–35]. Indeed, defects in repair processes that allow oxidative damage to accumulate can contribute to disease development and the ageing process [5, 33–35]. In recent years, there have been major advances in the methodology to measure accurately the end-products of oxidative damage to proteins, lipids and DNA, especially the use of measurements of isoprostanes as a robust biomarker of lipid peroxidation [5, 36–44]. Measurements of isoprostanes and certain other biomarkers are now giving insights into how oxidative damage can be modulated *in vivo* in humans (as summarized in Table 1). In several (but not all) clinical studies, high levels of certain oxidative damage biomarkers seem to correlate with higher risk of disease (discussed in [4, 5, 36–46]). It should be noted that accurate measurements of biomarkers of oxidative damage require suitable and rigorously validated methodology, usually based on mass spectrometry [37, 39, 40, 42, 43]. Please be wary of 'kit-based' methods, where the reliability and validity are often uncertain (e.g. discussed in [5, 47–51]).

Why does oxidative damage occur? Why have aerobes not simply evolved better antioxidant defences to prevent it? Perhaps they cannot. For example, $OH^{\bullet}$ reacts so fast with biomolecules that any putative scavenger of it would have to be present at unfeasibly high concentrations to compete with endogenous biomolecules for any $OH^{\bullet}$ generated [4, 5]. A better strategy to minimize damage by $OH^{\bullet}$ is to remove H_2O_2 when it is not needed, or to sequester safely the transition metal catalysts needed for $OH^{\bullet}$ formation by the Fenton reaction [5, 52]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe(III) + OH^{\bullet} + OH^{-}$$

so as to decrease $OH^{\bullet}$ formation as far as possible. A second reason for ongoing oxidative damage relates to the increasing evidence that ROS, especially H_2O_2, play important metabolic and signalling roles *in vivo* (reviewed in [4,

Table 1

What can alter levels of oxidative damage levels in humans or other animals?

Obesity (humans, rodents)
Hyperglycaemia (humans, rodents)
High plasma low-density lipoprotein cholesterol (humans, rodents)
High-cholesterol diet (rabbits and rats; probably not humans)
Zinc intake (rabbits, some other animals; human data inconclusive)
Body iron levels (rabbits, rats, mice, maybe humans)
Certain foods (humans, e.g. dark soy sauce, tomato; rodents)*
Diabetes (in some human studies, not others)†, but probably not the metabolic syndrome
Intake of polyunsaturated fatty acids‡ (docosahexaenoic acid, possibly eicosapentaenoic acid, humans)

*It is essential to carry out appropriate controls in testing effects of foods, because the consumption of any food (antioxidant or not) can sometimes alter levels of certain biomarkers. †May depend on how well glucose and lipids have been normalized in the diabetic cohorts studied, or on the degree of obesity, because hyperglycaemia, hyperlipidaemia and obesity can all increase F_2-isoprostane levels, i.e. it may not be diabetes *per se* but its sequelae or predisposing factors that cause the oxidative stress (at least as revealed by studies of F_2-isoprostanes). ‡Despite the propensity of polyunsaturated fatty acids to oxidize *in vitro*, growing evidence suggests that they minimize oxidative damage *in vivo*. This table is adapted from references [4] and [44] with permission. For full details and references, please see [4, 44].

5, 53–57]. Hence, humans and other animals appear to have evolved an integrated network of ROS-generating systems and antioxidant defences that allows some ROS to do useful work while minimizing (but not eliminating) their potential to cause oxidative damage to biomolecules. To quote [4], 'in order to allow extra H_2O_2 to be quickly generated, perform its signalling function and be removed, the subcellular location and activities of NADPH oxidases, dual oxidases and other sources of H_2O_2 such as mitochondria must be carefully aligned on a second by second basis with the location and activities of antioxidant defence systems'.

Oxidative damage contributes to human disease (partly true)

The criteria for deciding whether oxidative damage plays any role in human disease have been set out in several publications [5, 36, 44, 58] and need not be repeated here. Disease-related tissue injury (indeed, tissue injury by any mechanism) leads to increased ROS production that may (or may not) contribute significantly to the disease pathology [4, 5, 58]. My current view (for reasons explored in detail in the references cited below) is that ROS are significant contributors to the origin and progression of cancer [4, 5, 59–61] and of neurodegenerative diseases, especially Alzheimer's disease [5, 43, 62–64]. In atherosclerosis, the role of ROS is less clear. There is certainly increased oxidative damage, but ROS may do harm in some ways and good in others; hence, their overall contribution to the origin and progression of atherosclerosis remains uncertain (reviewed in [5, 65, 66]). In chronic inflammatory diseases, ROS cause tissue damage [4, 5] but can also act as modulators of inflammation, helping to resolve it [67–70], so their overall contribution is even less clear.

Let us therefore modify the statement at the beginning of this section to read, 'ROS are significant contributors to certain human diseases', cancer and dementia being frontline candidates. Neurodegenerative diseases have the problem, of course, that any active antioxidant agents to be tested for treatment or prevention need to cross the blood–brain barrier; several diet-derived antioxidants are thought not to do so (e.g. carotenoids) or to do so only to a very limited extent (e.g. polyphenols; discussed in [5, 63, 71].

Diminishing oxidative damage by administering antioxidants will therefore decrease disease incidence (yes, it would in certain diseases if the antioxidants did diminish oxidative damage)

There is an extensive literature on the effects of administering high doses (pharmacological rather than nutritional) of dietary antioxidants (usually carotenoids, ascorbate or vitamin E) on cancer development. It may be broadly summarized as 'no evidence of effectiveness unless there is pre-existing dietary deficiency (e.g. in some studies in China or Africa) and a suggestion of harm in some cases' [5, 72–75]. For dementia, the conclusion is not quite so bleak; there is some evidence consistent with limited effectiveness of vitamin E in slowing progression of dementia, but it is very mixed and inconclusive [76, 77]. Of course, vitamin E supplementation has great difficulty in raising its levels in the brain. Perhaps, if more of it could get in then it would have greater effectiveness [78, 79]. Or perhaps not!

What explains this lack of effectiveness of dietary antioxidants? One hypothesis would be that ROS do not matter in cancer and dementia, but the bulk of evidence seems inconsistent with that view [5, 59–64]. One assumption behind all these intervention studies is that administration of high doses of ascorbate, carotenoids, vitamin E etc. to humans will indeed decrease levels of oxidative damage. Sadly, it generally does not; a convincing explanation for their lack of effectiveness [4, 5, 80]. It has recently been argued that the alleged anticancer actions of ascorbate may be due not to antioxidant but to pro-oxidant activity, an interesting reversal of concepts by some of the proponents of mega-C supplementation [81]. Certainly, ascorbate oxidizes readily *in vitro* to generate H_2O_2, e.g. in cell culture media [5, 82, 83]. However, such claims are moot until a convincing therapeutic effect of ascorbate in cancer is actually demonstrated, which it has not been to date. To quote [4], 'we are perhaps fortunate that diet-derived "antioxidants" do not markedly decrease oxidative damage in humans – because otherwise they might sometimes have caused harm rather than good', given the important biological roles of certain ROS.

Three further points are worth emphasizing. One is that the levels of oxidative damage measured in laboratory animals seem more responsive to being decreased by dietary antioxidants than they are in humans [4, 5, 44]. Thus, it is necessary to be cautious when attempting to extrapolate positive effects of antioxidants in rats, mice etc. (e.g. in models of atherosclerosis, stroke or neurodegeneration) to humans; they are unlikely to work as well, if indeed they work at all [4, 5, 44, 66]. Indeed, the antioxidant content of animal feed can significantly affect experimental results [84].

A second point is that many studies of antioxidants have been carried out on large groups of human subjects without much attention being paid to their baseline nutritional status (e.g. if they are already well nourished, with optimal levels of vitamins C, E etc., so that extra will not give any benefit) and no attention at all being paid to their 'oxidative damage status' (discussed in [80]). Many studies have revealed a wide variation in levels of antioxidants and of biomarkers of oxidative damage (e.g. F_2-isoprostanes and urinary 8-hydroxydeoxyguanosine levels) between individuals [40, 41, 85–89]. Perhaps, as mentioned in [4, 32], we should only test the effects of antioxidants on the most 'rancid' people, who may be those at greatest risk of disease [40–43, 90–96].

Point three is that whereas nutritional antioxidants do not seem to decrease systemic oxidative damage, they have the potential to exert effects in the gastrointestinal tract, for instance because polyphenols, carotenoids and tocopherols can reach high concentrations there if the diet is rich in them [15, 97, 98]. Equally, however, some 'antioxidants' (e.g. polyphenols and ascorbate) could exert pro-oxidant effects, because transition metals, such as iron and copper, that can catalyse oxidation reactions are present in stomach and intestinal contents (reviewed in [97]). Nevertheless, it is possible to argue that mild pro-oxidant effects could even be beneficial, perhaps by increasing the levels of antioxidant defences, such as reduced glutathione, in the cells lining the gastrointestinal tract [4, 5, 22].

Conclusion

There is no good evidence in human populations 'overall' that in the absence of deficiency, consuming high levels of nutritional antioxidants will protect against disease development. Whether they would benefit the 'rancid' subset of the population is uncertain, and the topic needs further study. More-established ways to decrease one's oxidative damage level seem to be by consuming diets rich in certain polyunsaturated fatty acids (an ironic observation, given the ready tendency of polyunsaturated fatty acids to oxidize *in vitro* [5]; or is that why they work, perhaps?). Avoiding obesity, hyperglycaemia and hypercholesterolaemia and perhaps keeping body iron stores low seem to minimize levels of oxidative damage (Table 1). Of course, these interventions are likely to work by multiple mechanisms, but the fact that they do work is consistent with the view that lowering oxidative damage would decrease risk of development of certain diseases; it is simply that supplements of diet-derived antioxidants do not generally decrease oxidative damage. There is also limited evidence from certain trials that mixtures of low doses of antioxidants [99] or antioxidants plus other nutrients [100] may be more beneficial, but again the data are mixed and confusing (e.g. references [101–103]).

One fairly well-established (despite recent challenges) 'fact' is that diets rich in plant products (grains, fruits and vegetables) help to maintain human health. Multiple reasons have been advanced to account for this (reviewed in [5, 104], including the presence of mild 'toxins' that activate the nuclear factor erythroid 2 p4-5-related factor 2 system [21, 22, 104]. Indeed, Mattson and Cheng [22] have used the term 'neurohormetic phytochemicals'. Do antioxidants contribute to the health-promoting effects of plant-rich diets? The currently available data do not give a clear-cut answer. Some foods rich in antioxidants, such as tomato and dark soy sauce, do seem to exert a degree of antioxidant effect in the human body, but many others do not, or give confusing and contradictory results [105–112]. One possible reason for the variability is that many studies did not use controls with antioxidant-free food equivalents. This is not always easy to do, but it is essential to at least attempt it, because the simple act of eating can itself alter levels of some biomarkers of oxidative damage [105, 108, 113, 114].

So, eat well, including plenty of fruits, grains and vegetables, avoid obesity, don't smoke, exercise regularly (also a mild pro-oxidant challenge that triggers beneficial adaptation [115]) and your oxidative damage should be minimized!

Competing Interests

There are no competing interests to declare.

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