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

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’.
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 absolutely not capable of absolutely protecting and their efficiency decreases with 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 all 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) 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 vegetables and their functions, with different chemical properties that contribute 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 vegetables and their 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 bioavailability and methabolism 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 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.

**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...
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} terpenes (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 connexin 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

Polyphenolics 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_{6}) 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-
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, pecans), 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 the same servings of fruits and vegetables, yet indicating 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).

Breeder 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 (ORACv71 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 (ORACv71 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 (ORACv71 R=0.6743; 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 foods 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. Organisms use an antioxidant network to limit 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. Harmonic 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 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 or detoxified and protected from oxidative damage and the rationale for the consumption of dietary antioxidants is that these compounds will supplement and assist endogenous antioxidants.

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 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 or detoxified and protected from oxidative damage and the rationale for the consumption of dietary antioxidants is that these compounds will supplement and assist endogenous antioxidants.

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...
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’.

**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.

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

**Dietary 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. 2003; Padayatty et al. 2003; Duarte and Luneç 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 significantly lowers F2-isoprostanes, 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 proteins, lipids, and DNA was assessed by measuring plasma carbonyls, malondialdehyde, and urinary 8-oxo-2′-deoxyguanosine; plasma antioxidant status (ORAC<sub>50</sub>) 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. 0.29 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 antioxidant 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 dietary intake 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 ADN and vegetable consumption of adolescents found that oxidative stress (measured as F2-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 possible 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, antioxidants are defined as a biological antioxidant (that is a biological antioxidant) that has overt chemical antioxidant effects. 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 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 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 compounds donate electrons or hydrogen atoms.
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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REFERENCES

Lotito SB, Frei B (2006) Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon? Free Radical Biology and Medicine 41, 1727-1745
McGhee TK, Ainge GD, Barnett LE, Cooney JM, Jensen DJ (2003) Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolized by both rabbits and rats. Journal of Agricultural and Food Chemistry 51, 4539-4548
McGhee TK, Currie AJ (2008) Traditional breeding to enhance the phyto-
nutrient content of fruit and vegetables. In: Tomas-Barberan FA, Gil MI (Eds) Improving the Health-Promoting Properties of Fruit and Vegetable Products, Woodhead Publishing, Boca Raton, pp 301-325


