IFIS core food information

Antioxidants and 21st century nutrition

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About the author

After graduating from Anglia Polytechnic University in Cell and Molecular Sciences, Siân went to work as a technician at the University of Leeds/ Leeds General Infirmary Pathology unit. As well as developing her technical skills, the generous support of the department allowed her to undertake an MSc in Clinical Medicine (Pathology) part time. She joined the Institute of Food Research on a Food and Drink Federation PhD fellowship in 1992; her task to try to understand how carotenoids and vitamin E affect DNA damage. Siân was awarded her PhD in 1997, and with now long-standing colleagues this work was expanded to include effects of dietary compounds in human volunteers. In 2000, she was asked to coordinate an EU-funded concerted action reviewing antioxidant research in Europe (EUROFEDA). New collaborators and a different perspective led to the discovery of a new skill – science communication. At the beginning of 2004, Siân became the communications manager for the Network of Excellence "The European Nutrigenomics Organisation: linking genomics, nutrition and health research" (NuGO, http://www.nugo.org).

Abstract

The potential for the dietary supplements and functional foods market is considerable. It is generally acknowledged that consumers have accepted the ability of supplements to offer protection from a variety of illnesses, from the common cold to cancer, and are willing to embrace the concept of functional foods under the same premise. Although there are clinically demonstrated uses for many dietary supplements, little overwhelming evidence from human studies exists to support the widespread use of antioxidants as functional food components. The antioxidant hypothesis is based on the assumption that antioxidants have the capacity to limit the adverse effects of oxidative damage. Although this hypothesis was first proposed nearly fifty years ago, issues relating to the types and quantities of antioxidant-rich foods that we should consume still remains under debate today; however, it is generally agreed that a diet containing significant amounts of antioxidant-rich foods is important to prevent the development of age-related diseases. This review briefly examines the relevant literature relating to antioxidants, and suggests new routes for provision of specific nutritional advice to consumers, namely nutrigenomics.

The antioxidant hypothesis is based on the assumption that antioxidants have the capacity to limit the adverse effects of oxidative damage

1. Introduction

All cells in the body produce free radicals, together with other reactive oxygen or nitrogen species (ROS or RNS), in the normal course of metabolism; these radicals have an essential role in energy derivation, chemical signaling and detoxification as well as the proper functioning of our immune system.

If this rate of ROS production outstrips its tightly regulated consumption, oxidative stress (an imbalance in favour of oxidation) occurs (Sies 1985), which leads to oxidative damage to cellular components including lipids, proteins and DNA. Excessive generation of ROS – whether as a result of physiological stress (e.g. disease) or environment (e.g. smoking) – or failure to control ROS activity can ultimately lead to cell death (i.e. apoptosis). Whilst undesirable for the individual cell, this protective mechanism ensures the long-term survival of the whole organism. The risk, however, with oxidative stress is that disruption of normal cellular structure and function, caused by oxidative damage, prevents apoptosis allowing injured cells to proliferate. Many common conditions are associated with both oxidative stress and the resulting oxidative damage to cellular structure and function, including cancer, cardiovascular disease (CVD), dementia, cataract, diabetes and autoimmune diseases. Assuming that ageing also occurs as a result of oxidative damage, this could explain the correlation between growing older and increased risk of degenerative diseases. Indeed, ageing is coupled with increased cellular oxidative damage (Beckman and Ames 1998; Fraga et al. 1990; Hamilton et al. 2001; Mecocci et al. 1999; Richter et al. 1988), and a substantial body of evidence has accumulated that supports the hypothesis that this is due to the effects of oxidative stress (Harman 1956; Beckman and Ames 1998).

The inherent need for ROS during aerobic metabolism means that a variety of antioxidant defences in vivo are essential. The role of such 'antioxidants' is to ensure that the normal redox balance (reduced: oxidized ratio of cell components) of a cell is maintained and that ROS generation is transitory. The precise action of antioxidants varies according to the need, location and risk to the organism. Antioxidant defences include a large number of proteins as well as chemical antioxidants, which remove ROS, control their chemistry, and repair oxidative damage. In turn, oxidants and other chemicals coordinate and control the action of antioxidants, although the effectiveness of these processes can be determined by an individual's genotype for the various proteins involved in each biochemical pathway. Chemical antioxidants can be either hydrophilic or lipophilic, with many being obtained directly from food sorces. Increasingly, however, it is recognized that not all food components

that contribute to our antioxidant defences do so simply because of their antioxidant chemistry, but also because of their ability to interact with cell signaling mechanisms. Alteration in the cellular redox state can act as a powerful trigger for signal transduction, gene transcription and post-translational modification of proteins.

The antioxidant hypothesis is based on the assumption that antioxidants have a capacity to limit the adverse effects of oxidative damage

The antioxidant hypothesis is based on the assumption that antioxidants have a capacity to limit the adverse effects of oxidative damage, with the first experiments in this field being conducted over 40 years ago. Although many researchers have claimed success in reducing oxidative damage and disease, few have been rigorous in either their experimental design or their interpretation (Lindsay 1999). In spite of this, it has been demonstrated that diets rich in fruits, vegetables, cereals, nuts and plant-derived oils provide a rich source of food components (many of which have antioxidant characteristics) that may play important roles in augmenting cellular defences against oxidative stress, thereby preventing the development of age-related disease. However, the issue of optimum intake of these components (i.e. how much and what we should eat) still remains under debate. Although recommended dietary intakes exist for some of these food-derived components (e.g. vitamins and trace elements such as selenium; Standing Committee on the Scientific Evaluation of Dietary Reference 2000), these recommendations exist only to avoid the onset of deficiency diseases. Dietary reference intakes for other bioactive compounds (e.g. antioxidants such as carotenoids and polyphenols) do not exist and there seems to have been little progress in determining what they might be.

In 2004 alone, over 500 reviews were published examining the effect of antioxidants on subjects as diverse as atherosclerosis, cataract, mitochondrial function, neuroprotection, and gene transcription. In addition, numerous food components have been shown to offer beneficial 'antioxidant' properties. Some of the more well known of these are the vitamins C and E, carotenoids, polyphenols and glucosinolates, although there is also considerable interest in selenium and glutathione because of their role in endogenous antioxidant systems. One of the most comprehensive reviews of dietary antioxidants and their role in human health can be found in European Research on the Functional Effects of Dietary Antioxidants (EUROFEDA 2002).

2. A brief overview of key antioxidants

2.1 Vitamin C

Vitamin C promotes a healthy immune system, helps wounds to heal, and maintains connective tissue. It is not synthesized in humans but is obtained solely from the diet. This water-soluble antioxidant has a high reducing power and is able to quench a variety of ROS.

2.2 Vitamin E

Vitamin E is a generic term for a series of naturally occurring tocopherols and tocotrienols. Among them, a-tocopherol displays the highest antioxidant biological activity in vivo. Vitamin E is generally accepted as the primary lipid-soluble antioxidant in the human body, promoting the function of a healthy circulatory system, and having a role in the formation of erythrocytes and the use of vitamin K. Peculiarly, there is no known human deficiency disease associated with vitamin E.

2.3 Carotenoids

At least 60 carotenoids occur in those fruits and vegetable consumed by man. Besides the provitamin A carotenoids (a- and b-carotene and b-cryptoxanthin), lycopene and the hydroxy-carotenoids or xanthophylls (lutein and zeaxanthin) are the main carotenoids present in the human diet. Carotenoids are important not only for their provitamin A activity but also for a variety of other actions in vivo, including cell-cell communication (Stahl and Sies 1998).

2.4 Other bioactive compounds

2.4.1 Phenols

Phenols are ubiquitous in the plant kingdom. They are synthesized by the phenyl propanoid biosynthetic pathway, which produces the large variety of plant phenols that exists, including cinnamic and benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins. Within each family of plant phenols, there are many more compounds; for example, over 4000 different flavonoids alone have been isolated from plants. Plant phenols are antioxidants by virtue of their phenolic hydroxyl groups.

2.4.2 Glucosinolates

Glucosinolates are also widespread plant constituents. Investigations into mechanisms surrounding the preventative action of Brassica vegetables, with regard to incidence of cancer, have shown that glucosinolate breakdown products (e.g. indoles and isothiocyanates) are most likely to be the active agents responsible (van Poppel et al. 1999; Verhoeven et al. 1996). Depending on the reaction conditions involved, individual glucosinolates form structurally different breakdown products that have different biological activities. Some of these (e.g. goitrin, several isothiocyanates and nitriles) may exert adverse effects and can even be toxic in higher doses. Others, however, (e.g. isothiocyanates such as sulforaphane) are very likely to be responsible for the protective anticarcinogenic effects resulting from consumption of a diet high in Brassica vegetables (Heaney and Fenwick 1987; Stoewsand 1995; Hecht 1999; Kelloff et al. 2000). Indeed, the US National Cancer Institute classifies sulforaphane as 'one of the 40 most promising anticarcinogens' (van Lieshout et al. 1998). Taking into account these promising results, together with the commercial and consumer desire for finding non-toxic chemopreventative agents, especially of dietary origin, it is not surprising that glucosinolates are being considered as principal components in functional foods or nutraceuticals. A summary of the results of case studies concerning an association between consumption of Brassica vegetables and cancer development can be found in Verhoeven et al. (1996). These data give impressive epidemiological support for the continuing controversial effects of glucosinolate hydrolysis products on cancer development.

2.4.3 Glutathione

Glutathione (GSH) is an important cellular antioxidant. It is not absorbed in its native form as such from the diet but is broken down into its constituent amino acids on digestion; however, it constitutes one of the most abundant non-protein thiols in cells. GSH synthesis is tightly regulated and takes place in two stages. The first step links glutamic acid and cysteine and is catalysed by g-glutamyl cysteine synthetase to form g-glutamyl cysteine; this step is rate-limited by the amount of cysteine available. GSH is then assembled from g-glutamyl cysteine by means of GSH synthetase, which catalyses its addition to glycine. The overall pool of GSH available is regulated by a negative feedback control mechanism on g-glutamyl cysteine synthetase (Meister 1991). Thus, the dietary availability of sulphur-containing amino acids (i.e. cysteine and methionine) can influence cellular GSH concentrations. In response to the generation of ROS, the cell can adapt to generate GSH

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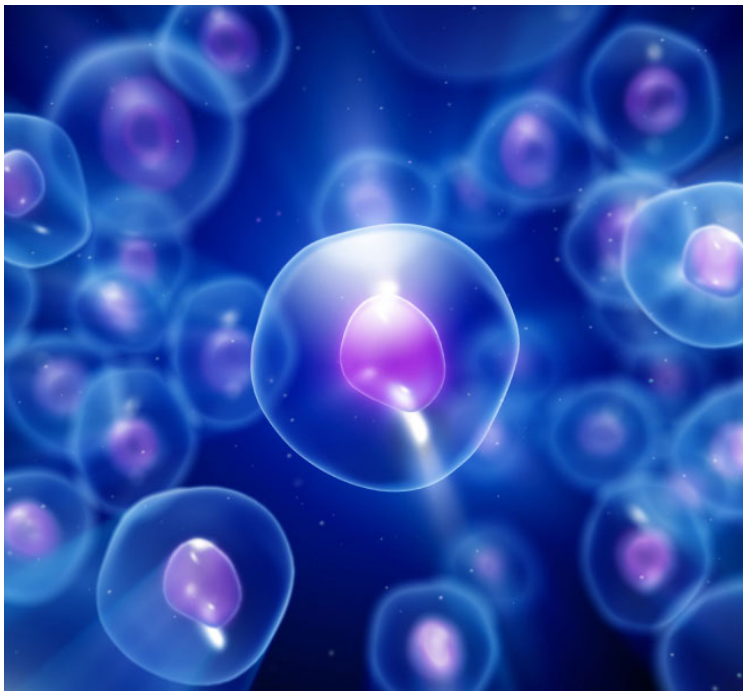
and GSH S-conjugate efflux pumps. Co-ordination of this response is achieved, at least in part, through the antioxidant responsive element (ARE) found in the promoters of many genes, which are inducible by oxidative stress. Many cancer chemopreventative compounds are thought to act through the ARE mechanism by enhancing the amount of antioxidants available, and, consequently, the detoxification capacity of normal cells (Hayes and McLellan 1999). Some of the phytochemicals in foods that behave as antioxidants may also act through a similar mechanism.



2.4.4 Bioactive compounds in allium vegetables

Allium vegetables, such as onions, garlic and leeks, contain a number of bioactive compounds such as cysteine sulphoxides, allyl sulphides and dithiolthiones that induce several phase I and II enzyme systems (Haber et al. 1994). Like the glucosinolates, the active principles found in allium vegetables result from enzymatic degradation and heat transformation. A constituent of garlic, for example, is S-2-propenyl-L-cysteine S-oxide (alliin). Action of the enzyme alliinase present in garlic on this compound gives rise to the distinctive garlic odour generated by formation of S-2-propenyl 2-propenethiosulfinate (allicin). Cooking mainly converts the allicin into diallylsulphide and related polysulphides (Block 1998), while ingestion converts these compounds into volatile dimethylsulphides.

2.4.5 Co-enzymes

There are many co-enzymes or factors that may contribute to maximizing antioxidant enzyme responses. Such compounds include NADPH (Kirsch and de Groot 2001) or lipoic acid (Biewenga et al. 1997), which are synthesized in vivo. This means foods such as fruits

and vegetables are not major sources of the precursors necessary for their biosynthesis. Fruit and vegetables, however, are a rich source of folates (Holland et al. 1996). Although these compounds are not antioxidants, it is likely that they play a role in DNA repair and that their intake reduces levels of plasma homocysteine, a risk factor in CVD (Wald et al. 1998). It has also been proposed that vitamin B6 is an important antioxidant in the plant cell (Ehrenshaft et al. 1999) and may play a role in maintaining the cellular redox state since it is an important co-enzyme in the transulphuration pathway (Lu 2000). Although fruit and vegetables are not the most important dietary source of vitamin B6, cereals and pulses are rich sources.

2.4.6 Selenium

Selenium is an essential nutrient that is present in some amino acids. Selenium intakes in Europe have fallen over recent years with the replacement of US wheat by European wheat, which contains less selenium, in bread manufacture. Selenium is intrinsically important as a component of glutathione peroxidase – one of a group of important cellular antioxidant enzymes that also includes superoxide dismutase and catalase – as well as at least 30 other proteins (Flohe et al. 1973). The identification of various types of so-called selenoproteins has shown that this element is involved in three main areas of metabolism, namely the antioxidant system, the redox system and thyroid hormone metabolism (Arthur 1999).

3. Direct evidence of benefit of dietary antioxidants from human studies

3.1 Epidemiology

Epidemiological surveys have provided a large body of evidence that suggests diets rich in specific antioxidants (from sources such as vegetables, fruits and some vegetable oils) diminish the relative risk of premature death from CVD and cancer. Relative risk from these diseases seems to be lowered in individuals consuming such diets when their blood plasma concentrations reach _50 mmol/l for vitamin C, _30 mmol/l

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lipid-standardized vitamin E (a-tocopherol:cholesterol ratio _5:1), _2.2 mmol/l vitamin A, _0.4 mmol/l b-carotene, and _0.4–0.5 mmol/l a-plus b-carotene (Standing Committee on the Scientific Evaluation of Dietary Reference 2000). Individuals with plasma levels that are 25–35% below these thresholds will have at least a two-fold higher risk of developing these diseases. Furthermore, sub-optimal levels of any of these compounds may increase relative disease risk independently, although lower levels of more than one compound may act synergistically to increase disease risk disproportionately.

3.2 Observational and case-control studies

Observational and case-control studies have shown that diets low in fat and high in fruits and vegetables are protective against heart disease (Gey et al. 1993; Diaz et al. 1997). Carotenoid plasma levels in general, and b-carotene in particular, have been correlated negatively with the risk of CVD (Street et al. 1994; Tavani and La Vecchia 1999; Gaziano and Hennekens 1993), while an inverse relationship has been found between cardiovascular risk and diets rich in vitamins C and E (Gey et al. 1987, 1993). The evidence suggests that vitamin E is a more important discriminator with regard to the prevention of CVD (Gey 1998). High levels of vitamin C in blood plasma are, however, also associated with a significant reduction in mortality arising from myocardial infarction, stroke and other forms of CVD, particularly in the UK. A number of studies have examined the possible link between flavonoid intake and prevention of CVD, with many of these concentrating on red wine and tea consumption. Most of these studies have demonstrated a significant reduction in coronary heart disease and risk of stroke (Hertog et al. 1993; Keli et al. 1996; Peters et al. 2001; Knekt et al. 1996; Yochum et al. 1999; Rimm et al. 1996) although one UK study could not find any association (Hertog et al. 1997).

Higher consumption of fruits and vegetables is associated with reduced risk of cancer at most sites throughout the body

In a similar manner, higher consumption of fruits and vegetables is associated with reduced risk of cancer at most sites throughout the body, although particularly in respiratory and gastrointestinal tract cancers (Steinmetz and Potter 1991a, 1991b; Block et al. 1992; World Cancer Research Fund 1997; Potter and Steinmetz 1996). Since it is likely that intake of vitamin C and other antioxidants are good indicators of fruit and vegetable consumption, it is hardly surprising that observational studies show such a similar pattern. Vitamin E, however, is an exception (Standing Committee on the Scientific Evaluation of Dietary Reference 2000) – there is no obvious link between vitamin E intake alone and reduced risk of cancer. Numerous other observational studies have described a correlation between intake of yellow-orange and leafy green vegetables and reduction in cancer risk. Moreover, plasma b-carotene

concentration is a good biomarker of fruit and vegetable intake, with low plasma levels of b-carotene being indicative of increased cancer incidence (Stahelin et al. 1991a, 1991b; Nomura et al. 1985). Meta-analysis of observational studies suggests that carotenoid-rich diets may specifically prevent cancer of the stomach, oesophagus, lung, oral cavity and pharynx, endometrium, pancreas and colon (World Cancer Research Fund 1997; Woutersen et al. 1999).

3.3 Intervention studies

Although intervention studies provide a more rigorous form of investigation than observational studies, they are not without their weaknesses. Many intervention studies have been designed as chemopreventative studies, using doses that are impossible to achieve except by supplementation of the diet (Lee et al. 1999; Redlich et al. 1999; Albanes et al. 2000; Arab and Steck 2000; Christen et al. 2000; Devaraj and Jialal 2000; Sasaki et al. 2000; Pryor 2000; van Zandwijk et al. 2000). Similarly, studies using single supplements or simple mixtures, in an attempt to avoid confounding synergistic effects, have been undertaken with individuals who are likely to have a pre-existing disease, albeit asymptomatic, or suffer high levels of oxidative stress (e.g. smokers). Because intervention studies involving long-term disease end-points are difficult and expensive, so-called surrogate biomarkers (e.g. DNA strand breaks or oxidized bases) are used in surrogate tissues (tissues that are readily accessible, e.g. lymphocytes or urine), which are not normally affected by the disease process but are nevertheless assumed to respond in the same way as those that do. Some biomarkers have been well validated in analytical terms although, in general, they lack a direct relationship with the disease under examination. Thus, it does not follow that changes detected by individual biomarkers during or following antioxidant administration indicate an impact on disease progression or outcome. Furthermore, little is known about the levels of oxidative damage that might be considered normal or about changes in biomarkers (e.g. increased concentration of specific oxidized DNA bases in urine) that reflect a reduction, or for that matter an increase, in disease risk. Like disease risk and antioxidant protein regulation, biomarker formation is affected by genotype; any potential benefits associated with antioxidant consumption are more likely to be detected in a population under oxidative stress or with impaired antioxidant status than in a healthy population. Moreover, discernment of benefit will be different in apparently healthy individuals compared with those suffering from degenerative diseases. Finally, an appropriate biomarker for healthy individuals may not be suitable for individuals with a particular disease.

Although double-blind, placebo controlled studies using nutritionally-relevant doses have yet to be undertaken for the majority of dietary antioxidants, sufficient evidence exists with vitamin E for the recommendation of modest dietary supplementation (100–400 IU/day) as part of a general programme of heart-healthy behaviour; this includes a fruit- and vegetable-rich diet and regular exercise (Pryor 2000). The fact that vitamin E intake from fruits and vegetables is low, however, raises the question of whether this antioxidant is important in explaining the protective effects observed in connection with consuming such a diet.

Few intervention trials have been undertaken in relation to studying effects of vitamin E on incidence of cancer only; those that have been performed have demonstrated no protective effect (Greenberg et al. 1994). It is difficult to obtain more than 20–30 IU/day of vitamin E by dietary means (Machlin 1980) and most clinical trials involving vitamin E have used supplements at significantly higher levels. The alpha-tocopherol-beta-carotene (ATBC) study showed no effect of vitamin E supplementation on lung cancer risk in heavy smokers (50 IU vitamin E/day), although a 34% lower incidence of prostate cancer was observed (Heinonen et al. 1998). Furthermore, several trials investigating preventative effects against recurrence of colorectal adenoma using vitamin E supplements showed no effect (Chen et al. 1988; DeCosse et al. 1989; Greenberg et al. 1994; Hofstad et al. 1998).

Trials involving b-carotene supplementation, or more frequently the combined effects of b-carotene and a-tocopherol supplementation, are complex. Many of these studies show no protective effects derived from such supplementation (Greenberg et al. 1990), whilst others suggest major benefits only where nutritional deficiencies were known to be prevalent (e.g. Linxiang study in China; Blot et al. 1993). The ATBC study involved smokers in a two-by-two factorial design in doses (25 mg b-carotene and/or 50 mg vitamin E). This level of intake represented a tenfold excess above median intakes of

b-carotene in an 'at risk' population. After two years, serum b-carotene levels rose 17.5-fold, and participants who received b-carotene alone or in combination with vitamin E had significantly higher rates of lung cancer and mortality. The greatest increase was seen in the heaviest smokers (Albanes et al. 1996; Rautalahti et al. 1997; ATBC Cancer Prevention Study Group 1994). The CARET study also involved people at high risk of developing lung cancer (i.e. smokers and workers exposed to asbestos; Redlich et al. 1999). Individuals in this intervention group received combined doses of b-carotene and vitamin A (25 000 IU retinyl palmitate) and also displayed an increased risk of lung cancer. However, ex-smokers and those with the highest initial levels of serum b-carotene had a much decreased risk of lung cancer (Omenn et al. 1996).

In short, human intervention studies, in contrast to the observational and case-control studies, do not provide overwhelming or consistent evidence for the beneficial effects of antioxidants.

4. Antioxidants and functional foods

The potential for supplements and functional foods, in terms of product development and available markets, is considerable. There are accepted, clinically demonstrated, uses for some dietary supplements, e.g. use of folic acid in pregnant women to prevent possible neural tube defects in the foetus, and use of B-vitamin supplements to counteract the malabsorption from the diet caused by treatment with biguanide (Metformin) in diabetics. In addition, there is demonstrable value in the inclusion of specific plant sterols within a healthy diet in order to reduce overall cholesterol content, particularly levels of low-density lipoprotein (LDL)-cholesterol. There is perhaps also sufficient evidence to include fish oils in the diet in order to treat and prevent heart disease and arthritis, as well as perhaps controlling metabolic syndrome (e.g. obesity, non-insulin dependent diabetes, abnormal blood lipid patterns and high blood pressure) and improving concentration in younger children. From a consumer viewpoint, the idea that supplements offer protection from chronic illnesses, be they the common cold or cancer, has caught on. Functional foods appear to have been accepted under a similar 'body of scientific evidence'. There is not, however, any overwhelming evidence from human studies in favour of antioxidants that in vitro studies had promised.

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Relatively few studies have examined the effects of antioxidants at levels likely to be relevant in vivo to nutritional studies on healthy people using validated biomarkers. By implication, the diverse group of compounds known

as antioxidants may have the capacity to regulate multiple key cellular processes such as cell proliferation and apoptosis, with important implications for understanding major disorders such as coronary heart diseases and cancer, and their prevention. However, although oxidative compounds have been frequently reported to cause cellular damage, they may also be mediators of change (e.g. cell signals) in cell function, proliferation and differentiation. Thus, there is still a need to understand the relative importance of the disparate roles of oxidants as well as the action of so-called antioxidants on oxidant behaviour and cellular response. To do this, however, requires a change of approach. It is no longer simply a matter of how dietary antioxidants affect the cellular process but also how cellular processes interact with food components at the molecular level. Nutritional genomics – how food compounds interact with our DNA, RNA and proteins – has tremendous potential to change the future of dietary guidelines with regard to recommendations for the individual.

5. Cardiovascular disease: an example of integrated thinking?

Epidemiological, together with observational and casecontrol studies, suggest a reduced risk of CVD following elevated consumption rates of vitamin E. However, as has already been noted, evidence from intervention studies regarding potential benefits of supplementation with this vitamin is, by and large, inconclusive. However, if genetic variability were taken out of the equation, might the evidence from intervention studies using vitamin E be more conclusive?

Dietary cholesterol concentrations can be modulated in a predictable manner by intake of different dietary fats. Consumption of cis-unsaturated fatty acids in particular, as well as fruits, vegetables and fibre reduces an individual's risk of contracting CVD. Similarly, the proportion of LDL-cholesterol and high-density lipoprotein (HDL)- cholesterol can be manipulated, to a limited extent, by dietary behaviour.

Blood pressure and HDL:LDL ratio are accepted clinical biomarkers for CVD risk (Zock et al. 1995; Goldstein and Brown 1977; Craig et al. 1980; Wierzbicki and Mikhailidis 2002; Sacks et al. 2002). As individuals become older, the balance between health and disease shifts to favour the development of disease; biomarkers such as HDL:LDL ratio, develop late in the degenerative process leading to CVD. It is very unlikely that dietary compounds will reverse the disease process, although they might slow down its effects. An important aim,

therefore, is to use food to prevent development of degenerative disease; however, securing early biomarkers for the prevention of CVD is much more difficult.

A healthy lipid profile is thought to be achieved by the coordinated action of a large number of biochemical pathways, involving hundreds of genes. Genetic variability has been demonstrated for the majority of these pathways, which explains the variability between individuals in cholesterol concentrations and HDL:LDL ratios, and their responses to dietary intervention. Some genotypes predispose an individual towards an abnormal lipid metabolism and lipoprotein profiles (Weggemans et al. 2001), leading development of atherosclerosis and CVD. Thus, simply determining disease risk on the basis of either a single factor (e.g. total cholesterol) or protection by a single compound (e.g. vitamin E) is misleading, particularly when other factors such as lifestyle choice exert such an influence.

The role of lipoproteins and vitamin E on the development and prevention, respectively, of CVD is supported by mechanistic evidence, which has been accumulated over a long period of time. Quite apart from the effect of lipid genotype on CVD phenotype, there is an obvious relationship with glucose metabolism (i.e. the insulin-glucagon regulatory mechanism) and transporter mechanisms (Horton et al. 2002; Chen et al. 2001; van Ommen and Stierum 2002); retinoids, and therefore some carotenoids, which affect the lipid profile by their action on triglyceride regulation via retinoic acid receptors (RAR) and retinoid X receptors (RXR; Staels 2001); and adipocytes, which regulate fatty acid metabolism, in part, through peroxisome proliferator-activated receptor (PPAR)-related systems (Seedorf and Assmann 2001; Debril et al. 2001). Satiety affects disease risk since, unlike protein consumption, eating a diet rich in saturated fats does not promote the function of leptin and other hormone-like functions controlling appetite (Soukas et al. 2000; van Ommen and Stierum 2002). Although, for mechanistic purposes, the study of isolated genes or proteins and metabolite concentrations is extremely useful, in complex situations the entire metabolic system needs to be considered. For other diseases like colorectal cancer or food allergy, mechanistic information on the effects of diet on disease prevention, occurrence and progression is not as readily available. The identification of predictive biomarkers for early biological changes, with a view to disease prevention, is lacking and the influence of genetic variability on each relevant biochemical pathway is even less well understood. The complex relationship between diet and a variety of key known biochemical pathways means that systems biology, using post-genomics technologies, appears to be the only way to make progress.

6. Nutrigenomics – not a panacea

Even with the use of post-genomics technologies, there are still enormous challenges to be faced in understanding how food or food compounds affect health. The technologies are relatively new, and researchers are rethinking their approaches to procedures such as study design, sample collection, data storage, and volunteer selection for practical, theoretical and, not least, logistical reasons. One factor is the volume of data that these techniques generate. For example, a single analytical procedure using, for example DNA microarrays, can easily generate tens of thousands of data points, each relating to the expression of a single gene, some of which will be linked – often in unknown ways – whilst others are independent. These data are also derived from only a single method of analysis and results should be confirmed, albeit on a more limited scale, with at least one alternative approach.

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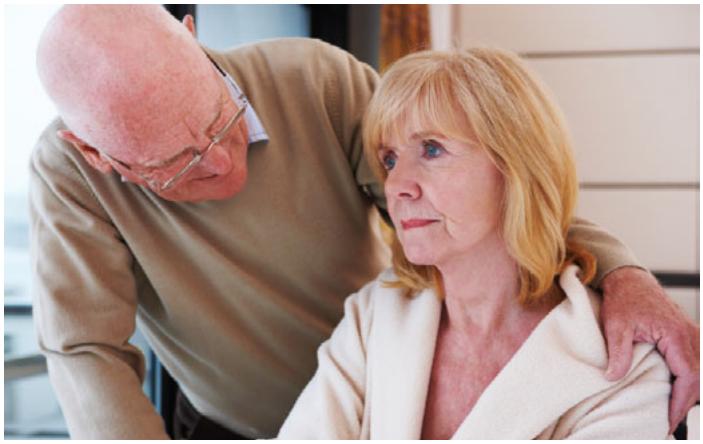
Whilst clinical symptoms of a disease are biomarkers in themselves, they are not suitable for early detection and therefore prevention. A series of biomarkers, in tissues that are easily accessible from apparently healthy individuals, as well as those at different stages of disease progression, is desirable. Early biomarkers can yield information on the nature of ROS damage and the action of food compounds – including antioxidants – in vivo. Initial results have indicated that genomic 'fingerprints' can be obtained, which can be used as biomarkers (Tang et al. 2001; Walker et al. 2002). However, more work is needed to determine whether these patterns can serve as valid biomarkers in relation to consumption of certain food compounds for the maintenance of good health or prevention of disease. The majority of work undertaken to date has typically been performed using surrogate tissues. The validity of using such tissues has yet to be demonstrated, as does control of the effects of processing techniques (e.g. post-genomic technologies) on these tissues; such effects could include changes in gene expression (e.g. inducing heat-shock proteins).

Another issue facing nutrition research in the post-genomic era is the prospect of working from the point of ignorance. Although the human genome has now been mapped and considerable headway has been made in interpreting the information contained therein, the implications of subtle differences in the DNA code between individuals is not, as yet, understood. We have also come to understand that some apparently 'abnormal' genotypes may confirm some other benefit to the individual, e.g. protection from malaria. When armed only with the DNA code or with very limited understanding of the interaction between a given genotype and its effect on human health, it is difficult to make accurate predictions regarding risk of disease or benefit from

a particular treatment. Chronic diseases such as cancer, CVD and diabetes involve many different genes and biochemical pathways, and are linked to a variety of external factors such as lifestyle. This means nutrigenomics is currently unable to offer any definitive conclusions regarding dietary regimens that minimize disease risk in later life. In fact, it may never be able to do so, since these diseases are about relative risk. However, as with genetic disorders, some of the information that is emerging from the systems biology approach is proving difficult to handle. For example, a mutation in apolipoprotein E (e4/e4), which is found in 1–3% of the UK population, is associated with increased risk of early CVD; changes in dietary fat intake are successful in reducing this risk. However, this genotype is also linked with a 60% increased risk of developing Alzheimer's disease. It is not clear whether a reduced risk of CVD is concomitant with a reduced risk of Alzheimer's disease following modification of an individual's dietary lipid intake. Such dilemmas are inevitable with increasing understanding of the link between diet and disease, and the role of genotype in risk.



7. Conclusions

There is compelling epidemiological evidence that links diets containing large quantities of antioxidant-rich foods with a reduced risk of degenerative disease. This has led to the suggestion that dietary antioxidants may augment cellular defences and help protect components of the cell from oxidative damage. However, a large amount of global research generated in this field has produced data that both support and challenge this hypothesis. Observational studies in humans have demonstrated an association between high plasma levels of dietary antioxidants and protection against a number of chronic disorders. However, plasma antioxidant concentrations reflect dietary antioxidant intake, and an association is not necessarily causal. Moreover, human intervention studies have shown no consistent benefit.

Dietary antioxidants may augment cellular defences and help protect components of the cell from oxidative damage

It is now clear that the relationship between ROS, antioxidants, and the individual are far more complex than originally envisaged. There is no evidence to support the view that any one antioxidant is more essential than another. Indeed, there is evidence that shows some synergistic mechanism in operation, as derived from all components of a diet rich in fruits, vegetables, cereals and plant derived fats. In this way, good health is maintained and the

risk of developing disease is minimized. However, as humans become older, the chances of developing degenerative diseases become higher. Consumers believe that consumption of antioxidants can promote good health and prevent incidence of disease, whether they are in the form of supplements or functional foods. The body of available evidence supporting this, however, is not so certain.

As a consequence of genetic differences (e.g. MTHFR gene and folates), there are distinct population differences regarding absorption and/or turnover of dietary antioxidants. However, there is insufficient information available regarding antioxidant metabolism, their distribution within tissues and cells, and the nature of their metabolites to know whether these differences are important, what the relevant target sites for these compounds are, and whether or not these will be sensitive to changes in intake. If the active compound cannot be measured at a target site, information on the relationship between a surrogate tissue (e.g. blood) and concentrations at the target site will have to be obtained from animal studies or other suitable models. In addition, not all bioactive dietary components exhibit such properties when present in foods, and risk-benefit analyses will also be needed for the metabolites of dietary antioxidants. Future human dietary intervention studies need to be conducted more rigorously, and should be modelled on pharmaceutical trials in order to discover the fate of dietary antioxidant compounds in vivo, together with factors that influence their bioavailability. This information can then be used to design and interpret studies that prove whether a given dose/range of intake is beneficial, as well as allowing risk-benefit evaluation to be carried out based on exposure to a specific compound for a given genotype in target tissues.

There are inherent strengths and weaknesses in the different biomarkers for the antioxidants currently available. Experts in the EU-funded FP5 Concerted Action European Research on the Functional Effects of Dietary Antioxidants (EUROFEDA 2002) used a specific set of criteria to establish the validity of biomarker methods currently used, and more importantly, to identify areas for further study. The European Standards Committee on DNA damage (ESCODD 2000, 2002a, 2002b, 2003) validated different methods of measuring 8-oxo-guanine (the most commonly oxidized DNA base) in pure DNA as well as in DNA isolated from cells and tissues (Collins et al. 2004; Lunec 1998; Riis 2002; Rodrı´guez et al. 2000); many potential biomarkers for determining oxidative damage would benefit from the same scrutiny. Since biomarker formation is affected by genotype, there is an urgent need to establish intra- and inter- individual variation in biomarkers that represents normal values, and the factors influencing them. Currently, the relationship between

biomarkers and onset of degenerative disease is poorly validated. Benefits to health are more likely to be detected in a population under oxidative stress, or with impaired antioxidant status, but any conclusions reached in such a group may not be applicable to healthy individuals earlier in the disease process.

A limited number of papers relating to nutrigenomics have appeared so far, with most of them focusing on analysis of multiple gene expression (transcriptomics). Of these studies, only a few have been conducted in humans. Changes in gene expression associated with age can be eliminated by caloric restriction (Lee et al. 1999), which also enhances the transcription of genes involved in endogenous antioxidant scavenging mechanisms as well as tissue development and energy metabolism. Caloric restriction decreases simultaneously the expression of genes involved in stress responses, signal transduction, and structural protein manufacture (Sreekumar et al. 2002a). In rats, it has been shown that gene expression, induced by a high lipid diet, can be modified by antioxidant supplementation (Sreekumar et al. 2002b). Similarly, low levels of dietary magnesium induce expression of genes involved in protection from, and repair of, oxidative damage (Petrault et al. 2002; van Ommen and Stierum 2002). Furthermore, mice fed a low selenium diet showed increased expression of genes involved in DNA damage processing, control of oxidative stress and the cell-cycle, and decreased expression of genes involved in detoxification (Rao et al. 2001). These studies indicate that gene expression profiling can be used to detect sub-optimal intakes of essential food compounds and that genes already known to be associated with antioxidant intake can be identified (van Ommen and Stierum 2002). The advantage of this approach is the mechanistic prediction of action, which will lead to a better understanding of health effects of certain diets or physiological factors (e.g. ageing) and the beneficial effects of dietary intervention (e.g. antioxidant treatment).

Although it is clear that nutrigenomics is capable of providing new insights into the effects of antioxidants on health, post-genomics technologies are not yet fully developed and need further technological improvements as well as supporting bioinformatics and databases. It should also be remembered that hypotheses postulated using transcriptomics, proteomics and metabolomics need to be confirmed using conventional biochemical or molecular biological tools. Furthermore, most biologists and nutritional scientists need to be trained in the appropriate use of these technologies, together with interpretation of the data and associated ethical considerations underlying disclosure of the associated information.

Post-genomics technologies are not yet fully developed and need further technological improvements as well as supporting bioinformatics and databases

Long-term human intervention studies aimed at measuring the effect of antioxidants on biological end-points in relation to health or disease remain the ultimate aim for nutritional research, even though such studies are generally not possible. However, consumers need to be offered information that allows them to make informed decisions regarding their lifestyle, including dietary behaviour. Therefore, nutritional sciences need to embrace nutritional genomics and systems biology in order to determine whether observations that dietary antioxidant consumption from food contributes significantly to our health are indeed correct.

Consumers need to be offered information that allows them to make informed decisions regarding their lifestyle

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