25 Natural Antioxidants in Land- and Marine-Based Wild-Type Food
Risk Reduction

Peter F. Surai, Ambrose J. Spinnler Benadé, and Brian K. Speake

Abstract

The relationship between health and food choices and the factors that determine our health have attracted the attention of scientists and health professionals for many years. However, during the last decade it has become obvious that the diet has a pivotal role in maintaining our health. It is generally accepted that natural antioxidants play important roles in risk reduction of many degenerative diseases, including two major killers of modern society cardiovascular diseases and cancer. In fact, a delicate balance between antioxidants and pro-oxidants in cells is an important determinant of various physiological processes and maintenance of this balance is the main aim of so called an integrated antioxidant system built in the human body. This system was developed during evolution to provide an antioxidant defence and give a chance for animals and humans to survive in an oxygenated atmosphere. It is now widely accepted that fruits and vegetables are important dietary components responsible for maintenance of good health. However, molecular mechanisms of protective effects of fruits and vegetables have not been fully elucidated. One of the attractive ideas is that various antioxidant compounds of fruits and vegetables are responsible for prevention of oxidative damage in the digestive tract.

Key Words: Antioxidants; vitamin E; carotenoids; flavonoids; ascorbic acid; health.

1. INTRODUCTION

The relationship between health and food choices and the factors that determine our health have attracted the attention of scientists and health professionals for many years. However, during the last decade it has become obvious that the diet has a pivotal role in maintaining our health. Therefore, in most developed countries, nutritional practice has changed focus from combating nutrient deficiencies to addressing nutrient requirements for maintaining good health throughout life. Indeed, collectively, cardiovascular disease (CVD) (including stroke), cancer, and diabetes account for approx two thirds of all deaths in the United States (US) and about $700 billion in direct and indirect economic costs each year *(1)*. They accounted for close to 1.5 million deaths in the US in 2001 *(2)*. The economic costs of cardiovascular disease, cancer, and diabetes in the US in 2003 were estimated to be $351.8 billion, $189.5 billion, and $132.0 billion, respectively *(3–4)*. It is generally accepted that natural antioxidants play important roles in risk reduction of many degenerative diseases, including two major killers of modern society cardiovascular diseases and cancer.

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2. ANTIOXIDANTS AS COMMON ELEMENTS IN IDEAL DIETS

The last 50 yr have been characterized by the understanding of the impact of nutrition and dietary patterns on health. Epidemiological findings, supported by animal studies, have led to recommendations that people should consume at least two servings of fruit and three servings of vegetable daily *(5)* in addition to at least two servings of fish weekly *(6)*. Whereas findings and reports such as these have had an impact on the type and quantity of the food that many of us eat *(7)* the majority of adults in developed countries fall well short of meeting healthy eating guidelines.

In the scientific literature, three major so-called "ideal diets" have received substantial attention over the last few years. They are Mediterranean, Japanese and hunter-gatherer diets *(8)*. The Mediterranean diet is associated with decreased rates of various diseases. For example, this diet, based on green and yellow vegetables, fruits, grains and olive oil, has been observed to have a protective effect against development of atherosclerosis. The Japanese diet reflects the highest life expectancy whereas the hunter-gatherer diet is based on a nutrient consumption similar to that of our ancestors during human evolution.

The evolution of man is connected with a life-style of hunting and gathering. The food sources (animal and plant) remained the same during this evolution, but the proportions of foods, preferences, preparations, and the attainability changed *(9)*. It has been suggested that the nutritional patterns of Palaeolithic humans influenced genetic evolution during the time period within which defining characteristics of contemporary humans were selected *(10)*. Because the human genome has not changed much since the beginnings of agriculture, genetically, humans remain adapted for a Palaeolithic dietary regimen. Such diets were based mainly on wild game, fish, and uncultivated plant foods. In comparison with Palaeolithic man, today our diet is characterized by substantial increases of some substances and decreases of others. For example, in comparison with the Palaeolithic period, current US intake of vitamin E decreased by 4 times (7–10 vs 32.8 mg/d), carotene by 2 times (2.05–2.57 vs 5.56 mg/d), and ascorbate by 6 times (77–109 vs 604 mg/d) *(11)*. In the history of mankind, anthropology shows that humans were gatherers of fruits and vegetables for their daily nutritional needs. This tradition has changed dramatically with the development of agricultural industries *(12)*. Furthermore, the relocation of people from rural areas to cities also decreased fruit and vegetable consumption. Therefore, in contrast with our ancestors who used to eat a variety of various wild plants in large amounts, today's diet includes a limited number of plants, consumed in limited amounts, and as a result is comparatively poor in antioxidant compounds *(11)*. In general it seems likely that the Palaeolithic diet contained quite high concentrations of various antioxidants, including vitamin E, carotenoids, ascorbic acid, flavonoids, and selenium.

The main advantage of the described diet is an adaptation of the human digestive system to most of those nutrients. This means that high efficiency of assimilation from the diet and distribution in the body could be a driving force in health promoting properties of various compounds, including antioxidants and phytochemicals. Possible implications of this kind of diet on the digestive tract need further investigations. Details of two other ideal diets are presented elsewhere *(13,14)*, but the main conclusion that can be drawn from analysis of "ideal" diets is that natural antioxidants are among the major players in these diets. Indeed, these three diets are different in composition but they have some similarities in terms of low saturated fat, high proportions of ω-3 fatty acids,

and high levels of naturally occurring antioxidants. It is generally accepted that antioxidant nutrients, especially those from food sources, have important roles in preventing pathogenic processes related to cancer, CVD, macular degeneration, cataracts, and asthma, and may enhance immune function *(15)*. Recent research on phytochemicals has changed a view on dietary factors affecting our health. In particular it seems likely that there are hundreds and even thousands of such factors and now we are dealing just with the top of iceberg. Based on this information a concept of the integrated antioxidant system in the human body was developed *(13,14)*. Indeed, all antioxidants in the body are working in concert as a team, which we call "the antioxidant system" and in this team every member has its own job to do and interactions between the antioxidants is a key for effective antioxidant defence and health-promoting properties of various antioxidants.

3. NATURAL ANTIOXIDANTS AND HUMAN HEALTH

The most important food related cause of disease is free radical overproduction. Free radicals are constantly produced in vivo in the course of the physiological metabolism in tissues. It is generally accepted that the electron-transport chain in the mitochondria is responsible for major part of superoxide production in the body *(16)*. Mitochondrial electron transport system consumes more than 85% of all oxygen used by the cell and, because the efficiency of electron transport is not 100%, about 1 to 3% of electrons escape from the chain and the univalent reduction of molecular oxygen results in superoxide anion formation *(17)*. About 10^{12} O_2 molecules are processed daily by each rat cell and if the leakage of partially reduced oxygen molecules is about 2%, this will yield about 2×10^{10} molecules of reactive oxygen species/cell/d *(18)*. An interesting calculation was made by Halliwell *(19)* showing that, in the human body, about 1.72 kg/yr of superoxide radical is produced. In stress conditions it would be substantially increased. Clearly, these calculations show that free radical production in the body is substantial and many thousand biological molecules can be easily damaged if they are not protected. The activation of macrophages in stress conditions is another important source of free radical generation. Immune cells produce free radicals and use them as an important weapon to destroy pathogens *(13,14)*.

Free radicals are implicated in the initiation or progression phase of various diseases, including CVD, some forms of cancer, cataracts, age-related macular degeneration, rheumatoid arthritis and a variety of neurodegenerative diseases *(20)* (Table 1). In general, it is widely believed that most human diseases at different stages of their development are associated with free radical production and metabolism. Normally, there is a delicate balance between the amount of free radicals generated in the body and the antioxidants to protect against them *(13,14)*. However, an excess of free radicals, or lack of antioxidant protection, will shift this balance producing oxidative stress. Food components can modulate this balance and may thereby influence the rate of aging *(21)* as well as disease resistance of the human *(5)*. The most important step in balancing oxidative damage and antioxidant defence in the human body would be to enhance the antioxidant capacity by optimising the dietary intake of antioxidants through, for example, increased consumption of antioxidant-rich foods. These could be foods natural rich in antioxidants as in the case with some fruits and vegetables *(5)* (Table 2) or through modification as with so-called modified or functional foods.

Table 1
Free Radical Involvement in the Development of Human Diseases*

- Liver
  - Reperfusion
  - Toxic effects of chemicals: halogenated hydrocarbons, quinones, iron, acetaminophen, ethanol
  - Endotoxin
- Kidney
  - Autoimmune nephrosis:inflammation
  - Toxic effects of chemicals: aminoglycosides, heavy metals
- Lung
  - Normobaric hyperoxic injury
  - Bronchopulmonary displasia
  - Toxic effects of chemicals: paraquat, bleomicin
  - Emphysema
  - Asbestosis
  - Idiopathic pulmonary fibrosis
- Heart and cardiovascular system
  - Atherosclerosis
  - Hemochromatosis
  - Reperfusion: after infraction or transplant
  - Selenium deficiency (Keishan disease)
  - Toxic effects of chemicals: ethanol, doxorubicin
  - Myocardial infarction
- Gastrointestinal tract
  - Reperfusion
  - Toxic effects of chemicals: nonsteroidal and anti-inflammatory agents, alloxan, iron
  - Pancreatitis, Colitis, Intestinal ischemia, Gastric ulcers
- Blood
  - Malaria
  - Various anemias
  - Protoporphyrin photooxidation
  - Toxic effects of chemicals: phenylhydrazine, primaquine and related drugs, sulfonamides, lead etc.
  - Favism
  - Fanconi's anemia
- Eye
  - Retinopathy of prematurity
  - Photic retinopathy
  - Macular degeneration
  - Ocular hemorrhage
  - Cataracts
- Muscle
  - Muscular dystrophy
  - Overexercising
- Skin
  - Radiation (UV or ionizing)
  - Thermal injury
  - Toxic effects of chemicals: tetracyclines stimulating photosensitization
  - Contact dermatitis
  - Porphyria
- Brain and nervous system
  - Parkinson's disease
  - Alzheimer's disease
  - Tardive dyskinesia
  - Neuronal ceroid lipofuscinosis
  - Neurotoxins
  - Hypertensive cerebrovascular injury
  - Allergic encephalomyelitis
  - Multiple sclerosis
- Inflammatory-immune system
  - Glomerulonephritis
  - Vasculitis
  - Autoimmune disease
  - Lupus erythermatosus
  - Rheumatoid arthritis
- Miscellaneous/general
  - Aging
  - AIDS, Cancer, Diabetes
  - Inflammation
  - Trauma
  - Ischemia/reperfusion
  - Radiation injury
  - Toxic effects of chemicals: alloxan (diabetes), iron overload
  - Acute pancreatitis, Amyloidosis

*Adapted with permission from ref. *13*.

Table 2
Natural Food Sources of Some Antioxidants

Compounds	*Source*
Vitamin E (tocopherols and tocotrienols)	Oilseeds, vegetable oils, nuts, whole grains, cereals, margarine.
Vitamin C	Fruits and vegetables, berries, citrus fruits, green papers.
Carotenoids	Dark leafy vegetables, carrots, sweet potatoes, tomatoes, apricots, citrus fruits, kale.
Flavonoids/isoflavonoids	Fruits and vegetables, oilseeds, berries, peppers, citrus fruits, tomatoes, onions.
Phenolic acids/derivatives	Oilseeds, cereals, grains.
Catechins	Green tea, berries, certain oilseeds.
Extracts/essential oils	Green tea, rosemary, sage, clove, oregano, thyme, oat, rice bran.

Adapted with permission from ref. *123*.

3.1. Vitamin E

Vitamin E is the main biological chain-breaking antioxidant, found in food in the form of 4 tocopherols and 4 tocotrienols. Biological activity of vitamin E in tissues results mainly from α-tocopherol, but in food the main form of vitamin E is γ-tocopherol. It is possible that the gut is a special place for γ-tocopherol and tocotrienols to play their antioxidant role. Vitamin E is not stable and is easily oxidized during food processing. Synthetic antioxidants added to the food can inhibit vitamin E oxidation. Vitamin E (α-tocopherol) in the tablet or capsular form is mainly produced in the stable esterified form or as a mixture of tocopherols. The major vitamin E sources in the diet are vegetable oils (e.g., wheat, soybean, sunflower, and corn) and some other plant-derived foods (Table 3). For example, in middle-aged Japanese, vitamin E was mainly of vegetable origin with main contributions coming from spinach, safflower oil, and pumpkin *(22)*. In the United Kingdom (UK), the average daily vitamin E intake is 11.7 mg in men and 8.6 mg in women. Similar consumption was reported in the US and other countries with margarines and mayonnaise supplying 23% of total vitamin E consumed. These levels are in the line with the recommended daily allowances (RDA). In fact the Food and Nutrition Board of the Institute of Medicine recently published dietary reference intakes for vitamin E, which is 15 mg for adults being 50% greater than the generous allowance in the 10th edition of Recommended Dietary Allowances published in 1989. It has been concluded that, according to the RDA, the intake of antioxidants is adequate in healthy subjects *(5)*. However, the recent data of Bodner et al. *(23)* indicate that vitamin E intake in Scotland is 6.6 mg/y for women and 7.3 mg/d for men, comprising only 50% of the RDA and being lower than that in other European countries. In addition, there are several categories of people whose vitamin E consumption is lower than the RDA.

Vitamin E deficiency is associated with a development of a range of specific diseases involving major tissues of the organism including immune system incompetence, impairment of lipid metabolism, fertility problems, and increased susceptibility to common and specific diseases. There are also several clinical conditions where vitamin E deficiency

Table 3
Vitamin E Content of Some Plant-Derived Foods

Product	*Vitamin E activity as α-TE*[a]
Vegetable oils	
Canola	21.5
Coconut	0.7
Corn	19.8
Cottonseed	42.8
Olive	12.0
Palm	33.5
Palm kernel	6.2
Peanut	15.2
Safflower	34.9
Sesame	16.5
Soybean	17.1
Sunflower	49.2
Walnut	63.6
Wheat germ	173.6
Vegetables, fruits and berries	
Potato	0.05
Carrot	0.37
Broccoli	0.69
White cabbage	0.04
Lettuce	0.66
Spinach	1.22
Tomato	0.68
Sweet paper	2.21
Apple (flesh only)	0.24
Orange	0.36
Banana	0.21
Peach (flesh only)	0.96
Blackcurrant	2.30
Bread	
French	0.38
Rye	0.52
White	0.06
Margarine, Stick	
Soybean	9.0
Corn	20.9
Sunflower	25.9
Dressings	
Blue cheese	11.2
French	9.8
Italian	10.8
Mayonnaise	7.4

[a]mg α-tocopherol equivalents/100 g product. Adapted with permission from ref. *124* and *125*.

states are described: vitamin E malabsorption syndrome, abetalipoproteinemia, chronic childhood cholestasis, cystic fibrosis, total parenteral nutrition, and prematurity *(24)*. Recently, it has been suggested that detrimental consequences of vitamin E inadequacy could be a result of changes in gene expression. For example, it has been shown that vitamin E-deficiency induces significant molecular regulatory properties in liver cells with an altered expression of both antioxidant-defense genes and genes that control the cell-cycle and demonstrate that liver nuclear factor (NF)-κB activation is involved in this response *(25)*. Therefore, it is important to maintain an adequate vitamin E consumption not only to prevent liver oxidative damage but also in modulating signal transduction. Recently, it has been shown that vitamin E is able to directly influence gene activity and potentially can affect drug metabolism in humans *(26)*. In fact, it has been found that vitamin E potently regulates the expression of about 230 genes, functioning in metabolism, cell-cycle progression, and transcriptional regulation *(27)*.

Vitamin E can not be synthesized in the human and its adequate intake relies upon adherence to a well balanced diet. It has been suggested that by enhancing the intake of vitamin E by fortification of foods or by dietary supplements it may be possible to reduce the risk of many common, yet disabling human diseases. Furthermore, there are many studies suggesting that intake of vitamin E in amounts much higher than RDA are associated with reduced risk of various diseases *(5)* and with enhancement of certain immune responses *(28)*. Results from large-scale human observational studies suggest that antioxidant consumption reduces the risk of developing cardiovascular disease. However, the American Heart Association (AHA) maintains that there are insufficient efficacy data from completed randomized trials to justify population-wide recommendations for use of vitamin E supplements in disease prevention *(29)*. It is interesting that about one half of American cardiologists take supplemental vitamin E *(30)*. Even so, results of clinical trials with vitamin E supplementation were not as successful as expected.

During the past decade, the health benefits of vitamin E have been shown in several epidemiological studies *(21)*. For example, epidemiological evidence shows a lower incidence of infectious disease in subjects with high plasma tocopherol concentrations *(31,32)*. In this respect, Lachance *(33)* has shown that the optimal daily antioxidant intakes are 23 mg for vitamin E and 3.2 mg for carotene.

It seems likely that there are important differences in molecular mechanisms of action of various tocopherols and tocotrienols. Indeed, the unique vitamin action of α-tocopherol, combined with its prevalence in the human body and the similar efficiency of tocopherols as chain-breaking antioxidants, led biologists and health professionals to almost completely discount the "minor" tocopherols as topics for basic and clinical research *(34)*. However, recent discoveries have forced a serious reconsideration of this concept. For example, new and unexpected biological activities have been reported for γ-tocopherol which are not related directly to their chemical antioxidant activities but showing anti-inflammatory, antineoplastic, and natriuretic functions possibly mediated through specific binding interactions. Furthermore, a great body of epidemiological evidence suggests that γ-tocopherol is a better negative risk factor for certain types of cancer and myocardial infarction than is a α-tocopherol *(34,35)*.

Vitamin E is considered to be not toxic for humans and a daily dosage of 100 to 300 mg vitamin E can be considered harmless from a toxicological perspective and therapeutic

vitamin E doses start at several hundred mg/d and end at approx 1600 mg/d *(36)*. Clearly, vitamin E can be considered as a main contributor to the antioxidant potential of the digesta.

3.2. Carotenoids

Carotenoids recently were included into family of natural antioxidants. They exhibit their maximum antioxidant activity at low-oxygen pressures, which prevail in healthy tissues. It has been recently hypothesized that carotenoids are not the major antioxidant players themselves but rather are an important part of the antioxidant system *(13)*. Therefore antioxidant interactions including their recycling provide an effective and reliable system of defence from free radicals and toxic products of their metabolism.

Carotenoids comprise a family of more than 600 compounds responsible for a variety of bright colours in fall leaves, flowers (e.g., narcissus, marigold), fruits (e.g., pineapple, citrus fruits, paprika), vegetables (e.g., carrots, tomatoes), insects (e.g., ladybird), bird plumage (e.g., flamingo, cock of the rock, ibis, canary), and marine animals (e.g., crustaceans, salmon) *(37)*. These pigments provide different colours from light yellow to dark red and, when complexed with proteins, they can produce green and blue colorations. Yellow, orange and green fruits and vegetables provide a range of carotenoids.β-carotene, α-carotene, and β-cryptoxanthin are the major provitamin A carotenoids in human and lutein, zeaxanthin, and lycopene are major carotenoids in the diet which are not converted to vitamin A. Biological functions of these natural pigments in relation to animals or humans are not well defined but their antioxidant properties seem to be of major importance. In mixture with other antioxidants they could be much more effective than on their own, and the GIT could be a major place for these compounds to exert their activity. In some conditions, carotenoids can be prooxidants. However, it is well recognized that this possibility is not likely to be the case in physiological conditions, including in the GIT when an array of other antioxidants is present.

There are species and tissue-specificity in carotenoid actions. For example, tomatoes are rich sources of lycopene, an antioxidant carotenoid reported to be a potent singlet oxygen-quenching agent. In addition to its antioxidant properties, lycopene shows an array of biological effects including cardioprotective, anti-inflammatory, antimutagenic, and anticarcinogenic activities *(38,43)*. Whereas a great body of experimental evidence has been accumulated to demonstrate the potency and nature of the biological effects of carotenoids, in most cases their underlying mechanisms of action remain uncertain. This is the result of a range of biological effects observed and their tissue specificity, time- and dose-dependency, and limitations of the available model and delivery systems *(39)*. For example, the anticancer activity of lycopene has been demonstrated in both in vitro and in vivo studies. The mechanisms underlying the inhibitory effects of lycopene on carcinogenesis needs further investigations and could involve *(40,41)*: reactive oxygen speces (ROS) scavenging, upregulation of detoxification systems, interference with cell proliferation, induction of gap-junctional communication, inhibition of cell cycle progression, modulation of signal transduction pathways, and effects on the genes governing the androgen stimulation of cell growth, cytokines and on the enzymes producing reactive oxygen species.

Approximately 40 carotenoids are commonly consumed in the US diet and approx 20 can be detected in human serum and tissues *(42)*. Most nutrition research was concentrated

on the six carotenoids found in the highest concentrations in human blood: β-carotene, lycopene, α-carotene, lutein, zeaxanthin, and β-cryptoxanthin. The major dietary lutein sources in the human diet are green vegetables and fruits. Carotenoid consumption and their serum profile vary substantially depending on the origin of the population studied. For example France presents the highest levels of serum lutein and β-carotene and Spain shows the lowest level of β-carotene, along with the highest levels of β-cryptoxanthin *(43)*. American women consume approximately 6 mg of total carotenoids/d *(44)*, the average daily intake of major carotenoids in Spanish population is 3.5 mg/d *(43)* and in Germany total carotenoid intake amounts to 5.33 mg/d with average lutein intake being 1.91 mg/d *(45)*. Daily consumption of lutein and zeaxanthin in American elderly subjects was 2.7 mg for men and 3.09 mg for women *(46)*. In general, the recommended daily intake of carotenoids can only be achieved by consuming 100 to 200 g/d of vegetables and fruits with a particularly high carotenoid content *(47)*.

Low lutein consumption reflects low consumption of fresh vegetable and fruits, changes in nutritional habits, and use of highly processed food. According to National Health Interview Surveys, the intake of lutein declined among different categories of people in the US between 1987 and 1992 *(48)*. There were also significant seasonal differences in plasma carotenoid concentrations in the UK, reflecting a higher intake of lutein during the spring compared with summer and autumn *(49)*. It is interesting to mention that there is also a high positive correlation of lutein ($r = 0.889$) between maternal plasma concentrations and cord plasma *(50)* indicating that the nutritional status of mothers is the major determinant of the lutein status of their babies. In addition it has been shown that breast milk is the major source of lutein to the infants *(51)*. An increased intake of another carotenoid, β-carotene, by lactating women increases the supply of milk β-carotene available to their breast-fed infants.

Carotenoid assimilation from the diet varies significantly depending on many various conditions. However, it seems likely that a substantial proportion of ingested carotenoids could be found in all segments of the digestive tract. Therefore, in combination with other dietary antioxidants carotenoids could promote antioxidant defence in the gastrointestinal tract. Furthermore, carotenoid activities related to the promotion of cell differentiation, regulation of cell proliferation and intracellular communication via gap junctions, as well as regulation of the detoxifying enzymes and enhancement of immune system *(13)*, could also be of great importance in the gastro-intestinal tract.

Carotenoids are known to influence diverse molecular and cellular processes that could be instrumental in their role in reducing the risk for chronic diseases such as CVD and cancer *(52–54)*. Epidemiologic and clinical data showed an inverse association between serum levels of β-carotene and other carotenoids and coronary heart disease (CHD) *(55–57)*. Dietary carotenoids may thus protect against CVD.

The relation between CVD risk and fruit and vegetable consumption was demonstrated by Joshipura et al. *(58)*, who reported a 20% reduction in the incidence of CVD in individuals who consumed the highest quintile of these foods, compared with individuals in the lowest quintile. It is now well established that levels of serum carotenoids may be readily altered by either increasing or decreasing the consumption of fruit and vegetables *(59)*. An increase in serum carotenoids was reported to be accompanied by a significant decrease in serum oxidizability *(59,60)*. Oxidizability in serum can therefore be modified by diet and is related to the carotenoid content of the serum.

In terms of CVD, β-carotene has been the most widely studied because it is one of the most abundant carotenoids *(61)*. Several studies examined the effects of β-carotene in the context of fruit and vegetable intake and also confirmed an inverse association between β-carotene intake and the risk of CVD *(62–64)*. Data from the Rotterdam study on 4802 men and women free of baseline CVD showed that those in the highest tertile of dietary β-carotene intake had a relative CVD risk half of those in the lowest tertile *(65)*.

The mechanisms of action of carotenoids in reducing CVD risk include inhibition of cholesterol synthesis, and an increase in degredation of low-density lipoprotein (LDL) particles through an enhancement of the macrophage LDL receptor activity *(66)*. There is also evidence that carotenoids may reduce the risk of atherosclerosis through inhibition of oxidative damage to LDL; oxidative damage to LDL promotes several key steps in atherogenesis *(67)*. Carotenoids may also reduce the risk of CVD by reducing inflammation as suggested by the inverse association between serum/plasma C-reactive protein (CRP) concentrations and serum/plasma concentrations of β-carotene, α-carotene, and lycopene concentrations *(68,69)*.

As for cancer, several epidemiologic studies also showed that an increased consumption of foods rich in carotenoids is inversely associated with the incidence of major types of cancer in the western world (e.g., carcinoma of the lung, stomach, prostate, mouth, esophagus, colon, or rectum) *(70)*. A similar association was also reported between the concentration of β-carotene in plasma and the risk for cancer *(71)*. Although the biological mechanism for such protection is unknown, various possibilities exist. Carotenoids are potent antioxidants and oxidative stress is known to be involved in carcinogenesis. In a model in vitro system, Bertram and Bortkiewicz showed that carotenoids both with and without provitamin A activity inhibit carcinogen-induced neoplastic transformation. Their results strongly suggest that carotenoids have intrinsic cancer chemo- preventive action in humans *(72)*.

Clinical studies with carotenoid supplementation and some major clinical trials with β-carotene supplementation showed either no or negative effects on CVD and cancer *(73–76)*. Although the reasons for the discrepancy between the results from supplementation and epidemiologic studies are not clear, it has been postulated that supplementation with a single carotenoid at high doses is not sufficient to elicit effects. It has been suggested that a combination of low concentrations of various carotenoids and other micronutrients—as found in fruits and vegetables rather than in individual supplements—appear to be necessary to effect the diverse molecular and cellular processes which form the basis for human health and disease prevention *(77)*. In addition various dietary compounds may provide synergistic effects required for protection against disease *(78)*.

There is no documented evidence that when β-carotene containing natural food sources are consumed in moderation, has negative health implications. Even when very large amounts of carotene rich foods are consumed, the only observed side effect was the occasional appearance of carotenodermia that appears to be harmless and is characterized by yellow or orange tinted skin. The condition disappears spontaneously shortly after the high intake of carotenoid is discontinued. Ingestion of 270 g/d cooked carrots, 180 g/d tomato juice, 300 g/d cooked broccoli or 12 mg/d β-carotene for six wk did not result in the development of carotenodermia *(79)*.

Whereas vitamin A has the potential for acute and chronic toxicity, provitamin A carotenoids do not share the same toxic potential *(80,81)*. As pointed out earlier the

degree of bioconversion of provitamin A in physiological systems is regulated and determined by the vitamin A status of the host *(82–84)*. No adverse effects were reported for any one of the studies in which the value of red palm oil as provitamin A was evaluated *(85–94)*. The duration of these studies varied from two weeks to ten months during which time participants in the study received an estimated 2 to 5 mg of total carotenoids daily.

Data from animal, human and laboratory research suggested that a chronically elevated intake of vitamin A, in the order of 3000 μg/d (about 4 times RDI) may increase the risk of osteoporotic bone disease and fracture, at least in older men and women *(95,96)*. As bioconversion of provitamin A to vitamin A is regulated and determined by the vitamin a status of the host, it could be assumed that the intake of provitamin A carotenoids from fruit, vegetables or red palm oil by older people with an adequate vitamin a status will not pose a risk for the loss of bone mineral density.

β-carotene is the only carotenoid that has been studied extensively in several large-scale primary and secondary prevention trials. Questions as to the safety of the ingestion of high doses of β-carotene have been raised by the α-tocopherol β-carotene cancer prevention study in Finland *(97)*. A statistically significant 18% higher incidence of lung cancer was reported in subjects given 20 mg β-carotene daily compared to subjects receiving a placebo.

Based on a review of published studies the US Preventative Service Task Force does not recommend that people take β-carotene supplements to lower their risk of developing CVD or cancer *(98)*.

3.3. Ascorbic Acid

Vitamin C refers to L-ascorbic acid (AA) and its two-electron reduction product dehydro-L-ascorbic acid. Most animal species synthesize AA from glucose, but human subjects are not able to synthesize it. Therefore AA is an essential dietary component playing an important role in many physiological processes. It is a hydrophilic antioxidant functioning in an aqueous environment and possessing high free-radical-scavenging activity. It can participate in vitamin E recycling thus maintaining efficient antioxidant defence. Fresh green fruits and vegetables are good sources of AA. However, during food processing AA is easily oxidized and as a result AA concentration in such foods is substantially decreased. As a result of its high reducing potential, in combination with iron ions AA can also be a prooxidant. However, it is believed that in physiological conditions, and in the intestinal tract, ascorbic acid performs mainly antioxidant functions. In fact ascorbic acid inhibits chemical synthesis of nitrosamines (animal carcinogens) in the gastric contents and there are suggestions that intakes of ascorbic acid much higher than the RDA may reduce the risk of such diseases as heart disease and cancer *(99)*.

The major advantages of ascorbate as an antioxidant have been described as follows *(100)*:

- Both ascorbate and ascorbyl radical have low reduction potentials and can react with most other biologically relevant radicals and oxidants.
- Ascorbyl radical has a low reactivity as a result of resonance stabilisation of unpaired electron and readily dismutates to ascorbate and dehydroascorbic acid (DAA).

- Ascorbyl radical and DAA can be converted into active ascorbate form by enzyme-dependent or independent pathways. In particular, the ascorbyl radical can be reduced by NADH-dependent semidehydroascorbate reductase or by thioredoxin reductase. At the same time DAA can be reduced to AA by GSH, lipoic acid or glutaredoxin.

The current recommended dietary allowance (RDA) of vitamin C is 75 mg for women and 90 mg for men, based on the vitamin's role as an antioxidant as well as protection from deficiency *(101)*. Recently reviewed data have suggested that an intake of 90 to 100 mg vitamin C/d is required for optimum reduction of chronic disease risk in nonsmoking men and women suggesting a new RDA of 120 mg vitamin C/d *(100,102)*. Therefore, it was suggested that five servings of fruits and vegetables/d may be beneficial in preventing cancer and providing sufficient vitamin C intake for healthy people.

High intakes of the vitamin are generally well tolerated, however, a Tolerable Upper Level (TUL) was recently set at 2 g based on gastrointestinal upset that sometimes accompanies excessive dosages. Indeed, the most common adverse effects of high vitamin C intakes (>2 g/d) are gastrointestinal symptoms such as nausea, abdominal cramps, and diarrhoea *(99)*. After exclusion of the vitamin supplements the symptoms usually disappear within a week or two with no further consequences. Several populations warrant special attention with respect to vitamin C requirements. These include patients with periodontal disease, smokers, pregnant and lactating women, and the elderly *(101)*.

3.4. Flavonoids

Flavonoids are low-molecular-weight polyphenolic substances based on the flavan nucleus. They are widespread in nature, occurring in all plant families, and are found in considerable quantities in fruits, vegetables, grains, cola, tea, coffee, cocoa, beer, and red wine *(103)*. The list of known flavonoids substantially increased from more than 4000 in 1996 *(104)* to over 8000 individual compounds known in 2000 *(105)*. The major flavonoid classes include flavonols, flavones, flavanones, flavanols (catechins), anthocyanidins, isoflavones, dihidroflavonols, and chalcones *(104)*. Representatives of major groups of flavonols were characterized as having antioxidant properties in vitro and in vivo *(105)*.

These compounds have received substantial attention in recent years. The major driving forces of research in the field were the positive effects of fruits and vegetables on human health and their preventive role in the development of various diseases, especially cancers. The flavonoid content in fruits and vegetables can be as high as 300 mg/kg fresh weight *(106)*. In fact, alleged health-promoting effects of flavonoids are usually attributed to their powerful antioxidant activities, but evidence for in vivo antioxidant effects of flavonoids is confusing and equivocal *(107)*. The major problem with antioxidant properties of these compounds is their low availability from the dietary sources. For example, in human blood or urine polyphenol concentrations was shown to be in a range 1 to 2 μM *(108,109)* in comparison with the general concentration of antioxidants in human plasma to be about 1000 μM *(110)*.

Therefore, it has been suggested that the digestive tract is the major site of antioxidant defence afforded by polyphenolic compounds such as flavonoids *(111–113)*. Indeed, phenols might exert direct effects within the gastrointestinal tract, because of the high concentrations present. These effects could include binding of prooxidant iron,

scavenging of reactive nitrogen, chlorine, and oxygen species, and perhaps inhibition of cyclooxygenases and lipoxygenases *(107)*.

Daily intake of flavonoids varies substantially between different countries and is highest in Asian population and in vegetarians. In particular, average daily intake of flavonols in Asian countries comprised about 68 mg and isoflavones 20 to 240 mg *(103)*. In contrast, the mean intake of flavonols of the German population was about 11.5 mg, mainly derived from fruits and vegetables, but also from black tea and red wine *(114)*. Indeed, naturally occurring polyphenolic compounds may play a role in the protective effects of fruits and vegetables against cancers in general, and they appear to have considerable potential as chemopreventive agents against neoplastic changes in the alimentary tract *(115)*. In general flavonoids can prevent LDL oxidative modification by scavenging ROS, chelating transition metal ions, or inhibiting lipoxygenase and this leads to the prevention of atherosclerosis. For example, a number of studies have shown that consumption of soy is antiatherogenic and that the isoflavones genistein, diadzein, and biochanin, which inhibit lipoprotein oxidation in vitro and suppress formation of plasma lipid oxidation products in vivo, are most likely responsible for this effect *(116)*.

However, there are no data available on the long-term effects of flavonoid dietary supplementation on humans. A serious problem with flavonoids is that, depending on conditions, they could be antioxidants or prooxidants, antimutagens or promutagens. Therefore unregulated use of flavonoid-containing supplements can have a detrimental effect on human health. For example, the results obtained by Silva et al. *(117)* suggest that there is a range of flavonols whose genotoxicity in eukaryotic cells depends on their autooxidation. These flavonols can autooxidize when the pH value is slightly alkaline, such as in the intestine, and therefore can induce genotoxicity in humans. Clearly more research is needed to clarify health benefit and potential dangers of these compounds.

Comparatively low bioavailability and antioxidant potential of various flavonoids could be beneficial for the human providing antioxidant protection in various part of the digestive tract, including the large intestine where levels of other antioxidants would be quite low.

3.5. Other Polyphenolics

Cereal brans contain significant quantities of the phenolic ferulic acid and diferulic acid and their potential health benefits (protection of LDL from oxidative modification and reduction in atherogenesis as well as inhibitory effects on tumor promotion and chemopreventive properties) have been related mostly to their antioxidant activity *(118)*.

3.6. Spices and Essential Oils

Addition of spices to food is a common procedure in most cultures. The seasonings contribute a pleasant flavor and recently it has been shown that they contain a range of antioxidant compounds and it seems likely that only a small proportion of them have been isolated and identified *(119)*. They include such phenolic diterpenes as carnosoic acid, carnosol, rosmaridiphenol, and rosmariquinone from rosemary, sage, and summer savory. In other spices a range of flavonoids have been identified. In general, spices and herbs have been shown to have over 100 compounds with high antioxidant activity including 26 active compounds from the *Labiatae* family, *Rosmarinus officinalis*, *Thymus*

vulgaris, *Origanum vulgare* and *O. majorana*, over 40 antioxidative compounds from *Zingiber officinale* and 26 compounds from *Curcuma domestica (120)*. Spices are effective in prevention food deterioration during storage and this explains why traditional diets in countries with high temperature (e.g., India, Thailand, Mexico) are usually rich in spices.Essential oils from aromatic and medicinal plants have been shown to have antibacterial, antimycotic, and antioxidant properties. Recently the essential oils from black pepper, clove, geranium, melissa, nutmeg, oregano, and thyme and 33 phytoconstituents have been assessed in vitro *(121)*. All the compounds demonstrated antioxidant capacities superior to the water-soluble α-tocopherol analogue Trolox with the exception of the essential oil melissa and three phytoconstituents. The best results were obtained with clove, oregano, and thyme oils and their corresponding phytoconstituents namely eugenol, carvacrol, and thymol. Again in the GIT antioxidant properties of various compounds from spices and herbs would contribute to total antioxidant potential.

4. SYNTHETIC ANTIOXIDANTS

Antioxidants in foods may be endogenous origin or may be added externally to preserve their lipid components from peroxidation. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) are commonly used in food formulations. However, because of safety concerns, public interest shifted from synthetic to natural antioxidants. As a result mixed tocopherols, herbal extracts such as those of rosemary and sage, as well as tea extracts have been commercialized for food and nutraceutical applications *(122)*.

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