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Fruit Antioxidants: Are they Beneficial for Human Health?

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ABSTRACT

There is substantial evidence that links increased consumption of fruits with increased protection from various degenerative diseases, such as cardiovascular disease, some cancers, and the onset of dementia. Fruits contain numerous components that could contribute to this health enhancing effect; many fruit components have the capacity to function as antioxidants, at least *in vitro*, and the antioxidant properties of fruit foods are often viewed as contributing substantially to the health effect. This review will attempt to describe and summarise the current knowledge about the antioxidant capacity of the major fruit types and the components that are consumed when these fruits are eaten. Although fruit components have substantial antioxidant capacity, as measured by *in vitro* assays, the significance *in vivo* of the consumption of fruit antioxidants is far from clear. The review will consider and summarise the research on the effect of fruit antioxidants on human health, with the aim of presenting an overall picture of the current state of knowledge in this area.

Keywords: carotenoids, flavonoids, oxidative stress phytochemicals, polyphenolics, vitamin C

CONTENTS

INTRODUCTION

There is robust evidence from human studies that diets with greater amounts of fruits and vegetables lead to a reduced risk of several diseases, such as cardiovascular disease (CVD), some cancers, and the onset of neurodegenerative diseases (Steinmetz and Potter 1996; Ness and Powles 1997; Joshipura *et al.* 2001; Youdim and Joseph 2001; Knekt *et al.* 2002; Dai *et al.* 2006; Morris *et al.* 2006). This has led to the recommendation that individuals consume more than 400 g (or 5 servings) of fruits and vegetables per day (Anon 2003).

Fruits and vegetables contain a diverse range of phytochemicals that may act individually or in concert to produce disease-protective effects. Some of the phytochemicals thought to be associated with health benefits are carotenoids, polyphenolics, vitamins, folate, calcium, selenium, potassium, iron and dietary fibre. Even though it has long been recognised that increased fruit and vegetable consumption reduces the risk of chronic disease, it is not known definitively which component (or components) of fruits and vegetables are the main contributors of the health effect.

Many of the phytochemicals present in fruits and vegetables have demonstrable antioxidant properties and it is widely thought that it is these antioxidant properties that convey the health benefits of a diet rich in fruits and vegetables. The antioxidant phytochemicals present in fruits are the focus of this review and following introductory comments about antioxidants and the variety of antioxidant phytochemicals found in fruits, the current status of our knowledge on the relevance of fruit antioxidants to human health will be discussed.

ANTIOXIDANTS

Antioxidants and health

The term 'antioxidant' has become well known over the last decade and the consumption of antioxidants is widely regarded as being beneficial to health and well-being (Waterhouse 2006). This interest has led to a large increase in the number of foods that are promoted as high in 'antioxidants'.

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Invited Review

The interest in antioxidants arises from the convergence of two lines of investigation. Firstly, humans as aerobic organisms utilise oxygen for the production of energy and are therefore continuously exposed to potentially damaging free radicals that may produce oxidative damage in critical biochemicals leading to dysfunctional processes. Although humans, and in fact all aerobic organisms, rely on built-in mechanisms for protection from oxidative damage, these mechanisms are not absolutely effective and their decreasing efficacy with ageing is believed to be responsible for age-related declines in function and the increased risk of many degenerative diseases. There is accumulating evidence that oxidative damage is associated with many, if not most degenerative diseases (Halliwell and Gutteridge 2007; Valko *et al.* 2007). Secondly, many population-based (epidemiological) studies have shown that the consumption of fruits and vegetables is beneficial to health and may provide protection against conditions such as cardiovascular disease, some cancers, neurodegenerative diseases, and others. As fruits and vegetables contain substantial amounts of antioxidant phytochemicals, an often stated hypothesis is that antioxidants are, at least partly, responsible for the health benefit, and therefore it is desirable to consume more antioxidants. Consequently, there is a view that consumption of foods (or supplements) with high concentrations of antioxidants will provide protection from the inevitable oxidative damage, with concomitant protection from diseases. Here we consider the antioxidant phytochemicals of fruits and the evidence that fruit preparations may have biological antioxidant properties that protect against oxidative damage *in vivo* and may provide protection of oxidative stress-related diseases.

What is a food antioxidant? Properties and functions

Chemically 'antioxidants' are compounds that are oxidised by readily donating an electron or hydrogen atom to another molecule. Therefore, by definition, potent antioxidants are reactive and relatively unstable compounds. Biologically, 'antioxidants' use this chemical reactivity to protect other molecules from oxidation by acting through a variety of mechanisms. Antioxidants may: 1) react preferentially with reactive oxygen and nitrogen species (ROS/RNS) to protect labile molecules from oxidation; 2) regulate the redox environment; 3) bind transition metals; and 4) induce the production of endogenous antioxidants. It is interesting to note that these last two biological definitions of antioxidants need not relate directly to the chemical definition for antioxidants. Although antioxidant properties are due to specific chemical features of molecules, many different types of compounds exhibit both chemical and biological antioxidant properties. The chemical antioxidant properties of compounds (or mixtures) can conveniently be measured *in vitro* by a wide variety of antioxidant assays and the relative methodologies and mechanisms have recently been reviewed (Huang *et al.* 2005; Moon and Shibamoto 2009). It is important to realise that these assays measure chemical antioxidant properties and they therefore represent the potential **capacity** of a food to function as a dietary antioxidant. Clearly foods that have low antioxidant capacity values (as measured by *in vitro* methods) are less likely to function as *in vivo* antioxidants as foods that have high antioxidant capacity. However, a high antioxidant capacity measurement for a food may not produce *in vivo* antioxidant effects since other factors such as bioavailablity and metabolism also need to be considered. Furthermore, any given plant-based food is likely to contain a variety of antioxidant components, with different chemical properties that combine to represent the antioxidant capacity of that food, and these compounds may behave entirely differently *in vivo* depending on their chemical characteristics. The most obvious of these chemical properties is solubility, with water-soluble antioxidants (e.g. vitamin C) tending to be present in hydrophilic *in vivo* compartments, whereas fat-soluble antioxidants (e.g. carotenoids) will be present in lipophilic compartments.

ANTIOXIDANT COMPONENTS OF FRUITS

The world fruit bowl is very large and global fruit consumption has been estimated at approximately 503 million tonnes per year (FAOSTAT 2005, www.fao.org). The composition of the global fruit bowl is depicted in **Fig. 1** and comprises bananas (14.2%), grapes (13.2%), oranges (12.5%), apples (12.3%), berries (1.5%), other tropical fruits (21.1%), other deciduous fruits (15.9%), and other citrus fruits (9.1%). It is interesting to note that although there is a great variety of fruits potentially available; bananas, grapes, oranges, and apples represent 52% of the fruits consumed globally. The antioxidant capacity and the concentrations of the antioxidant phytochemicals present in the most widely consumed fruit are presented in **Table 1**. These data show that there is substantial variation in antioxidant components and the corresponding *in vitro* antioxidant capacities for the different fruit types. Even though this discussion will concentrate on the major phytochemicals present in the most widely consumed fruits, it is recognised that some potentially important phytochemicals are present in fruits with relatively low global consumption. An excellent review of the health-promoting compounds in fruits has recently been published (Jaganath and Crozier 2008). Following is a brief description of the main antioxidant phytochemicals present in fruits.

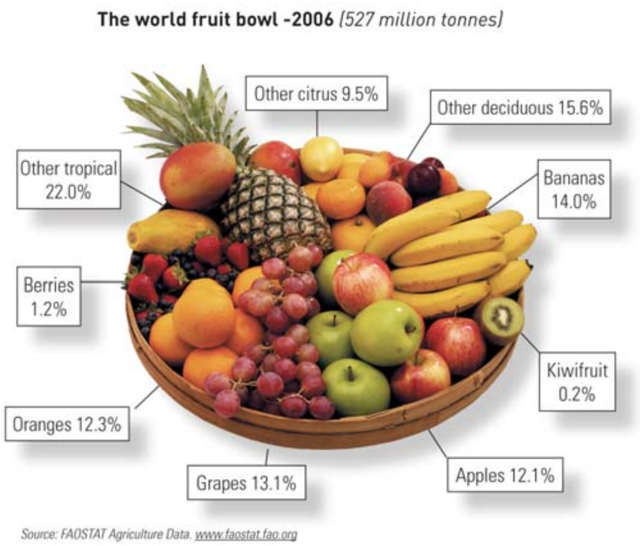

Fig. 1 The global fruit bowl, showing the major fruits consumed globally and the relative proportions of each fruit type.

Antioxidant vitamins

Vitamins A, C and E are probably the best known dietary antioxidants whose values have been appreciated for a considerable time. Vitamin C (ascorbic acid, **Fig. 2**) will only be discussed here, as fruits are relatively poor sources of vitamin E, and vitamin A will be mentioned in the discussion on provitamin A carotenoids below.

Vitamins, by definition, are essential for human health and well-being and much has been written about ascorbic acid. For an comprehensive discussion of vitamin C, readers are referred to the book The Antioxidant Vitamins C and E (Packer *et al.* 2002).

Ascorbic acid (**Fig. 2**) has many important roles including direct antioxidant activity and as a co-factor for enzymes involved in the biosynthesis of collagen, catecholamines, carnitine, cholesterol, amino acid and some peptide hormones (Padayatty *et al.* 2003). In all these functions, the activity of ascorbic acid is mediated through an ability to donate a single electron, resulting in the production of the ascorbate free radical. Dehydroascorbic acid is generated by

Table 1 Concentrations of a selection of the antioxidant compounds present some of the major fruits consumed globally.

Antioxidant component	Banana	Grape	Orange	Apple	Peaches	Strawberries	Raspberries	Blackberries	Kiwifruit
Antioxidant capacity[A] (umol TE/100 g)	879	1260	1819	3082	1814	3577	4882	5347	882
Vitamin C[B] (mg/100 g)	8.7	4	45	4.6	6.6	58.8	26.2	21	75
Vitamin A[B] (IU/100 g)	64	100	225	54	326	12	33	214	175
Vitamin E[B] (mg/100 g)	0.10	0.19	0.18	0.18	0.73	0.29	0.87	1.17	1.46
Carotenoids[C] (μg/100 g)									
lutein	22	49	92	51	60	17	n.d.	n.d.	138
β-carotene	38	23	25	22	84	8	20	78	30
β-cryptoxanthin	n.d.	n.d.	266	10	70	n.d.	n.d.	n.d.	n.d.
Flavonoids[D] (mg/100 g)									
quercetin	n.d.	1.38	0.58	4.27	0.68	1.14	1.23	1.76	n.d.
kaempferol	n.d.	0.01	0.01	0.02	n.d.	0.46	0.09	0.06	n.d.
myricetin	n.d.	n.d.	0.02	n.d.	n.d.	n.d.	n.d.	0.67	n.d.
hesperetin	n.d.	n.a.	27.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
naringenin	n.d.	n.a.	15.32	n.d.	n.d.	0.26	n.d.	n.d.	n.d.
Total ACNs[D] (mg/100 g)	7.39	44.86	n.d.	2.44	1.61	33.63	38.38	90.46	n.d.
Total PP[E] (mg GAE/100 g)	51.5	195.5	31.0	179.1	59.3	263.8	51.7[F]	49.5[F]	28.1
Total PA[G] (mg/100 g)	3.4	61.6		127.8	71.8	141.7	25.1	23.3	3.2

[A] – USDA Oxygen Radical Absorbance Capacity (ORAC of Selected Foods – 2007 (http://fnic.nal.usda.gov/)
[B] – USDA National Nutrient Database for Standard Reference Release 18 (http://fnic.nal.usda.gov/)
[C] - Holden *et al.* 1999; O'Neill *et al.* 2001
[D] – USDA Database for the Flavonoid Content of Selected Foods Release 2.1 (http://fnic.nal.usda.gov/) ACN = anthocyanidins
[E]- Brat *et al.* 2006; PP= polyphenols; GAE = gallic acid equivalents
[F] - Wada and Ou 2002
[G] – USDA Database for the Proanthocyanidin Content of Selected Foods (http://fnic.nal.usda.gov/); PA = pranthocyanidins
TE = Trolox® Equivalents; n.d. = no data

a further single-electron oxidation of the ascorbate free radical, which may be reduced back to ascorbic acid by intracellular processes such as nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductases (Harrison and May 2009). Ascorbic acid is highly bioavailable and the transport mechanisms into cells have been studied in detail (Harrison and May 2009). It is interesting to note that the ascorbic acid concentration is many times higher in most tissues and organs than in the plasma – evidence that cells accumulate ascorbic acid against a substantial concentration gradient, which serves to underline the importance of this compound (Harrison and May 2009). The potential role of vitamin C in human disease has been recently reviewed (Padayatty *et al.* 2003).

Carotenoids

The many orange and red colours of fruits are due to the presence of carotenoid pigments. Carotenoids are C_{40} tetraterpenes (**Fig. 2**) and are highly coloured, fat soluble and powerful chemical antioxidants. Both the highly coloured nature and the antioxidant properties of carotenoids are due to the extended electronic conjugation of the multiple double bonds present in the carbon backbone of the molecule (**Fig. 2**). Although over 600 carotenoid compounds are known, only about 40 are normally present in the human diet. From a dietary perspective the most important carotenoids are lycopene, β-carotene, lutein, and β-cryptoxanthin (Rao and Rao 2007).

Carotenoids have a variety of natural functions including ecological roles of pigmentation for attraction and pollination of flowers, in addition to biochemical roles for protection during light-harvesting and electron transport in the photosynthetic structures in chloroplasts (Britton 1995). As dietary components, carotenoids are also thought to have a protective role in humans not only because of their antioxidant properties, but also through their ability to induce gap junction communication by increasing expression of connexion 43 (Bertram 1999), and a role in protecting specific tissues from light-induced damage, for example the macular region of the eye (Rao and Rao 2007). Of special importance is the ability of some (but not all) carotenoids to function as provitamin A. Vitamin A (retinol) has a specific role in vision and gene expression, and vitamin A deficiency is a leading cause of blindness. It is estimated that 250 million preschool children are vitamin A deficient and that up to 500,000 vitamin A deficient children become blind each year, with half dying within 12 months (World Health Organisation 2009).

Polyphenolics

Polyphenols are molecules that contain aromatic groups with more than one hydroxyl constituent. Many polyphenolics are pigmented, such as the red/blue anthocyanins, but most are non-coloured.

1. Phenolic acids

The phenolic acids found in fruits are mainly derivatives of benzoic (e.g. gallic acid) or cinnamic acids (e.g. chlorogenic acid) (Macheix *et al.* 1990) (**Fig. 2**) and are relatively abundant especially in the Rosaceae fruits (apple, strawberry, raspberry and blackberry). Phenolic compounds have antioxidant activity and therefore contribute to the total antioxidant capacity of fruits. Often polyphenolics are measured as a total as in the Folin-Ciocalteu assay (Brat *et al.* 2006) not as individual compounds. Unfortunately the Folin-Ciocalteu assay often referred to erroneously as 'total phenolic assay' but is in fact a type of antioxidant assay and suffers from interference from other non-phenolic antioxidant fruit components, in particular ascorbic acid (Singleton *et al.* 1999; Huang *et al.* 2005). Nevertheless the 'total phenolic' content of fruits is often presented and associated with their health benefits. Brat and colleagues have recently shown that fruits contribute more 'total phenolics' to the French diet than vegetables, and that apples, of all fruits, make the largest contribution to 'total phenolics' in the diet (Brat *et al.* 2006).

2. Flavonoids

Flavonoids are a large group of polycyclic, polyphenolic compounds based on a C_6-C_3-C_6 skeleton containing a heterocyclic ring (C_3) between two aromatic groups (**Fig. 2**). The flavonoid group is further subdivided depending on the degree of saturation of the heterocyclic ring; subgroups include flavonols, flavanones, flavan-3-ols, anthocyanins, flavones, and isoflavones (**Fig. 2**). The antioxidant capacity of flavonoids is due to the presence of hydroxyl groups and is largely due to an *ortho* dihydroxy moiety on the B ring, a 2,3 double bond in combination with 4-oxo, and the pre-

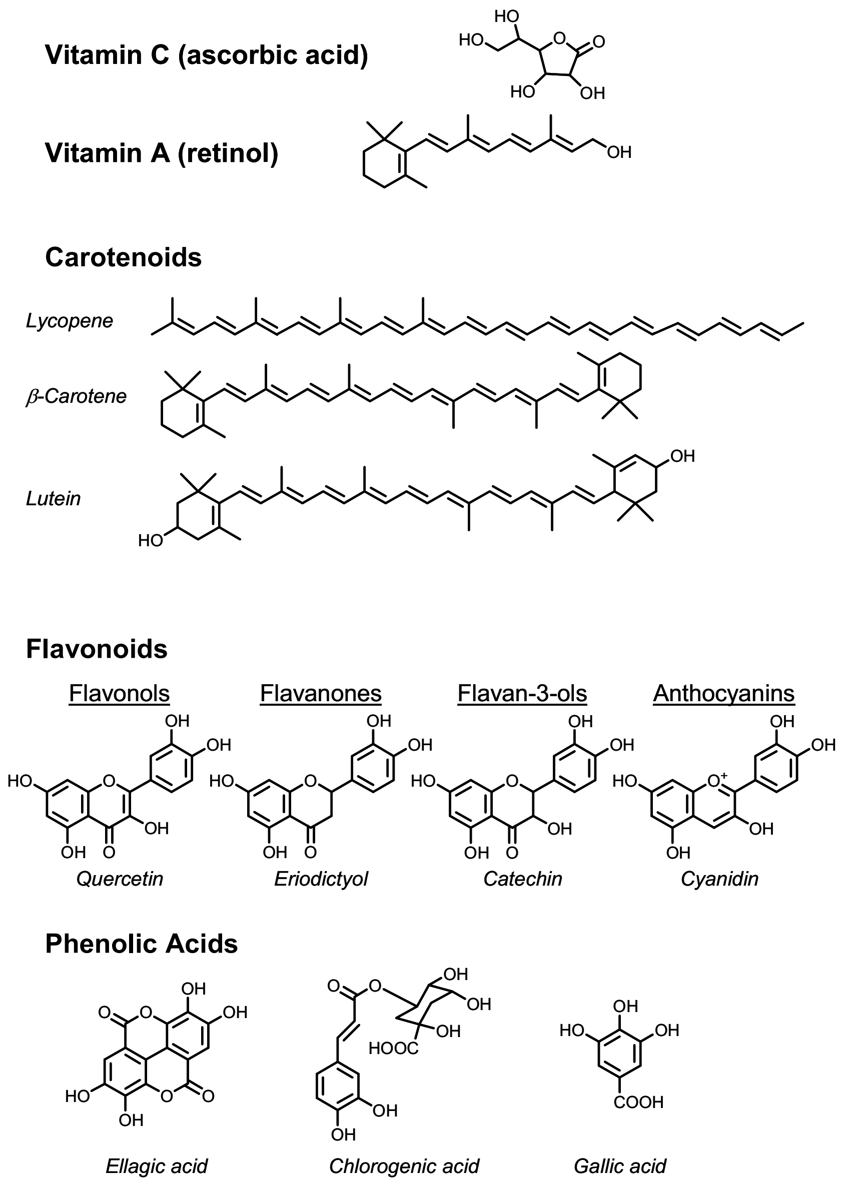

Fig. 2 Chemical structures of the main antioxidant components of fruits.

sence of an OH on carbons 3 and 5 (Rice-Evans *et al.* 1996). Quercetin has all these features and is considered one of the most powerful antioxidants of all the flavonoids and is frequently studied (Boots *et al.* 2007). Although flavonoids have substantial antioxidant capacity, their bioavailability in animals and humans is relatively low and the concentrations obtained in plasma, organs and tissues may be too low to exert a chemical 'antioxidant' effect (Manach *et al.* 2005; Lotito and Frei 2006). Flavonoids are produced in plants and have a variety of functions, including signalling and defence against pathogens. It is becoming clear that in humans and animals flavonoids also modulate signalling cascades, especially intracellularly (Williams *et al.* 2004) and the bioactivity of flavonoids may be only partly related to their antioxidant activity. For example, in a recent study it was shown that three potent flavonoid antioxidants (quercetin, myricetin, and delphinidin; (Rice-Evans *et al.* 1996)) all provided protection against ischemia/reperfusion-injury in isolated rats' hearts, but that myricetin and delphinidin were more effective because of an ability to inhibit signal transducers and activators of transcription 1 (STAT1) activation. This ability appears to be due to a direct molecular interaction of myricetin and delphinidin with STAT1, which was absent for quercetin (Scarabelli *et al.* 2009). Thus it is becoming more apparent that the health benefits associated with flavonoids may be related to a variety of mechanisms.

Variability of antioxidant phytochemicals in fruits

Fruits contain substantial quantities of antioxidants and approximately 20% of the 50 foods with the highest antioxidant contents are fruits (Halvorsen *et al.* 2006). Other foods with high antioxidant contents are herbs and spices (cloves, oregano, ginger, cinnamon, tumeric powder), nuts (walnuts, peacans), whole grain cereal, and beverages (tea, coffee). Furthermore, fruits are often consumed in relatively high amounts suggesting that fruit consumption makes a substantial contribution to the intake of antioxidant phytochemicals in human populations. There are now abundant data describing both the antioxidant capacities (as measured by antioxidant assays such as the Oxygen Radical Absorption Capacity (ORAC), and Ferric Reducing Antioxidant Power (FRAP)) (Halvorsen *et al.* 2006; Anon 2007) and the composition concentrations of antioxidant phytochemicals in fruits. However, it needs to be kept in mind that not all types of fruit are equivalent with respect to antioxidant phytochemicals, as shown by the data in **Table 1**. Although many studies have attempted to measure antioxidant capacities to compare fruit types, other studies have shown that there are also considerable differences in the phytochemical contents between cultivars of the same fruit species, and within germplasm populations used by breeders to develop new fruit cultivars. For example, we recently showed that the variation in antioxidant polyphenolics in apple cultivars commercially grown in New Zealand is almost three-fold (McGhie *et al.* 2005). Thus although the simple message of '5+ A Day' has considerable merit for the consumer, the judicious selection of fruit type and specific cultivars of each fruit type also has the potential to markedly modify the intake of fruit antioxidants by consumers. For example, a recent human intervention study investigating the effects of high and low antioxidant diets, used diets that both contained 5.0 and 5.3 servings of fruit and vegetable, illustrating that the consumption of antioxidants by the general population is likely to vary depending on *which* fruits and vegetables are selected by individuals for inclusion in their diet (Valtuena *et al.* 2009).

Breeders use large germplasm collections to develop new fruit cultivars with improved qualities. Increasingly fruit breeding programmes are targeting quality parameters associated with health (McGhie and Currie 2008), particularly antioxidant phytochemicals. The first step in breeding for a new parameter is to establish that sufficient variation exists within the germplasm and breeding populations. For example, in our research with New Zealand blackcurrants the analysis of 250 genotypes of blackcurrant showed that antioxidant capacities ($ORAC_{FL}$: 71-194 μmol TE/g FW, and FRAP 25-86 μmol TE/g FW), vitamin C (68-274 mg/100 g FW) and total anthocyanins (180-732 mg/100 g FW) all varied sufficiently to justify including these parameters as breeding targets. Furthermore anthocyanins were found to be the primary contributors to antioxidant capacity in blackcurrant ($ORAC_{FL}$ R=0.8655; FRAP R=0.7197). Surprisingly, although the blackcurrant genotypes studied contained substantial amounts of vitamin C, the correlation between vitamin C concentrations and antioxidant capacity was not high ($ORAC_{FL}$ R=0.0743; FRAP R=0.2551) suggesting that vitamin C makes a minor contribution to the antioxidant capacity of blackcurrant. The outcomes of studies such as these show that there are substantial amounts of variation in antioxidant phytochemical concentrations between genotypes of the same species, and indicate that progress can be made towards increasing the phytochemical contents of fruit by breeding new fruit cultivars.

Bioavailability and metabolism of fruit antioxidant phytochemicals

The type of fruits consumed, together with any manufacturing and storage processes used during food production, determine both the quantity and types of fruit phytochemicals consumed by humans. However, it is the chemical structures of the compounds that are responsible for the beneficial health effects, and these are also modified by metabolic processes that occur during consumption, bioabsorption, and transport to the location where bioactivity occurs. Tissues in the upper gastrointestinal tract will be exposed to many of the compounds present in consumed fruits, whereas tissues in the lower gastrointestinal tract may be exposed to the original phytochemicals and additionally to metabolites generated during passage through the intestinal tract. When phytochemicals are absorbed from the gut into the portal vein, they often undergo metabolic conversion during the absorption process and further modification may occur in organs, particularly in the liver. The actual mechanisms of bioabsorption and metabolism that occur are a function of the chemical properties of the individual compounds and may vary substantially between phytochemical types and between similar compounds within each sub-class. For example, carotenoids are fat-soluble compounds and absorbed in micelles that also contain food lipids and bile acids; therefore co-consumption of fat is necessary for optimal bioabsorption of carotenoids (Van het Hof *et al.* 2000; Yeum and Russell 2002). Flavonoids are water-soluble compounds and require specific cellular transportation; however, differences exist between related flavonoids. For example, quercetin exists primarily as glycosides in fruits that are hydrolysed before the quercetin aglycone is transported into enterocytes, where metabolism (e.g. glucuronidation), takes place. Further metabolism (e.g. methylation) takes place in the liver, resulting in the presence of numerous quercetin metabolites in plasma (Mullen *et al.* 2006). In contrast, anthocyanins are also present in fruits as glycosides, are absorbed into the circulatory system intact as glycosides, where they may be modified by the liver (e.g. methylation) (McGhie *et al.* 2003; McGhie and Walton 2007). Furthermore the degree of metabolism appears to differ among the anthocyanins, with pelargonidin glycosides being much more susceptible to metabolism than delphinidin glycosides (Prior and Wu 2006). An example of progressive metabolism during transit through the gut are the ellagitannins that are present in fruits such as strawberries, *Rubus* berryfruits, and pomegranates. Ellagitannins are phenolic polymers and are successively converted into ellagic acid, urolithin D, urolithin C, urolithin A, and finally urolithin B during transit through the gut (Espin *et al.* 2007).

Bioavailability and metabolism *in vivo* are modulated by various factors related to the human consumer. For example, individuals show substantial differences in the bioavailability of anthocyanins, which may be due to genetics, gut features (e.g. microbial), or a result of habitual consumption (McGhie *et al.* 2003). Bioabsorption is effected by co-consumed foods, not just for carotenoids, but also for anthocyanins (McDougall *et al.* 2005; Walton *et al.* 2008) and flavonols (Boyer *et al.* 2004). Finally, the gut contains substantial and diverse microbial populations and most fruit phytochemicals are subjected to considerable microbial metabolism with very little of the original phytochemical consumed being excreted unmodified in the faeces (Jenner *et al.* 2005). Colonic microbia are responsible for the generation of a large number of smaller molecular weight phenolic compounds, which may then be absorbed or contribute to bioactivity locally in the colon (Fleschhut *et al.* 2006; Kahle *et al.* 2007).

It is important to appreciate that the type of metabolism that occurs *in vivo*, and the degree of bioavailability, are essential determinants of beneficial bioactivity and are critical parameters to consider in the design and application of *in vitro* bioactivity studies.

FRUIT ANTIOXIDANTS AND HEALTH

Oxidative stress and disease

Diets rich in antioxidants are assumed to be beneficial to health because they protect the body from oxidative stress caused by an over-production of ROS/RNS, as depicted in **Fig. 3**. Both ROS and RNS are primarily produced as a result of normal cellular metabolism and function and although usually beneficial, can be harmful. Harmful effects develop when the available antioxidant network is overwhelmed by the production of ROS/RNS and lipids, DNA, and proteins are damaged. Beneficial effects of ROS/RNS are associated with signal transduction and responses to pathogens (Halliwell and Gutteridge 2007).

Organisms use an antioxidant network to limit oxidative stress and provide protection from oxidative damage and the rationale for the consumption of dietary antioxidants is that these compounds will supplement and assist endogenous antioxidants. The putative relationship between dietary antioxidants and oxidative stress is depicted in **Fig. 3**. In essence, harmful ROS/RNS generated from sources such as the metabolic utilisation of molecular oxygen, exposure to pollutants/UV radiation, and inflammation events are neutralised before damage to critical biomolecules such as lipids, proteins, and DNA occurs. While this notion is rather attractive, it is simplistic; it now appears that the effects of dietary antioxidants are rather more complex and much remains to be discovered about how, or indeed if, dietary antioxidants effectively combat oxidative damage.

Oxidatively damaged lipids, DNA and proteins are believed to lead to a variety of disease conditions and are proposed to be a key component of ageing. Although it has been unequivocally shown that oxidative stress is associated

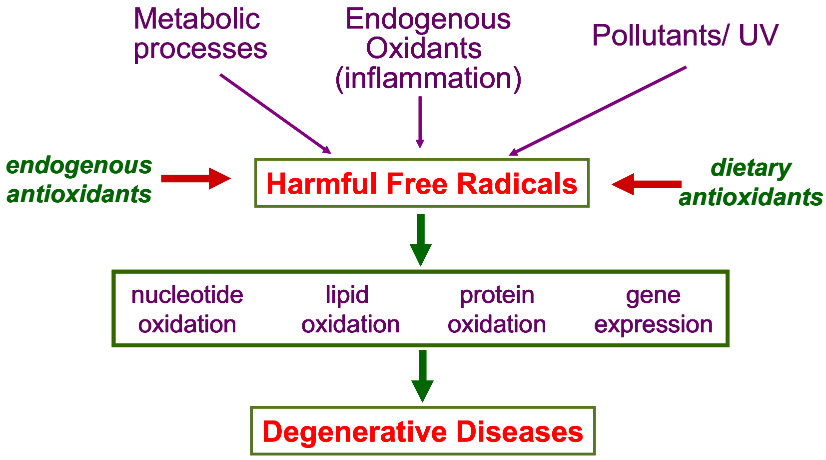

Fig. 3 Simplified view of how antioxidants might provide protection from disease. Damaging reactive oxygen (or nitrogen) species are produced and may damage critical biomolecules. Endogenous antioxidant systems intercept and destroy these reactive species before damage occurs. Dietary antioxidants are capable of enhancing and supplementing the endogenous antioxidant networks.

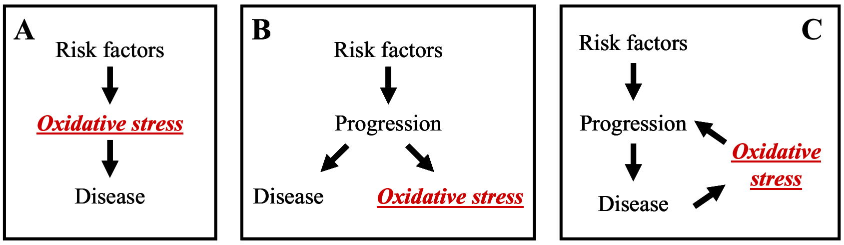

Fig. 4 Potential roles of oxidative stress in the pathogenesis of human disease. (**A**) Disease risk factors generate oxidative stress that leads directly to disease; (**B**) Disease risk factor initiates a progression to disease that generates oxidative stress unrelated to the disease; (**C**) Disease risk factors initiates a progression to disease that generates oxidative stress that directly enhances further progression of the disease.

with many diseases (Valko *et al.* 2007), the actual role of ROS/RNS in disease remains elusive and controversial for most diseases (Halliwell and Gutteridge 2007). As depicted in **Fig. 4**, oxidative stress can potentially participate in disease development in several different ways. It remains to be determined if oxidative stress is the cause of disease (**Fig. 4A**), a result of disease progression (**Fig. 4B**), or enhances the severity of the disease (**Fig. 4C**). It is more than likely that the type of contribution of oxidative stress to disease will vary with disease and it is important not to assume the one model of oxidative stress and disease is appropriate to all diseases. Progress in understanding the role of ROS/RNS in disease will be important in the development of antioxidant-based therapies as an improved understanding will enable causes and/or symptoms of diseases to be treated and identification of the correct molecular targets for protection by antioxidant therapy.

Dietary antioxidants: beneficial or not?

Initially population-based epidemiological studies discovered inverse relationships between the consumption of dietary antioxidants and some diseases, in particular cardiovascular disease, and were the cause of considerable optimism that diet-based approaches would help to reduce the rates of many degenerative diseases. These studies have recently been summarised by Willcox and co-workers (Willcox *et al.* 2008). Additionally the protective effects observed in these studies were supported by the increasingly held view that oxidative stress was closely associated with many diseases, in particular cardiovascular disease. To provide further evidence for the antioxidant hypothesis, a number of large-scale human intervention studies were initiated to test the beneficial effects of antioxidant vitamins. Meta analyses of these intervention studies have found no benefit for consuming antioxidant supplements (Bjelakovic *et al.* 2004; Bjelakovic *et al.* 2007), raising the possibility that the antioxidant hypothesis is incorrect. However, several authors have offered reasons for the apparent discrepancy between the intervention studies and the evidence supporting the antioxidant hypothesis (Steinhubl 2008; Willcox *et al.* 2008; Halliwell 2009). Reasons include 1) insufficient length of study - is a relatively short intervention sufficient to reverse a life-long dietary deficiency? 2) inappropriate study populations that may contain a substantial proportion of non-responders because of a satisfactory nutritional status or genetic predisposition; 3) the effectiveness of single (supplements) versus multi-component (whole foods) antioxidants; 4) insufficient power to detect differences in the primary outcomes, especially when large number of non-responders are present in the experimental populations; and 5) inadequate methods to assess the effects of dietary antioxidants, such as measures of oxidative damage. Some, or all, of these issues may have affected the outcomes of these studies and future studies will no doubt help to clarify what has become known as the 'antioxidant paradox'.

Fruit antioxidants (phytochemicals): Are they beneficial to health?

Do the largely negative results of the human intervention studies for vitamin supplementation mean that the beneficial effects so clearly apparent for fruit and vegetable consumption are not related to the antioxidant components? Or perhaps an alternative question to consider is – are the effects of large doses of single antioxidant vitamins comparable with the multi-antioxidant phytochemical exposure likely to occur with fruit consumption? Although the outcomes of the supplement intervention studies require that we adjust our understanding of the role of dietary antioxidants, the following issues need to be considered when applying the results of the supplement intervention studies to the benefit of fruit antioxidants.

A number of the human intervention studies using supplements have targeted vitamin E, but fruits are generally poor sources of vitamin E (except avocado), suggesting that the failure of vitamin E in supplement studies has little relevance to the antioxidant efficacy of most fruits. In contrast, fruits are good sources of vitamin C and several studies investigating diet composition have shown that increased consumption of fruits is correlated with increased plasma concentrations of vitamin C (Dauchet *et al.* 2008). Vitamin C is an excellent antioxidant and believed to be an essential component of the *in vivo* antioxidant network (Packer *et al.* 2002; Padayatty *et al.* 2003; Duarte and Lunec 2005; Winterborn 2008). Furthermore, recent studies continue to demonstrate that vitamin C acts as an antioxidant *in vivo*. For example, Block and colleagues have shown that treatment with vitamin C significant lowers F_2-isprostanes, a biomarker of lipid peroxidation (Block *et al.* 2008) in obese humans. More recently this group has also shown that vitamin C reduced elevated C-reactive protein, a inflammatory biomarker related to cardiovascular disease (Block *et al.* 2009).

Recent animal studies continue to show that the consumption of dietary antioxidants reduces oxidative stress. For example, our group has conducted studies on rats to determine if berry fruit antioxidants are able to reduce oxidative stress (Barnett *et al.* 2007). Oxidative damage to protein, lipids and DNA was assessed by measuring plasma carbonyls, malondialdehyde, and urinary 8-oxo-2'deoxyguanosine; plasma antioxidant status ($ORAC_{FL}$) and vitamin E concentrations were also included. Animals were conditioned to different base diets (chow, synthetic/soybean (SO), and synthetic/fish oil (FO)) and fed a Boysenberry extract, which contained substantial *in vitro* antioxidant capacity, at 2 and 10% of the diet for two weeks. The results showed that the Boysenberry extract raised the total antioxidant capacity of plasma while decreasing some biomarkers of oxidative damage, but the effect was highly modified by the base diet. The base diets had significant effects on the biomarkers of oxidative damage and antioxidant status, with rats fed the FO diet having the lowest levels of oxidative damage and the highest antioxidant status. For example, plasma malondialdehyde concentrations were significantly lower for the FO-fed rats than for SO-fed rats (45 v.182 ng/mL).

Addition of Boysenberry extract to the diet had little effect on 8-oxo-2'deoxyguanosine excretion in urine; however, protein carbonyls were significantly decreased, and plasma MDA either increased or decreased depending on the base diet. For example, the protein carbonyl concentration was significantly less for chow-fed rats with Boysenberry added to their diets than without Boysenberry (0.07 v. 0.21 nmol/mg protein). Interestingly, malondialdehyde concentrations decreased to 36% of the control for the SO-fed rats, increased by 256% for FO-fed rats, and remain unchanged for chow-fed rats when 10% Boysenberry extract was added to the diet. These results show that Boysenberry-derived antioxidants do function as *in vivo* antioxidants in some situations. Other studies using animals have also shown that antioxidant phytochemicals function as *in vivo* antioxidants, according to the definition of a biological antioxidant given above (Kolosova *et al.* 2006; Shin *et al.* 2006; Andrade and Burgess 2007; Luangaram *et al.* 2007; Dulebohn *et al.* 2008; Wong *et al.* 2009).

Despite the failure of large-scale human studies to show beneficial effects of some antioxidant vitamins, particularly vitamin E, a number of smaller studies have shown that fruit antioxidant phytochemicals function *in vivo* as antioxidants. For example, our group found that both blackcurrant or Boysenberry juice drinks consumed daily could improve measures of oxidative status in elderly humans (McGhie *et al.* 2007). In this parallel-design, placebo controlled study the plasma total antioxidant capacity was significantly increased for participants in both the Boysenberry and blackcurrant drink treatments compared with those taking a placebo, and there was a trend to reduced plasma malondialdehyde in participants receiving the Boysenberry and blackcurrant treatments, although the decrease was not statistically significant. Additionally, other studies have shown that treatments containing antioxidant phytochemicals can modulate measures of oxidative damage in humans, but the effects vary (Bub *et al.* 2003; Sanchez-Moreno *et al.* 2003; Dragsted *et al.* 2004; Sanchez-Moreno *et al.* 2006; Block *et al.* 2008; Guo *et al.* 2008; Block *et al.* 2009). Antioxidant effects are not always observed (Duthie *et al.* 2006). It appears that oxidative stress can be decreased by dietary antioxidants in specific target populations when oxidative stress is elevated, but that in well-nourished, healthy individuals, additional consumption of antioxidants has little effect on the currently used biomarkers of oxidative stress.

Finally, recent epidemiological studies using improved methodology and compositional databases to calculate dietary intakes continue to find inverse relationships between fruit antioxidant consumption and disease outcomes. For example, the relationships between the various flavonoid sub-classes and cardiovascular disease mortality were examined in the Iowa Women's Health Study (34,489 participants) and it was found that the intake of certain flavonoid types (flavanones, anthocyanins) were significantly inversely associated with reduced risk of death due to coronary heart disease, cardiovascular disease and all causes of death (Mink *et al.* 2007). Very recently, a study investigating the effects of fruit and vegetable consumption of adolescents found that oxidative stress (measured as F_2-isoprostanes) was inversely correlated with intakes of fruits and vegetables, vitamin C, β-carotene, and flavonoids. In addition, serum C-reactive protein (a marker for inflammation) was inversely associated with fruit and vitamin C intakes (Holt *et al.* 2009).

CONCLUDING COMMENTS

Despite the conflicting results between the results of the supplement-intervention studies with results from *in vitro*, animal, and epidemiological studies, evidence to date strongly suggests that it is beneficial to consume more fruits and vegetables. It remains to be determined if antioxidant vitamin supplementation is of any benefit in well-nourished populations. At present, the best approach is to obtain sufficient nutrition by means of an adequate diet (where this is available) rather than through supplementation, to obtain the full health benefits from fruit components. However, there is little doubt that in vitamin-deficient situations (which are relatively common globally), vitamin supplementation may be of substantial benefit.

Although it may be that the antioxidant components of fruits help to reduce oxidative stress, inflammation and the progression of many diseases, it is also probable that the health benefits of fruits are due to a variety of mechanisms, including the ability to modulate signalling pathways and direct molecular interactions through ligand-receptor processes.

The beneficial functions of fruit phytochemicals are obviously determined by molecular structures that define the chemical and physiochemical properties of the compounds. Chemically, antioxidant compounds donate electrons or hydrogen atoms. However, biologically 'antioxidants' ameliorate oxidative stress and reduce (or control) the level of oxidative damage to critical biomolecules and probably provide protection from many diseases and may reduce the speed of ageing. 'Biological antioxidants' are not necessarily compounds that have overt chemical antioxidant effects *in vivo*. The term 'antioxidant' has thus become confusing and consequently not useful in a scientific context. Phytochemical antioxidants need to be redefined according to the biochemical properties they exhibit and the specific mechanisms by which they are beneficial to health, as these become known. Large-scale studies designed to 'demonstrate' or prove that antioxidants are beneficial (or harmful) are not helpful for discovering the true identities of the bioactive components of fruit phytochemicals, or the mechanisms by which they enhance health. The actions of dietary phytochemicals are biochemical in nature and it appears that they are very specific. Future research must be directed at discovering the precise nature of the interaction of the components of fruits with the key determinants of health in animals and humans. This is a challenging task, as the composition of fruits and diets and the concepts of healthfulness and protection from disease are all complex areas of research. What is required are robust collaborations between medical, food, and plant researchers to enable better utilisation of fruit (and vegetable) phytochemicals for the enhancement of health.

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