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Commentary

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Interaction of dietary antioxidants *in vivo*: how fruit and vegetables prevent disease?

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Summary

Epidemiological studies indicate that fruit and vegetables are health-promoting and protective against disease, particularly cardiovascular disease and cancer. Possible plant nutrients providing this protection include antioxidants and dietary fibre. Clinical trials with antioxidant supplements give inconsistent results for protection against lung cancer in smokers, invasive cervical cancer, oesophageal and gastric cancers, colorectal polyps and coronary heart disease. The antioxidants used in trials may be contributing to a more complex system. Antioxidants have differing solubilities which partition across the phases of tissues, cells and macromolecular structures: water-soluble ascorbate, glutathione and urate; lipid-soluble tocopherols and carotenoids, and intermediatory-soluble flavonoids and hydroxycinnamic acids. The health protection provided by fruit and vegetables could arise through an integrated reductive environment delivered by plant antioxidants of differing solubility in each of the tissue, cellular and macromolecular phases.

Introduction

Diets which contain an abundance of fruit and vegetables are protective against a variety of diseases, particularly cardiovascular disease and epithelial (but not hormone-related) cancers. The principle nutrients thought to provide the protection afforded by fruit and vegetables are the antioxidants[1] and dietary fibre (non-starch polysaccharides[2]).

In trying to unravel the underlying mechanisms of this protection, various approaches have been taken. Epidemiological studies usually start with a measurement of dietary intake or serum concentrations of the studied antioxidant. Human supplementation trials are critical to testing any hypothetical merit for antioxidant supplementation. The subsequent disease incidence or pattern is followed in that identified population. Susceptible groups are often chosen for the study: e.g. smokers, who have a distinct chance of developing cancer of the lung, individuals with colonic polyps (a precursor of colonic cancer) and women with cervical dysplastic change which can progress to cancer of the cervix. Coronary heart disease and the complications of hypertension and strokes are more difficult to predict, and form multifactorial risk groups.

Potter[3] reviewed 200 epidemiological studies, the majority of which showed a protective effect of increased fruit and vegetable intake. When the role of individual antioxidants or dietary fibre is examined by epidemiological studies or supplementation trials, the results are not as clear-cut as those obtained for fruit and vegetables, and are often disappointing.

The conclusion drawn by Potter[3] was that fruit and vegetables provide the best polypharmacy against the development of malignancy in tissues. Van Poppel and van den Berg[4] and Rexrode and Manson[5] echo this sentiment, and recommend a diet

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Table 1 Partition coefficients for some important dietary antioxidants

Antioxidant	$\log_{10}$ Pc
α-Carotene	17.6
β-Carotene	17.6
Zeaxanthin	14.9
Lutein	14.8
α-Tocopherol	12.2
Retinol	7.6
Flavone	3.5
Flavonone	3.8
Apigenin	2.8
Luteolin	2.4
Kaempferol	2.0
Quercetin	1.5
Myricetin	1.4
Coumarin	1.5
Ferulic acid	1.4
p-Coumaric acid	1.6
L-Ascorbic acid (protonated)	−4.0
Theophylline	−0.4

Data from Cooper *et al.* (1997).[20]

rich in fruit and vegetables, seeing no merit in recommending vitamin supplements for disease prevention. This is a safe principle but avoids the issue of which fruit and vegetables to eat, whether the unknown beneficial nutrients are affected by conditions of growing, time of year and maturity of the plant when harvested, time of day of harvesting, method of cooking and freezing, etc.[6–9]

The absorption and delivery of nutrients from fruit and vegetables may not be the same when given as individual sources. For example, increasing the daily intake of dark-green leafy vegetables did not improve vitamin A status, whereas an equivalent dose supplementation of beta-carotene produced a marked increase.[10] In another study, fruit and vegetable consumption was increased and the plasma concentrations of vitamin C, α- and β-carotene, but not retinol and α-tocopherol nor lipoproteins, increased.[11] This variation may be due to the physical inaccessibility of the β-carotene, held within cells with consequent reduced bioavailability. On the other hand, the reduced bioavailability of antioxidants in foods compared with that of a supplement may be important in minimizing any potential metabolic disturbance arising from taking too much of the pure compound.

In large supplementation trials, individuals are treated as belonging to a homogeneous population. Within such study groups there may be susceptible genetically-defined at-risk subgroups for cardiovascular disease or carcinoma who would benefit from the supplement. Hyperhomocysteinaemia is a possible risk factor for premature cardiovascular death. Supplementation of the diet in hyperhomocysteinaemic individuals with the B group of vitamins (but not antioxidant vitamins) reduces homocysteine concentrations.[12]

Another important component of fruit and vegetables which has been proposed to have protective effects against disease is dietary fibre. Dietary fibre dilutes gastrointestinal contents, or may be fermented in the colon with release of low-molecular-mass chemicals of nutritional and biochemical significance, e.g. short chain fatty acids[13] and hydroxycinnamic acids.[14] The protective action of fibre against coronary heart disease could result from a reduction in serum cholesterol. The mechanism may be through the binding of bile acids to fibre resistant to colonic bacterial fermentation[15] or more certainly through adsorption to bacteria, which proliferate during colonic fibre fermentation.[16]

The action of fibre in reducing cancer is confined to colonic cancer, and is essentially a local or direct mucosal effect. The action of fibre may be secondary to the dilution of potential carcinogens in the colon through the water-binding capacity of fibre, e.g. moderately-fermented cereal fibre.[17] Alternatively, following fermentation in the colon, short-chain fatty acids, especially butyric acid, may act on cell turnover and growth in the colonic mucosa.

Despite all these contradictory results, there is a strong body of opinion that diseases may be caused by, or their development accelerated by, an imbalance between the overproduction of free radicals and antioxidant activity, the so called oxidative stress concept.

Oxidative stress

During oxidative metabolism, there is a continuous production of free radicals which are scavenged by antioxidants, thereby limiting damage.

A number of low-molecular-mass compounds with a range of solubility properties can act as chain-breaking antioxidants. Amongst these are ascorbate, glutathione and urate, acting as hydrophilic scavengers. Major lipophilic scavengers include carotenoids and sterically-hindered phenols e.g. α-tocopherol and flavonoids. The link between dietary fibre and these antioxidants, all constituents of fruit and vegetables, is provided by the hydroxycinnamic acids, p-coumaric acid and ferulic acid. These C6–C3 phenols form cross-linkage dimers in plant cell-wall structural polysaccharides of fruit and vegetables. Hydroxycinnamic acids, e.g. feruloyl β-glucoside, are also found as soluble conjugates in the vacuoles of plant cells.[18]

When fruit and vegetables are eaten, the hydroxycinnamic acids contained within vacuoles will be absorbed from the small intestine. After bacterial dissimilation of the plant cell wall in the colon, hydroxycinnamic acids will be released and absorbed from the colon.

Antioxidant activity: an integrated reaction?

The body, its structures, cells and macromolecules, consist of phases with a range of physical variables, anatomical divisions and water-soluble and lipid-soluble fluid phases. Within these phases, and at interphases, there will be variations in pH, ionic strength, osmolality, electrical charge, chemical concentration and redox potential. These variables will influence the ability of the phases to act as solvents for lipid- and water-soluble antioxidants, e.g. vitamin C in an aqueous phase, carotene in a lipid phase. Some water-soluble antioxidants, including transferrin, caeruloplasmin and albumin, have low partition coefficients. Entry into a lipid phase or retention in a water-soluble phase of these weak acid antioxidants will be dependent upon their pK_a and the pH gradient across the membrane.

The lipophilicity of a chemical can be estimated by the pK_a or by the octanol-water partition coefficient (kow) expressed in log units ($\log_{10}Pc$). This is a useful measurement for determining biological function.[19] The partition coefficients for a number of antioxidants are given in Table 1.[20] Of the range of phytochemicals described: among the carotenoids, 95% have a $\log_{10}Pc$ value of >9; among the flavonoids, 22% have a $\log_{10}Pc < 1.0$, 75% a $\log_{10}Pc$ between 1.1 and 5.0, and 2% have a $\log_{10}Pc$ between 5.1 and 7.5.

Chemicals with both hydrophobic and hydrophilic substitutions, and hence mixed solubility properties will be distributed between aqueous and non-aqueous systems, e.g. hydroxycinnamic acid and lipoic acid,[21] a partitioning dependent upon the relative contribution and stereochemistry of hydrophobic and hydrophilic substitutions. Solubility is further modified when the partition coefficient of a chemical is altered by conjugation, chemical combination or binding into more complex substances, e.g. proteins which facilitate miscibility into a phase.

This means that antioxidants of differing solubility will nestle next to each other in cellular structures and tissues. Adjacent antioxidants will recharge neighbouring antioxidants in an integrated manner which will also depend upon reaction stoichiometry and kinetics. The relative importance or significance of a given antioxidant may rely not only on its reaction rate and concentration in a given compartment or solving space, but also on its capability to interact with regenerating systems of varying types. This raises the prospect that a variety of antioxidants are necessary to maintain the antioxidant environment in a non-homogeneous biological state, that there is a continuum of antioxidants. There would thus be synergism between antioxidants dispersed in a non-uniform manner between different phases in the body cells and compartments. Water-soluble antioxidants would have an indirect role in membrane and lipoprotein protection. This can be achieved in a chain reaction through the activity of antioxidants of intermediary partition coefficient, polyphenols, tannins and hydroxycinnamic acids and lipoic acid.[21] A precedent for this idea is the concept of osmolality, a summation of a mixture of osmotically active chemicals.

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