Dietary antioxidants and cardiovascular disease

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Purpose of review

Oxidative damage is involved in cardiovascular diseases. Intervention with α-tocopherol, ascorbic acid and β-carotene does not appear to reduce pathogenesis. The purpose of this review is to describe alternative antioxidant mechanisms that may be involved.

Recent findings

Antioxidants with different chemical properties may recharge each other in an antioxidant network. The total antioxidant content of dietary plants may therefore be a useful tool for testing the 'antioxidant network' hypothesis. Several berries, fruits, nuts, seeds, vegetables, drinks and spices have been found to be high in total antioxidants. Initial studies in animals and humans are supportive as to the beneficial effects of dietary plants rich in total antioxidants. Additionally, antioxidants and other plant compounds may also improve the endogenous antioxidant defence through induction of antioxidant and phase 2 enzymes. Dietary plants rich in such compounds include broccoli, Brussel sprouts, cabbage, kale, cauliflower, carrots, onions, tomatoes, spinach and garlic.

Summary

Although initial studies have indicated that antioxidants may reduce oxidative stress, human intervention studies do not support a beneficial effect of antioxidant supplements. Further research is needed to clarify whether other plant antioxidants, plants rich in a combination of antioxidants, or plant compounds that induce the endogenous antioxidant defence can reduce pathogenesis of cardiovascular disease and other oxidative stress-related diseases.

Keywords

antioxidant network, redox active compounds, reduction potential

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Abbreviations

α-T•	α-tocopheroxyl radical
FRAP	ferric reducing ability of plasma
NF-κB	nuclear factor κB
ORAC	oxygen radical absorbance capacity
RNS	reactive nitrogen species
ROS	reactive oxygen species
TEAC	Trolox equivalent antioxidant capacity

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Introduction

The collection of solar energy and its conversion into chemical energy in plants would not have been possible without a mechanism that effectively eliminates hazardous excess energy and prevents oxidative damage of the plant cell [1]. Plants are therefore, in general, high in numerous antioxidant compounds such as polyphenols, carotenoids, tocopherols, tocotrienols, glutathione and ascorbic acid, as well as enzymes with antioxidant activity [1,2•]. Animal cells have a much more limited de-novo antioxidant production. Oxidative damage can therefore accumulate in animal cells when the critical balance between generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and antioxidant defence is unfavourable. Compelling evidence demonstrates that such oxidative damage is involved in the pathogenesis of cardiovascular diseases [3•–5•].

A diet rich in fruits and vegetables reduces the risk of cardiovascular diseases and some other diseases, and most countries have developed recommendations for an increased intake of fruit and vegetables [6,7]. It is perhaps a surprise for many that the mechanisms and the compounds involved in the protective effects of fruits and vegetables have not been established. Oxidative stress reduction by dietary antioxidants has been regarded by many as the most likely candidate. This hypothesis has however been difficult to prove. While many studies have demonstrated beneficial effects in experimental model systems [4•,6,8–10], and epidemiological studies [6, 10,11•], the final proof – the randomized intervention trials – have not been at all supportive [12•–15•].

These data may suggest that antioxidants do not contribute to the beneficial effects of fruits and vegetables. This conclusion is, however, premature. The complex chemistry and biology of antioxidants and oxidative stress have been largely neglected in many studies. The purpose of this review is to provide some background

chemistry and biology to promote the investigation of alternative antioxidant mechanisms that may be involved.

The chemistry of oxidative stress

ROS and RNS (including free radicals) are formed as a result of normal cellular metabolic reactions [16]. Such molecules are also formed as a consequence of diseases (e.g. inflammations) and from tobacco smoke, environmental pollutants, natural food constituents, drugs, ethanol and radiation. If not eliminated by antioxidants, these highly reactive compounds will react with and potentially alter the structure and function of several cellular components, such as cell membranes, lipoproteins, cellular proteins, carbohydrates, RNA, and DNA [16,17].

Redox reactions

The type of reaction ROS and RNS are engaged in is called a reduction–oxidation (redox) reaction. In general, redox reactions are at the core of our metabolic machinery as well as the source of energy driving all forms of life. Plants convert solar energy into reduced molecules. Then, living cells obtain energy to maintain themselves by oxidizing reduced molecules through many intermediate steps into oxidized forms of matter such as carbon dioxide and water [16,17]. The energy involved in redox reactions comes from the movement of electrons from oxidizable organic molecules to oxygen. Thus, in redox reactions there is a transfer of electrons from one reactant to another. The loss of electrons from a substance is called oxidation; the addition of electrons to another substance is called reduction. Not all redox reactions involve complete transfer of electrons from one substance to another; some change the degree of electron sharing in the covalent bond. A redox reaction that relocates electrons closer to oxygen releases chemical energy that can be put to work [17].

Reduction potential

The energy of molecules that participate in redox reactions can be interpreted by their reduction potential [17]. Because of their reactivity, most ROS and RNS undergo simple first and second-order reactions. The one-electron reduction potential can therefore be used to predict the direction of such reactions. In this hierarchy, the oxidized species is capable of stealing an electron or hydrogen atom from any reduced species below it in the list [17,18]. The most oxidizing radical that is likely to arise in a biological system is the hydroxyl radical (standard reduction potential of the HO•, H^+/H_2O couple is about 2300 mV), which can steal an electron from almost any molecule [18]. An oxidized molecular species with a high reduction potential is called an electrophile. Importantly, several antioxidants form reactive electrophiles ('antioxidant radicals') when they react with a ROS or RNS [17,18]. Thus, although the chemical energy has been reduced compared with the original free radical, the 'antioxidant radical' may still have substantial reduction potential which can be utilized in damaging reactions.

Activation energy

Activation energy is the energy needed to move an electron out of its orbit. Only the barrier of activation energy holds back the flood of electrons to lower energy states. Without this barrier, reduced molecules would spontaneously react with oxygen and release energy as heat in an enormous explosion. Due to the activation energy, most molecules therefore do not react when they collide, even if the potential product would have been energetically favourable. An important characteristic of ROS and RNS is that such molecules have quite low activation energy and can therefore react when they collide with other stable molecules [17].

Enzymatic versus nonenzymatic reactions

The chemistry of life is organized into metabolic pathways controlled by enzymes whose sole objective is to reduce activation energy of certain specific reactions characteristic of that organism. With the help of enzymes, a cell systematically degrades complex reduced organic molecules to simpler oxidized waste products. The oxidative damage that causes oxidative stress is not due to such enzymatic reactions. Oxidative stress is, however, due to nonenzymatic reactions involving ROS and RNS with low activation energy. If the reaction is energetically favourable, ROS and RNS will react with most molecules with which they collide [19].

Definitions of oxidative stress and antioxidants

The term 'oxidative stress' is today one of the most popular terms in biomedicine: more than 10 papers dealing with oxidative stress are published daily. The term is almost never defined and is probably misused quite often. From the considerations above, I would therefore like to suggest a new definition of oxidative stress: 'oxidative stress is a condition that is characterized by the accumulation of nonenzymatic oxidative damage to molecules that threatening the normal function of the cell or the organism'.

It is important to note that many enzymes can indirectly cause oxidative stress, but these enzymes do not produce oxidative stress by themselves: they produce ROS and RNS that subsequently can cause oxidative damage. The oxidative damage is always due to nonenzymatic redox reactions.

The term 'antioxidant' cannot be defined purely chemically; it is always related to the cellular or organismal context, and to oxidative stress. Furthermore, every molecule can be both an oxidant and a reductant; this

is determined by the reduction potential of the molecule with which it reacts. Thus, I suggest that antioxidant and antioxidant enzymes should be defined as follows: 'an antioxidant is a redox active compound that limits oxidative stress by reacting nonenzymatically with a reactive oxidant'; 'an antioxidant enzyme is a protein that limits oxidative stress by catalysing a redox reaction with a reactive oxidant'.

Antioxidant supplements apparently do not protect against cardiovascular disease

It was initially thought that supplementation of antioxidant such as ascorbic acid, α-tocopherol, β-carotene would neutralize ROS or RNS and thereby avoid any oxidative damage. The first strategy to test for the antioxidant hypothesis was therefore to study the ability of these to inhibit oxidative damage/stress in cell-free experiments (e.g. oxidation of LDL), cell cultures (e.g. cell signalling mediated by oxidized LDL), and experimental animals (e.g. atherosclerotic models based on null mutations of certain apolipoproteins or lipoprotein receptors). Such experiments have generated many positive results [4,6,8–10]. In addition, observational epidemiological studies do in general support the hypothesis that foods rich in these antioxidants are correlated with reduced cardiovascular disease [6,10,11•].

Large randomized double-blind intervention trials that have been conducted to finally prove the antioxidant hypothesis have not been supportive. Indeed, supplementation with antioxidants has often resulted in no effect or even adverse disease outcomes. Recently, several reviews and metaanalyses have concluded that there is now a strong body of evidence indicating that there is no beneficial effect of supplemental α-tocopherol, and probably also of supplemental β-carotene and ascorbic acid [12•–15•].

One possible explanation may be that the beneficial health effect is due to other antioxidants in fruits and vegetables: carotenoids are ubiquitous in the plant kingdom, and as many as 1000 naturally occurring variants have been identified [20]. Phenolic compounds are synthesized in large varieties belonging to several molecular families such as benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins. Over 8000 plant phenols have been isolated [20]. Plant phenols are antioxidants by virtue of the hydrogen-donating properties of the phenolic hydroxyl groups. In addition, the vitamin E family consists of α, β, γ and δ-tocopherols, and α, β, γ and δ-tocotrienols [9]. It is a distinct possibility that some of these antioxidants, whose role in plants is to reduce oxidative stress [1], can do better in randomized intervention trials than α-tocopherol, ascorbic acid and β-carotene.

A combination of antioxidants may be needed to reduce oxidative stress

Even though there are many antioxidants in each single dietary plant, knockout of a synthetic pathway for a single antioxidant may case a serious injury to the plant cell [1]. Therefore, it seems likely that a mixture of different antioxidants is needed to keep the plant cell healthy and protected against oxidative stress. Thus, maybe a combination of a variety of different antioxidants is needed to keep the animal cells protected from oxidative stress.

Antioxidant network

When antioxidants react with ROS or RNS, the antioxidant is itself often transformed into an 'antioxidant radical'. Although the resulting radical has a reduced ability to react with vital cellular targets, it can still cause damage [18]. The 'antioxidant radical' needs to react with another antioxidant to bring the reduction potential and the reactivity further down. These antioxidant reactions can continue in a stepwise fashion, involving a large number of antioxidant molecules, until the 'antioxidant radical' is no longer a threat to the cell, simply because it has been reduced to a product which does not contain enough reduction potential to react with lipids, protein, DNA and other important cellular molecules.

Thus, the α-tocopheroxyl radical (α-T•), which is formed when α-tocopherol reacts with HO•, has reduced ability to be involved in redox reactions. However, α-T• is still quite reactive. It has been observed that α-T• can participate in lipid peroxidation of LDL [21,22]. Accumulation of α-T• or other antioxidant radicals may in fact be one of the reasons for the adverse effects seen in some of the randomized intervention trials using antioxidant supplements.

Normally, however, the α-T can be regenerated by the reaction of α-T• with ascorbic acid, a reaction that generates the ascorbyl radical [18,23]. The reduction potential hierarchy demonstrates that ascorbic acid can regenerate α-tocopherol from the α-tocopheryl radical, but not vice versa.

The best protection for the animal cell, as for the plant cell, may be obtained by a combination of antioxidants. These antioxidants with different chemical properties may recharge each other in an integrated manner, and may be needed for proper protection of all compartments in a cell or an organism. Such interactions have indeed been proven *in vitro* for α-tocopherol, α-tocotrienol, ascorbic acid, lipoic acid and thiols by Packer and colleagues [23], but the concept could have much broader validity.

Identification of dietary plants rich in total antioxidants

The total concentration of redox active compounds with energy above a selected redox potential which is present

in dietary plants may be a useful tool for testing the antioxidant network hypothesis. Although, such an assay may pick up many reductants that are not absorbed by humans, it will identify many antioxidant-rich dietary plants which are very useful candidates when testing the antioxidant network hypothesis.

Initial studies have used different methods to assess total antioxidant concentration or capacity in dietary plants: the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay of Miller et al. [24], the oxygen radical absorbance capacity (ORAC) assay of Glazer's [25] and Cutler's laboratories [26], and the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain [27]. The TEAC and the ORAC assays are based on the antioxidant's ability to react with free radicals, while the FRAP assay measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron).

An appropriate assay for measuring total dietary antioxidants should be based on a reduction potential that is below the characteristic reduction potential for oxidative damage of lipid, proteins, DNA, and other cellular targets. While these reduction potentials are largely not known, and may vary across cellular targets, the reduction potential of the major cellular endogenous antioxidant, glutathione (GSH), is likely to indicate a 'safe' level. The standard reduction potential for the GSSG/2GSH couple is −264 mV. The actual cellular redox state of the GSSG/2GSH couple is slightly higher (about −200 mV) [28]. Thus, a useful cut-off value for determining total antioxidants relevant in a cellular context should probably be around −200 mV. The FRAP assay is based on a reduction potential slightly above −200 mV, while the ORAC and TEAC reactions are slightly below. Thus, all three assays have probably an appropriate reduction potential for picking up the most important antioxidants. However, ORAC and TEAC, but not FRAP, detect GSH or protein thiols. This is an advantage of FRAP since these molecules are for a large part degraded in the intestine and poorly absorbed.

A total antioxidant assay should have little selectivity and pick up all relevant reductants above the characteristic reduction potential. A complex molecule – but not a symmetrical molecule – must collide with the reductant in a proper orientation in order to react. Since it is more likely that a small symmetrical molecule such as Fe^{3+} will react when it collides with the reductant than the more complex free radicals used in the ORAC and TEAC assays, the FRAP assay is less selective than the other two assays. ORAC and TEAC may, however, be more useful when analysing reactivity against specific free radicals.

Table 1. Total antioxidant values in foods as determined by the ferric reducing ability of plasma assay (mmol/100 g)

Dietary supplements (range 0.1–1364.1)	
Body Wise Right Choice AM	530.6
Medox, anthoxyanins	455.9
Ocuvite ekstra	281.1
GNC Ultra Mega Gold	235.6
St John's Wort	118.5
Centrum Silver	40.6
Ginkgo Biloba	35.9
Theragran	30.0
Women's Ultra mega	11.3
Metamucil Orange	0.6
Chondroitin sulfate	0.1
Herbs and spices (range 0.3–465.3)	
Clove	465.3
Allspice	101.5
Cinnamon	98.4
Rosemary	66.9
Oregano	45.0
Curry	13.0
Coriander	2.8
Cardamom	0.5
Berries (range 1.0–39.5)	
Dog rose	39.5
Blueberry	8.2
Blackberry	5.1
Raspberry	3.1
Strawberry	2.2
Sweet cherry	1.0
Nuts and seeds (range 0.2–21.0)	
Walnut	21.0
Sunflower seed	5.4
Sesame seed	1.2
Hazelnut	0.5
Almond	0.3
Chocolate (range 0.4–13.4)	
Dark chocolate (70%)	13.4
Milk chocolate	1.8
Fruits (range 0.1–11.3)	
Pomegranate	11.3
Red grape	2.4
Orange	1.1
Lime	0.7
Apple	0.3
Pear	0.2
Banana	0.2
Watermelon	0.0
Vegetables (range 0.0–3.8)	
Kale	2.3
Red cabbage	1.9
Brussel sprouts	1.1
Spinach	1.0
Cauliflower	0.2
Squash	0.1
Zucchini	0.0
Wine (range 0.4–3.7)	
Montepulciano, red	3.7
Canepa, red	2.8
La Buvette, red	2.4
Muscato, white	0.4
Liebfraumilch, white	0.4
Caliterra, white	0.3
Fruit juices (range 0.1–3.2)	
Grape, blue	1.6
Orange	0.8
Apple	0.6
Pineapple	0.2

(*continued overleaf*)

Table 1. (*continued*)

Coffee (range 1.6–3.0)	
Black coffee, filter	2.8
Black coffee, boiled	2.2
Black coffee, instant	1.7
Tea (range 0.8–2.5)	
Green tea	2.5
Black tea	0.8
Cereals (range 0.0–1.1)	
Barley, wholemeal flour	1.1
Oats, rough oatmeal	0.6
Barley, white flour	0.6
Rye, wholemeal flour	0.5
Wheat, wholemeal flour	0.3
Rye, white flour	0.2
Wheat, white flour	0.1
Dairy produce (range 0.0–1.0)	
Butter	0.7
Cheese	0.1
Milk	0.0
Meat (range 0.0–0.1)	
Moose	0.1
Calf	0.0
Pork	0.0
Ox	0.0

The table shows a range of total antioxidants found in various food groups. Selected foods are also shown in each food group. As values may vary considerably for each food based on site of origin, manufacturer, botanical species, varieties etc., typical values are shown. The table presents some examples of total antioxidant values of dietary plants published in Refs [29,30•], and values to be published elsewhere (Blomhoff, unpublished results).

Another advantage of the FRAP method is its ability for absolute quantitative determination of the amounts of total antioxidants (or reductants) in samples. Thus, values can be used to calculate the total intake of antioxidants and the contribution of various food groups for total dietary intake. The FRAP assay is also the only assay that directly measures total reductants in a sample.

In order to test the antioxidant network hypothesis we have used the FRAP assay to generate a 'total antioxidant table' which contains more than 2000 food items collected from all over the world [29,30•] (Blomhoff *et al.*, unpublished data). We used a workup procedure that allowed analysis of both water-soluble and fat-soluble antioxidants [29,30•] (Blomhoff *et al.*, unpublished data).

Foods identified by the FRAP assay as containing high levels of total antioxidants include several berries (such as blueberries, blackberries, strawberries and raspberries), fruits (pomegranates, grapes and oranges), nuts (walnuts), seeds (sunflower seeds), vegetables (kale, red cabbage and pepper), drinks (green tea, red wine and coffee) and spices (oregano, sage, peppermint, garden thyme, lemon balm, clove, allspice and cinnamon) (Table 1) [29,30•] (Blomhoff *et al.*, unpublished data). Similar but somewhat varying results are also obtained in other smaller studies using the FRAP, ORAC and TEAC assays [31–33,34•,35,36].

With these results available, we are now able to test whether dietary plants rich in total antioxidants may protect against oxidative stress-related diseases such as cardiovascular disease. It should be kept in mind that these analyses include many hundreds, maybe thousands, of different antioxidant compounds belonging to several molecular families. These antioxidants may have very different absorption in humans, and their transport to, and within, tissues is likely to vary dramatically. It would therefore be interesting to test whether total antioxidants in specific botanical families or food groups, or specific combinations, are able to contribute to an antioxidant network.

Antioxidant-rich dietary plants protect against cardiovascular disease in initial studies

Some initial experimental dietary studies are supportive as to the beneficial effect of dietary plants rich in antioxidants. Pomegranate is the fruit containing the most antioxidants (about 11.5 mmol/100 g). Aviram and colleagues [37,38] have recently demonstrated that pomegranate juice administration to apolipoprotein E-deficient atherosclerotic mice reduced macrophage lipid peroxidation, decreased LDL susceptibility to oxidation, aggregation and retention, cellular cholesterol accumulation and development of atherosclerosis. In small-scale human studies they observed that pomegranate juice increased the activity of serum paraoxonase (an HDL-associated esterase that can protect against lipid peroxidation), inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure in hypertensive patients [37,39]. Finally, pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduced common carotid intima-media thickness, blood pressure and LDL oxidation [40•].

Walnuts contain even more antioxidants (about 21.0 mmol/100 g). Five human short-term walnut intervention trials, involving individuals at risk of coronary heart disease, consistently demonstrated that walnuts, as part of a heart-healthy diet, lower blood cholesterol concentrations [41,42]. These results are supported by several large prospective observational studies in humans, all demonstrating a dose–response-related inverse association of the relative risk of coronary heart disease with frequent daily consumption of small amounts of nuts, including walnuts [43]. In addition, Ros *et al.* [44•] recently demonstrated that a walnut diet improves endothelial function in hypercholesterolemic individuals. In March 2004, the US Food and Drug Association accepted the following qualified health claim on walnuts: 'Supportive but not conclusive research shows that eating 1.5 ounces per day of walnuts, as part of a low saturated fat and low cholesterol diet and not resulting in increased caloric intake, may reduce the risk of coronary heart disease' [45].

These preliminary studies are supportive of a beneficial effect of dietary plants rich in antioxidants. The overall evidence is, however, limited, and much more work is needed.

Fruits and vegetables may contain compounds that induce the endogenous antioxidant defence

A complex endogenous antioxidant defence system has developed through evolution to counteract oxidative damage. The antioxidant defence has both nonenzymatic (e.g. GSH and thioredoxin) and enzymatic components that prevent radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, and prevent mutations [2•,16,21, 28,46•]. The antioxidant enzymes include superoxide dismutases for the elimination of the superoxide radicals, and catalases and glutathione peroxidases for the elimination of hydrogen peroxide and organic peroxides. Additionally, detoxification enzymes, such as members of the glutathione S-transferase family, γ-glutamyl cysteine synthetase and NAD(P)H : quinone reductase [1,16], are also essential in endogenous antioxidant defence. These enzymes are generally referred to as phase 2 enzymes because they catalyse conversion of toxic metabolites to compounds that are more readily excreted.

Paul Talalay and colleagues [47,48] have demonstrated that glucosinolate breakdown products from brassica vegetables (such as the isothiocyanate sulphoraphane) and several other sulphur-containing plant compounds can induce antioxidant and phase 2 enzymes. Allium vegetables contain a number of other sulphur-containing compounds (e.g. cysteine sulphoxides and dithiolthiones) that may also induce phase 2 enzymes. Like the glucosinolates, the active compound from allium vegetables results from enzymatic degradation of the plant compounds. Dietary plants rich in compounds that induce antioxidant defence enzymes include broccoli, Brussel sprouts, cabbage, kale, cauliflower, carrots, onions, tomatoes, spinach and garlic [47,48].

The molecular mechanism by which these plant compounds can induce phase 2 and antioxidant enzymes is likely, at least partly, to be mediated by effects on protein kinases (e.g. phosphoinositide 3-kinase (PI3 kinase), protein kinase B (PKB, also termed Akt), extracellular signal-regulated kinase (ERK), protein kinase CK2 (formerly termed 'casein kinase 2') and 5′-AMP-activated protein kinase (AMPK)) and transcription factors (e.g. nuclear factor κB (NF-κB), activator protein-1 (AP-1), aryl hydrocarbon receptor/dioxin receptor (AhR) and NF-E2-related nuclear factors (Nrf1 and Nrf2) [46•,49••]. The Nrf transcription factors which bind to antioxidant response elements/electrophilic response elements are central in such induction [46•,50••].

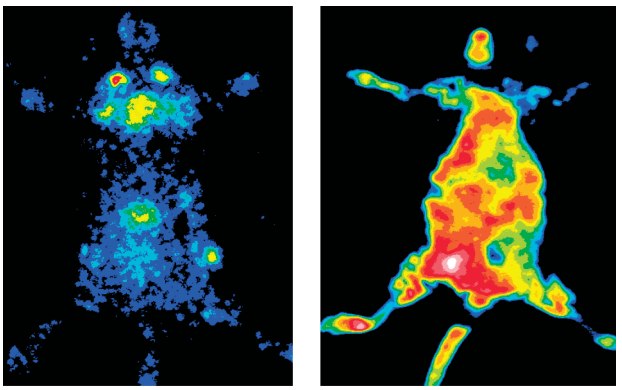
Interestingly, some of these biological activities seem to be related to redox active plant compounds (i.e. antioxidants). For example, the laboratories of René V. Bensasson and Paul Talalay have recently found that the tendency of certain plant compounds to release electrons correlates linearly with their potency in inducing the activity of NAD(P)H : quinone reductase [50••,51••,52]. Thus, the reduction potential of the plant compound determines its inducer potency.

These data suggest that some plant compounds (but not α-tocopherol, ascorbic acid and others) may have a dual role in supporting the antioxidant defence. First, the antioxidant can donate an electron to a ROS or RNS in a classical redox reaction. Then, the antioxidant radical which is formed in the reaction may additionally activate gene expression of antioxidant and phase 2 enzymes.

Noninvasive imaging of phytochemical modulated gene expression

Most of the studies demonstrating transcriptional regulation of phase 2 and antioxidant defence genes by plant compounds have been conducted in cell culture experiments. Therefore, we have developed transgenic reporter mouse models containing complete promoters or response elements coupled to the luciferase gene (Fig. 1) to test the hypothesis *in vivo* [46•,49••,53•,54]. The γ-glutamylcysteine synthetase heavy (GSHh) subunit promoter was selected (Fig. 1B) because it contains response elements for transcription factors such as NF-κB, AP-1 and Nrf1, and the gene product is an important phase 2 enzyme. Our initial data show that antioxidant-rich berries induce GCSh gene expression in brain and muscle [53•]. I believe such transgenic reporter models, which allow noninvasive imaging of gene expression in

Figure 1. In-vivo imaging of nuclear factor κB response element (a) and γ-glutamylcysteine synthethase promote (b) activity in luciferase based reporter mice



(a) (b)

Imaging of transgenic mice was performed with an ultra-sensitive camera consisting of an image intensifier coupled to a CCD camera (C2400-47 Hamamatsu, Japan). For details, see Refs [46•,49••,53•,54].

living mice, will be a useful tool to elucidate the ability of dietary plants to induce antioxidant and phase 2 enzymes in vivo.

Conclusion

Although experimental studies in cell cultures and animals have indicated that antioxidants such as β-carotene, ascorbic acid or α-tocopherol may reduce oxidative stress, human intervention studies do not support a beneficial effect. Governmental and nongovernmental organizations such as the US Food and Drug Administration [55], the US Institute of Medicine (Dietary Reference Intakes) [56], the American Heart Association [57] or the report by the World Cancer Research Fund [6], therefore, do not recommend intake of single or combinations of supplemental antioxidants.

It is suggested that the total antioxidant content of dietary plants may be a useful tool for testing the antioxidant network hypothesis. Several berries, fruits, nuts, seeds, vegetables, drinks and spices have been found to be high in total antioxidants. Additionally, some compounds found in brassica and allium vegetables may improve the endogenous antioxidant defence through induction of antioxidant and phase 2 enzymes. Further research is needed to clarify if such dietary plants can reduce pathogenesis related to cardiovascular disease.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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In this paper, different strategies of antioxidant defence are outlined, as well as evolution of dietary antioxidants from the perspectives of plant need and our current dietary requirements.

3 • Bandyopadhyay D, Chattopadhyay A, Ghosh G, Datta AG. Oxidative stress-induced ischemic heart disease: protection by antioxidants. Curr Med Chem 2004; 11:369–387.

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11 • Stanner SA, Hughes J, Kelly CN, Buttriss J. A review of the epidemiological evidence for the 'antioxidant hypothesis'. Public Health Nutr 2004; 7:407–422.

The British Nutrition Foundation was recently commissioned by the Food Standards Agency to conduct a review of the government's research programme on Antioxidants in Food. Part of this work is presented in this paper. The authors conclude that the suggestion that antioxidant supplements can prevent chronic diseases has not been proved by the findings of published intervention trials. Further evidence regarding the efficacy, safety and appropriate dosage of antioxidants is needed.

12 • Hasnain BI, Mooradian AD. Recent trials of antioxidant therapy: what should we be telling our patients? Cleve Clin J Med 2004; 71:327–334.

This paper reviews important studies on the effects of antioxidant supplements and examines whether the current evidence supports, confirms or rejects the presumed protective role. It is concluded that the current evidence does not support the indiscriminate use of supplements to prevent or reduce cardiovascular disease.

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A metaanalysis of seven large-scale randomized trials of the effectiveness of vitamin E in the treatment and prevention of cardiovascular disease.

14 • Shekelle PG, Morton SC, Jungvig LK, *et al.* Effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease. J Gen Intern Med 2004; 19:380–389.

A metaanalysis of placebo-controlled randomized trials. Eighty-four eligible trials were identified. For the outcomes of all-cause mortality, cardiovascular mortality, fatal or nonfatal myocardial infarction, and blood lipids, neither supplements of vitamin E alone nor vitamin E given with other agents yielded a statistically significant beneficial or adverse pooled relative risk.

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