Antioxidants and Cardiovascular Disease: Still a Topic of Interest

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Abstract

Cardiovascular disease constitutes a major public health concern in industrialised nations. Over recent decades, a large body of evidence has accumulated indicating that free radicals play a critical role in cellular processes implicated in atherosclerosis. Herein, we present a mechanism of oxidative stress, focusing mainly on the development of an oxidised low density lipoprotein, and the results of a clinical trial of antioxidant therapy and epidemiological studies on the relationships between nutrient antioxidants, such as vitamin E, vitamin C, β -carotene, coenzyme Q, flavonoids and L-arginine, and coronary events. These studies indicated that a diet high in antioxidants is associated with a reduced risk of cardiovascular disease, but did not confirm a strong causality link. With regard to vitamin E, observational studies suggested that the daily use of at least 400 International Units of vitamin E is associated with beneficial effects on coronary events. However, it is apparently too early to define the clinical benefits of vitamin E for cardiovascular disease. From the results of several randomised interventional trials, it appears that no single antioxidant given to subjects at high doses has substantial benefits, and the question of whether nutrient antioxidants truly protect against cardiovascular disease remains open. This article provides an overview of the epidemiological and clinical studies related to antioxidants and cardiovascular disease.

Key words: antioxidants, oxidative stress, atherosclerosis, cardiovascular disease, vitamins

1. Introduction

In recent decades, substantial interest has been focused on antioxidant therapeutic strategies for cardiovascular disease. Cardiovascular mortality rates are declining in Japan, but the mortality probability of the three leading causes of death (i.e., malignancy and heart and cerebrovascular diseases) accounts for over 50% of mortalities in both sexes, and cardiovascular disease remains a major cause of mortality and morbidity in Japan (1). It is imperative to emphasise effective preventive strategies for the cardiovascular disease epidemic.

In years past, vegetable-, fruit- and antioxidant-rich Mediterranean diets have been highlighted, since a number of epidemiological studies have shown a strong inverse relationship between cardiovascular disease and vegetable- and fruit-rich diets (2, 3). The World Health Organization recommends 500 g of fresh fruits and vegetables per day (4). Antioxidant micronu-

trients have attracted special attention, particularly vitamin E, vitamin C, β -carotene and other carotenoids, such as lutein, zeaxanthin and lycopene, which have the greatest singlet oxygen-quenching properties (5). More recently, there has been increased interest in putative dietary antioxidants like bioflavonoids, flavonols like quercetin or special phenol derivatives in red wine, and oxygen-sensitive B complexes, which are involved in the metabolism of homocysteine and L-arginine (6–9). Many epidemiological studies have shown some evidence of an association between cardiovascular disease and antioxidant intakes (10–14), but results have been conflicting. Numerous excellent reviews related to cardiovascular disease and antioxidants have been published in the last ten years (15–19). This article briefly summarises the biological effects of antioxidants and provides an updated review of epidemiological studies as well as randomised trials of antioxidant therapy, and explores possible ways to prevent cardiovascular disease.

2. Antioxidants in the human body

2.1. Atherosclerosis and oxidative stress

Oxidative stress is defined as an imbalance between the production of various reactive species and the capacity of the

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organism's natural defence mechanisms for coping with these reactive compounds and preventing adverse effects. Generally, free radicals are produced in the human body during normal metabolism, involving xanthine oxidase, aldehyde oxidase, membrane-associated nicotinamide adenine nucleotide phosphate oxidase and the cytochrome P450 system (20). Cigarette smoke, pollution, ultraviolet light, ionising radiation, ultrasound and chemical shearing forces also lead to radical production. These radicals are mainly highly reactive oxygen species and reactive nitrogen species, which include both radicals and other reactive species, as well as hydroxyl radicals, peroxy radicals, peroxy nitrile and singlet oxygen. Many free radicals have the potential to damage biological macromolecules, such as DNA, membrane lipids, proteins and carbohydrates, considered to be involved in the aetiology of many diseases, including cancer and atherosclerosis (21, 22).

Atherosclerosis and arteriosclerosis have both been considered to involve elements of oxidative stress (23, 24). It has been established that oxidative modification of lipoprotein occurs via the scavenger receptor on macrophages. After very low density lipoprotein is synthesised in the liver and enters the systemic circulation, a portion of triglycerol is removed from very low density lipoprotein and transformed into low-density lipoprotein (LDL) particles. Since the lipids in LDL are fatty acids very sensitive to oxidation, i.e., linoleic acid (18:2, n-6), arachidonic acid (20:4, n-6), and docosahexaenoic acid (22:6, n-6), some LDL particles undergo oxidation. As a result, LDL is no longer recognised by the normal (Apo B/E) LDL receptor. Macrophage LDL uptake is then mediated via the scavenger receptor family and oxidised LDL receptors, a pathway which is three to ten times more rapid than uptake of native LDL. As a consequence, macrophages in the subendothelium are initially converted into lipid-engorged foam cells, pathognomonic of fatty streaks (25, 26). Some biologically active phospholipid oxidation products, in oxidised forms of LDL, affect the pattern of gene expression via activation of the transcription factor nuclear factor- κ B in endothelial cells. This leads to changes in the expression of monocyte binding molecules, monocyte chemoattractant protein and macrophage colony stimulating factors, promoting the recruitment of monocytes and driving their phenotypic transformation into macrophages (27). Further oxidation leads to alteration of the structure of apolipoprotein B, which can not bind to the LDL receptor, binding instead to macrophages. It has been suggested that the accumulation of cytotoxic products from oxidised LDL on the endothelial wall induces platelet aggregation, release of growth factors, disturbance of eicosanoid homeostasis and immigration of inflammatory cells, thereby promoting the formation of atherosclerotic plaque (23). *In vitro* studies have suggested that oxidative stress may induce the production of superoxide radicals, which could inactivate endothelium-derived nitric oxide (28).

2.2. Antioxidants in extracellular fluids

Human plasma contains numerous antioxidants which protect against oxidative stress. These antioxidant proteins include 1) transition metal-binding proteins: transferrin and ceruloplasmin, 2) iron-complex binding proteins: hemopexin and haptoglobin, and 3) albumin, which may act as a sacrificial antioxidant (29).

There are very few antioxidant enzymes in plasma. Rather, these enzymes are mainly intracellular, constituting the cell's antioxidant armour. Copper or zinc containing super oxide dismutase has been found on the endothelial cell surface. Catalase is undetectable in plasma and other extracellular fluids. Furthermore, the physiological activity of selenium-dependent glutathione peroxidases is questionable, based on the low glutathione level (less than 10 μ M) (30).

Another type of antioxidant is nonenzymatic and resides in extracellular fluids. This type includes water-soluble antioxidants (vitamin C and uric acid). In particular, ascorbate interacts strongly with aqueous reactive species and thereby prevents LDL oxidation (31). Ascorbic acid radicals may then be reduced back to ascorbate by dismutation, chemical reduction by glutathione, or enzyme reduction by thioredoxin reductase (32). Bilirubin acts as an antioxidant, and may protect human LDL against oxidation by oxyradicals, including unconjugated bilirubin and conjugated bilirubin. Thiol groups, which are present mostly on albumin in plasma, could act by trapping aqueous peroxy radicals (33). In human LDL, lipid soluble antioxidants, including α -tocopherol, ubiquinol-10, and carotenoids and oxy-carotenoids, have a protective role against lipid peroxidation (34). The most abundant antioxidant in the LDL lipid phase is α -tocopherol, which plays a role by scavenging highly reactive lipid peroxy and alkoxy radicals. Other potentially useful antioxidants include polyphenolic flavonoids extracted from fruits, vegetables, tea and wine. Their antioxidant activities are attributable to the hydrogen-donating capacity of their phenolic moieties and their metal-chelating properties may be effective against free radical oxidation. Multiple polyphenolic flavonoids, such as quercetin and rutin (flavonoids), catechin and epicatechin (flavanols), cyanidin and apigenidin (anthocyanidins), chrysin (a flavone), and taxifolin (a flavanone) are currently being investigated in *in vitro* analyses (35, 36). Soybean products (genistein) and red wine (quercetin) are considered to have a cardio-protective effect (37), including inhibition of platelet aggregation and prevention of LDL oxidation, as are blueberries (aqueous extract of *Vaccinium myrtillus*) and Licorice root (Chinese licorice; *Glycyrrhiza glabra*) (38).

2.3. Vitamin Dose Requirements

Retinol is a preformed vitamin A which is converted to retinal and retinoic acid. α -carotene and cryptoxanthin are also converted to vitamin A in the body. Lycopene, lutein and zeaxanthin are all carotenoids, but are not converted to vitamin A and are strong antioxidants. The primary dietary sources of preformed vitamin A are liver, eggs and fortified foods and those of carotenoids are dark fruits and vegetables. The recommended daily allowances (RDA) for vitamin A are represented as Retinol Activity Equivalent (RE)/day, μ g/day, or International Units (IU). One RE is equivalent to 1 μ g retinol or 12 μ g β -carotene, α -carotene or β -cryptoxanthin. IU are often used in the literature and one RE is equal to 3.3 IU (39). Adverse effects of carotenoids may include birth defects, liver abnormalities and reduced bone mineral density leading to osteoporosis. The RDA for Vitamin A in the USA is 1,000 RE (5,000 IU) and the Recommended Nutritional Intake (RNI) in the UK is 700 RE for males (40–42). The set tolerable upper intake limit

of vitamin A is 16,000 to 20,000 RE (8,000 to 10,000 IU) for most adults in the USA (40). The Acceptable Daily Intake for β -carotene is 5 mg/kg body weight according to the World Health Organization Joint Expert Committee on Food Additives (43). Overdosing on vitamin A can affect nearly every part of the body, including the eyes, bones, blood, skin, central nervous system, liver, and genital and urinary tracts. Severe toxicity can cause blindness and may even be life-threatening (39).

Vitamin C is also known as ascorbic acid, and the main food sources of vitamin C are citrus fruits, papayas, broccoli, potatoes, cauliflower, hot chilli peppers and Brussel sprouts. Long-term high doses may increase the risk of kidney stones. Vitamin C deficiency is characterized by fatigue, weakness, shortness of breath, aching bones, joints and muscles and loss of appetite. The RDA for vitamin C in the USA is 90 mg and the RNI in the UK is 40 mg (40–42). The tolerable upper limit is 2,000 mg/day in the USA, and vitamin C overdose may cause headaches and diarrhoea and increased risk of kidney stones, in the long term (40).

The isomer of α -tocopherol from natural food sources is *RRR*- α -tocopherol, and a mixture of 8 stereoisomers in the synthetic form is referred to as *all-rac*- α -tocopherol. Other vitamin E compounds include tocotrienol, β - and γ -tocopherol. The vitamin E activity of 1 mg of *RRR*- α -tocopherol from natural sources is equivalent to 2 mg of *all-rac*- α -tocopherol from synthetic supplementations, and 1 IU is equivalent to 1 mg of absorption after oral administration to fetal rats. One IU can be converted to that of 0.67 mg of *RRR*- α -tocopherol or 0.91 mg of *all-rac*- α -tocopherol. The biological potency of α -tocopherol is 1, that of γ -tocopherol 0.08; α -tocotrienol 0.21 and γ -tocotrienol 0.01 (39, 40). In the USA, the RDA is 15 mg of α -tocopherol from natural sources, which is equivalent to 22 IU of *RRR*- α -tocopherol or 33 IU of *all-rac*- α -tocopherol. In Western countries, vitamin E deficiency is uncommon and this deficiency is only seen in special cases, such as very low birth weight infants and individuals with fat-absorbing or metabolising disorders. Vitamin E deficiency is believed to manifest as a membrane dysfunction and is likely to produce neurological problems due to poor nerve conditions (39). The RDA for vitamin E is 15 mg (22 IU) in the USA and the RNI in the UK is 4 mg (6 IU) (40–42). The RDA in Japan is 8–10 mg of α -tocopherol from natural sources (44). The tolerable upper limit is 1,100 IU of α -tocopherol in the USA and overdosing may cause bleeding problems, especially in individuals taking anti-clotting medications.

3. Epidemiological Evidence

3.1. Antioxidants and cardiovascular disease

Several ecological studies have indicated strong, inverse associations between antioxidant intakes and cardiovascular disease mortality. Gey et al., summarising data from 12 study populations, found the plasma status of vitamin E to be the most important factor explaining cross-cultural differences in ischaemic heart disease mortality (45). Of the antioxidant vitamins, the tocopherol component of vitamin E was considered to be strongly related to cardiovascular disease across Europe. The Seven Countries Study Research Group (USA, Finland, the

Netherlands, Italy, the former Yugoslavia, Greece and Japan) (46) investigated the associations between food-group intakes and 25-year coronary artery disease mortality in 12,763 middle-aged men, and found the average intake of antioxidant flavonoids to be inversely associated with mortality from cardiovascular disease. However, a random cross-sectional survey of four European populations (north Karelia (eastern Finland), southwest Finland, Scotland and southern Italy) revealed regional plasma vitamin C differences. In Pitkaranta, 93% of the men had plasma levels suggesting severe vitamin C deficiency, while in North Karelia only 2% had deficient levels, possibly explaining the high cardiovascular disease mortality in the former (47). These results require careful interpretation, given the possibility of ecological fallacy and the influences of confounders, which could not be measured.

Large prospective cohort studies have focused on the relationships between antioxidants and cardiovascular disease (Tables 1, 2 and 3). Several epidemiological studies have shown an inverse association between β -carotene intake levels and cardiovascular disease risk in Table 1 (48–55). In the Health Professionals' Study, a comparison of the highest quintile of carotene intake versus the lowest revealed an inverse association with risk among current smokers (Relative Risk (RR), 0.30, 95% Confidential Interval (CI), 0.11 to 0.82) (48). In the Nurses' Health Study (55), 73,286 female nurses were followed for 12 years and then assessed using a semi-quantitative food-frequency questionnaire, and the investigators detected modest but significant inverse associations between the highest quintiles of β -carotene and α -carotene intake and the risk of cardiovascular disease.

Table 2 shows major observational studies of the association of vitamin C and cardiovascular disease (48, 49, 53, 56–62). The Health Professionals' Follow-up Study showed no protective effect against cardiovascular disease after adjustment for cardiovascular disease risk factors and other vitamin intakes. Also, the Nurses' Health Study follow-up for 14 years found that vitamin C offered no significant protection against myocardial infarction or death from coronary heart disease (62). In contrast, the Eastern Finland Study and the Second National Health and Nutrition Examination Survey revealed a beneficial effect on cardiovascular disease (58, 60). These inconsistent results may be due to the difference in duration of vitamin C intake, or in the method of dealing with possible confounders which were not fully adjusted.

Table 3 shows some epidemiological cohort studies of the association between vitamin E intake and cardiovascular disease (48, 49, 57, 61, 63–65). In the Nurses' Health Study (63), it was concluded that the use of supplemental vitamin E for at least 2 years was needed to reduce cardiovascular risk, but low doses (less than 1,000 IU/day) and a short duration were insufficient. Kushi et al. studied 34,486 postmenopausal women in the USA and found the intake of vitamin E from food to be inversely associated with the risk of death from cardiovascular disease (57). In the Health Professionals' Follow-up Study (48), 39,910 male health professionals (40 to 75 years old) were followed for 4 years, and the multivariate relative risk for men consuming more than 60 IU per day of vitamin E was 0.64 (95% CI, 0.49 to 0.83) as compared with those consuming less than 7.5 IU per

Table 1 Prospective Observational Studies of β -carotene and Cardiovascular Disease

Study Country, Publication year	Study Population Age	Follow-up (years)	Comparison	Outcomes	Results RRR or RR (95% CI)
1. Health Professionals' Follow-up Study (48) USA, 1993	39,910 male health professionals 40–75 years old	4	Upper versus lower quintiles of daily intakes	667 coronary diseases	RR 0.30 (0.11–0.82) [†]
2. Longitudinal Population Study (49) Finland, 1994	5,133 Finnish 2,748 men, 2,385 women 30–69 years old	14	Upper versus lower tertiles of daily intakes	186 (men), 58 (women) coronary diseases	RRR –2% (–48% to 30%) [‡] RRR 38% (–29% to 70%)
3. Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study (50) USA, 1994	1,899 males 40–59 years old	13	Upper versus lower quartiles of serum β -carotene	282 nonfatal MI and coronary mortality	RR 0.64 (0.44–0.92) [‡]
4. Massachusetts Health Care Panel Study (51) USA, 1995	1,299 elderly subjects >66 years old	4.75	Upper versus lower quartiles of intakes of carotene-containing fruits and vegetables	161 CVD deaths 48 fatal MI	RR 0.54 (0.34–0.86) RR 0.25 (0.09–0.67)
5. Western Electric Study (52) USA, 1995 (Retrospective Cohort Study)	1,556 men (1958 and 1959 cohorts) 41–57 years old	32,935 person years	Upper versus lower tertiles of daily intakes	231 CHD deaths	RR 0.71 (0.49–0.98)
6. Rotterdam Study (53) Netherland, 1999	4,802 participants 55–95 years old	4	Upper versus lower tertiles of daily intakes	124 MI	RR 0.55 (0.34–0.83) ^{††}
7. National Health and Nutrition Examination Survey III (54) USA, 2000	11,327 subjects 35–90 years old	10	Upper versus lower quartiles of serum carotene level	Angina Pectoris	RR 0.57 (0.38–0.86) ^{§§}
8. Nurses' Health Study (55) USA, 2003	73,286 nurses 34–59 years old	12	Upper versus lower quartiles of daily intakes	998 CVD ^{‡‡}	RR 0.74 (0.59–0.93) ^{¶¶}

RRR=Relative Risk Reduction; RR=Relative risk; 95% CI=95% Confidence Interval; CVD=Cardiovascular Disease; MI=Myocardial Infarction.

[†] Adjusted for age, smoking, body mass index, caloric intake, fiber intake, alcohol use, hypertension, aspirin use, exercise, family history, profession, and vitamin use.

[‡] Adjusted for age, smoking, blood pressure, and cholesterol level.

[§] Adjusted for age, smoking, serum cholesterol level, hypertension, body mass index and energy intake.

[‡] Adjusted for age, body mass index, high-density lipoprotein cholesterol level, low-density lipoprotein cholesterol level, systolic blood pressure, aspirin use, diabetes, physical activity, current smoking and smoking history, and education.

^{††} Adjusted for age, sex, body mass index, pack-years, income, education, alcohol intake, energy-adjusted intakes of vitamin C and E, and use of antioxidative vitamin supplements.

^{§§} Adjusted for age, sex, race or ethnicity, education, smoking status, systolic blood pressure, serum cholesterol, high-density lipoprotein cholesterol, history of diabetes mellitus, body mass index, and physical activity.

^{¶¶} Adjusted for age, smoking, alcohol use, menopausal status, hormone use, exercise, aspirin use, hypertension, cholesterol level, diabetes, caloric intake, and vitamin intake.

^{‡‡} Nonfatal myocardial infarction and fatal CVD.

day, after controlling for cardiovascular risk factors.

The epidemiological studies discussed above suggested an inverse association between vitamin E and coronary events, while the results pertaining to vitamin C, β -carotene, and selenium did not consistently point to a protective effect at high intake levels. Regarding meta-analysis for vitamin intakes, Brubacher et al. estimated the intake-plasma relationship for vitamin C by means of meta-analysis, but did not find a relationship with cardiovascular disease (66). Vivekananthan et al. analysed seven randomised trials of vitamin E treatment and eight of β -carotene treatment and found that vitamin E did not reduce the risk of cardiovascular death while β -carotene was actually associated with a slight but significant increase in risk of cardiovascular death (67). However, we need to consider that such studies do not always follow the same protocol and that adherence to protocols and underreporting of clinical trial results or publication bias (or publication policy bias) can affect the validity of meta-analyses.

Generally, an extremely low plasma level of antioxidant may be a risk factor, however, we must be cautious when interpreting these findings, considering all possible confounders. For example, vitamin E supplement users might be more health-conscious than the general population, and other unmeasured or

unknown characteristics as possible confounding factors must be considered. These observed associations between antioxidant intake and disease risk could reflect other nutrients. Diets rich in antioxidants are also lower in saturated fat and cholesterol and higher in fiber. In large prospective studies, such difference has been considered by stratification or adjustment in statistical models. However, it is impossible to adjust for unmeasured health behaviours completely. In the cases of the Nurses' Health Study and Health Professionals' Follow-up Study, their risk estimates were adjusted for measured dietary factors such as fiber and caloric intake. However, vitamin E intake may still be correlated with intake of other undetermined factors. Furthermore, the health behaviour between the subjects who used and that of those who did not use vitamin supplements were quite similar, because the target populations in these studies were homogeneous occupational cohorts. This may limit generalization to broader populations, since health professionals may be more health-conscious than the general population. According to observational epidemiological studies, the results of which have been debated by those attempting to fully understand the multifaceted role of nutrients in cardiovascular disease, it remains uncertain whether adequate intake of both vitamin supplements and diet-derived antioxidants, and

Table 2 Prospective Observational Studies of Vitamin C and Cardiovascular Disease

Study Country, Publication year	Study Population Age	Follow-up (years)	Comparison	Outcomes	Results RR (95% CI) or OR
1. Health Professionals' Follow-up Study (48) USA, 1993	39,910 male health professionals 40–75 years old	4	Upper versus lower quintiles of supplementation	360 CABG/PTCA 201 nonfatal MI, 106 CVD	No significant effects
2. Longitudinal Population Study (49) Finland, 1994	5,133 Finnish men, 23,85 women 30–69 years old	14	Upper versus lower tertiles of daily intakes	186 (men), 58 (women) coronary diseases	No significant effects RR 0.49 (0.34–0.98)
3. Atherosclerosis Risk in Communities Study (56) USA, 1995 (Cross-sectional study)	4,989 men and 6,318 women 45–67 years old	—	Linear trend	Carotid artery intima-media wall thickness	No significant effects [†]
4. Menopausal Women Study (57) USA, 1996	34,486 menopausal women 55–69 years old	7	Upper versus lower quintiles of daily intakes	242 CHD	No significant effects [§]
5. Eastern Finland Study (58) Finland, 1997 (Prospective population study)	1,605 healthy men 42, 48, 54, 60 years old	8	Vitamin C deficiency versus non deficiency	58 MI	3.5 (1.8–6.71) [†]
6. Rotterdam Study (53) Netherlands, 1999	4,802 participants 55–95 years old	4	Upper versus lower tertiles of intakes	124 MI	No significant effects
7. National Health and Nutrition Examination Survey III (59) USA, 1999 (Cross-sectional study)	7,658 subjects >30 years old	—	Mortality rates in three biological vitamin C categories	CVD	0.48 (0.23 to 1.03) MI [‡]
8. National Health and Nutrition Examination Survey II (60) USA, 2001 (Cross-sectional study)	8,453 subjects >30 years old	—	Mortality rates in three biological vitamin C categories	CVD deaths	–21%–25% CVD ^{††}
9. Male Physicians' Study (61) USA, 2002	83,638 male physicians 44–64 years old	5.5	Upper versus lower quintiles of supplementation	1,037 CVD deaths (608 CHD deaths including)	RR 0.88 (0.61–1.27) ^{§§}
10. Nurses' Health Study (62) USA, 2003	85,118 nurses 38–63 years old	16	Upper versus lower quintiles of supplementation	1,356 nonfatal MIs and CVD	No significant effects

MI=Myocardial infarction; CABG=Coronary artery bypass graft; PTCA=Percutaneous transluminal coronary angioplasty; RRR=Relative Risk Reduction; RR=Relative risk; 95% CI=95% Confidence Interval; CVD=Cardiovascular Disease; CHD=Coronary Heart Disease

[†] Adjusted for age, gender, race, education, physical activity, smoking, alcohol use, cholesterol, body mass index, diabetes, hypertension, history of cardiovascular disease, aspirin use and vitamin use.

[§] Adjusted for age, energy intake, body mass index, waist-hip ratio, smoking status, diabetes, hypertension, physical activity, estrogen use, alcohol use, marital status, education.

[†] Adjusted for age, year of examination, season of year.

[‡] Among participants who consumed alcohol, association with a decreased prevalence of angina (multivariate odds ratio).

^{††} Adjusted for alcohol use, smoking history, aspirin use, and medical conditions.

^{§§} Adjusted for age, sex, cigarette smoking, education, and consumption of citrus fruits and vegetables.

individual oxidative stress status are vital for providing the full protective effect against coronary events.

3.2. Bioflavonoids, fruit intake and cardiovascular disease

van't Veer et al. reported fruits and vegetables to be beneficial in the prevention of cancer and cardiovascular disease, based on a review of more than 250 observational studies (68). Cardiovascular deaths were reduced by 16% (8,000 deaths annually) by increasing the current 250 g of fruits and vegetables per day to 400 g per day in the Dutch general population. One of the most famous epidemiological investigations yielding positive results was the Zutphen elderly study (69), in which the flavonoid intakes of 805 men (65–84 years old) were assessed using a cross-check dietary history, with 5 years of follow-up. Flavonoid intake (analysed in three parts) was significantly inversely associated with mortality from coronary heart disease (p for trend=0.015). In a study of 5,133 Finnish men and women, Knekt and colleagues found an inverse association between cardiovascular disease and intake of both vegetables and fruits, as assessed by a combinational dietary history which measures with better validity compared to the use of food-

frequency questionnaires (70, 71). Data from the Nurses' Health Study and the Health Professionals' Follow-up Study revealed that individuals in the highest quintile of fruit and vegetable intake had a RR for coronary heart disease of 0.80 (95% CI, 0.69 to 0.93) compared with those in the lowest quintile of intake (72). However, in several other large-scale prospective studies (e.g., the Caerphilly Study (73), the Adventist Health Study (74) and the Women's Health Study (75)), no significant association was found between coronary heart disease and the total amount of fruit consumed. The observed protective effects of fruits and vegetables on cardiovascular disease may be associated with other healthy behaviours, or the reduction of intake of putative nutrients such as salt or saturated fat. Furthermore, analysis in prospective population-based studies tends to be influenced by the effects of effect modifiers and other confounders, such as dietary patterns, lifestyle and nationality of the target population.

Studies of individuals consuming diets rich in fruits and vegetables containing lycopene have shown high lycopene levels to be associated with reductions in cardiovascular disease incidence (76). The prospective Kuopio Ischemic Heart Disease Risk Factor Study revealed that men in the lowest quartile of

Table 3 Prospective Observational Studies of Vitamin E and Cardiovascular Disease

Study Country, Publication year	Study Population Age	Follow-up (years)	Comparison	Outcomes	Results RR (95% CI)
1. Nurses' Health Study (63) USA, 1993	87,245 nurses 34–59 years old	8	Upper versus lower quintiles of supplementation	437 nonfatal MI 115 CHD	RR 0.66 (0.50–0.87) [†]
2. Health Professionals' Follow-up Study (48) USA, 1993	39,910 health professionals 40–75 years old	4	Upper versus lower quintiles of supplementation	667 CHD	RR 0.60 (0.44–0.81)
3. Longitudinal Population Study (49) Finland, 1994	5,133 Finnish 27,48 men, 23,85 women 30–69 years old	14	Upper versus lower quintiles of supplementation	186 CHD (men) 58 CHD (women)	RR 0.68 (men), RR 0.32 (women) both p for trend <0.01
4. Menopausal Women Study (57) USA, 1996	34,486 postmenopausal women 55–69 years old	7	Lowest to highest quintile of VE intakes	242 CHD deaths	RR 0.42 p for trend 0.008
5. Established Populations for Epidemiological Studies of the Elderly (64) USA, 1996	11,178 subjects 67–105 years old	9	Usage of VE	3,490 total deaths CHD deaths	RR 0.53 (0.34, 0.84) [‡]
6. Multiple Risk Factor Intervention Trial (65) USA, 1998 (Nested case-controlled study)	734 subjects 35–57 years old	—	Upper versus lower quartiles of intakes	MI and CVD deaths	No significant effects
7. Male Physicians' Study (61) USA, 2002	83,638 male physicians 44–64 years old	5.5	Upper versus lower quintiles of supplementation	1,037 CVD deaths	No significant effects

MI=Myocardial infarction; CABG=Coronary artery bypass graft; PTCA=Percutaneous transluminal coronary angioplasty; RR=Relative risk; 95% CI=95% Confidence Interval; CVD=Cardiovascular Diseases; CHD=Coronary Heart Diseases

[†] Adjusted for age and smoking.

[‡] Adjusted for age, smoking, body mass index, caloric intake, fiber intake, alcohol use, hypertension, aspirin use, exercise, family history, profession, and vitamin use.

[§] Adjusted for age, sex, alcohol use, smoking history, aspirin use, and medical conditions, cigarette smoking, education, and consumption of citrus fruits and vegetables.

serum lycopene levels had a 3.3-fold increased risk of an acute coronary event or stroke, as compared with the other 725 middle-aged men (77). In a cross-sectional analysis of antioxidant supplementation in the Atherosclerosis Prevention study, the association between the plasma lycopene concentration and intima-media thickness of the common carotid artery wall was assessed in 520 asymptomatic men and women, and low levels of plasma lycopene were associated with an 18% increase in intima-media thickness (78). Tea is also rich in antioxidant polyphenols (catechins, flavonols, theaflavin and thearubigins), and several epidemiological studies have been conducted to elucidate the protective role of tea against coronary artery disease (79–81). However, the interactions of other phytoestrogens, flavonoids, other carotenoid compounds, vitamins and phenol-derived substances in cardio-protection have been less extensively studied.

Rimm et al. (82) pointed out that the findings for their target population may not be applicable to the general population, since health professionals may differ in crucial ways from the general population. In addition, the aforementioned inconsistent results may arise from dietary assessment differences, and measurement errors in compiling data from dietary records. The above prospective epidemiological studies used self-reported dietary assessments, food records, 24-hour recalls, or dietary history questionnaires, but their authors should perhaps have recognised that the reproducibility test is vital due to the expected underestimation associated with questionnaire results. Furthermore, self-reports may lead to systematic bias, which is sometimes observed when an individual food intake report is affected by personal characteristics, such as body mass index, ethnicity, and age. Heitmann et al. pointed out that total energy consumption tended to be underreported among obese persons

in a study depended on a self-reporting style (83).

4. Clinical trials

4.1. Antioxidant therapy

To assess the effects of antioxidant intakes on restenosis (smooth muscle cell migration, replication and accumulation of extracellular matrix and vascular remodelling) following Percutaneous Transluminal Coronary Angioplasty (PTCA), several randomised clinical trials with probucol therapy, which also plays the role of a scavenger in elective angioplasty, were conducted. The Probulcol Angioplasty Restenosis Trial compared a 1000 mg/day dose with a control (no treatment) and found that probucol significantly reduced restenosis (84, 85). However, unexpectedly, a MultiVitamin and Probulcol trial, which examined the effects of probucol (1000 mg/day) alone versus in combination with vitamin C (1000 mg/day), vitamin E (1400 IU/day) and carotene (100 mg/day) on the rate and severity of restenosis, demonstrated that concomitant administration of antioxidant vitamins results in the loss of the beneficial effects of probucol (86, 87). These findings might be attributable to vitamins C and E with carotene interfering with the conversion of probucol to active metabolites. Recently, marketed non-prescription nutritional supplement products are used with the aim of improving health, but more rigorous clinical trials among high-risk groups are needed before antioxidant therapy can be routinely recommended to coronary artery disease patients.

4.2. Antioxidant capacity and cardiovascular disease

Once synergistic interactions among the nutrient antioxidants were suggested, a new concept of antioxidant capacity

emerged in the 1980s–1990s. Total antioxidant capacity is now considered to reflect the ability of the extracellular fluid compartment to scavenge oxygen free radicals, estimated by measuring the inhibition of an artificially generated oxidative process (88–92). The principal antioxidants in human plasma (or serum) are albumin and uric acid, and the remaining antioxidant capacity is attributed to vitamin C, bilirubin, vitamin E and flavonoids, as well as other relevant serum proteins such as transferrin and ceruloplasmin, therefore, the data on antioxidant capacity should be interpreted as total *in vivo* function, rather than in terms of individual antioxidant levels. Interestingly, global antioxidant capacity, including nutritional and endogenous components which are considered to interact synergistically in plasma, usually manifests at sites of endothelial injury. Some authors have used antioxidant capacity methods to examine oxidative damage or reperfusion damage in myocardium upon PTCA. Rigattieri et al. investigated the oxidative state using the Total Radical Antioxidant Potential method, in great cardiac vein blood from 5 patients undergoing PTCA. Great cardiac vein Total Antioxidant Radical Potential levels were significantly lower than aortic levels before single balloon inflation (93). Tomasetti et al. examined the extent of ischaemic damage and measured certain biochemical indexes of reperfusion damage in 25 patients undergoing PTCA, and found that antioxidant capacity and uric acid decreased upon reperfusion, which lowers hydro-soluble antioxidants, representing a mild oxidative insult (94). These data indicated that short episodes of myocardial ischaemia during PTCA induce a sustained oxidative stress, which is detectable in the venous effluent of the reperfused myocardium. Other clinical studies also used the antioxidant capacity method to find the possible roles of prevention of cardiovascular disease, on the assumption that the oxidation of LDL cholesterol in vessel walls is one of the steps involved in atherogenesis. Kummerow et al. conducted a case-controlled study in 1,200 angina patients and healthy subjects in the USA and found a decrease in antioxidant capacity in the plasma of catheterised patients (95). Nojiri et al. investigated the relationship between coronary artery disease and serum antioxidative status in a case-controlled study (96). A multiple regression analysis of data from the coronary artery disease patients revealed that the Total Antioxidant Status is correlated most negatively with the number of diseased vessels. The concentrations of carotenoids and glutathione peroxidase, as well as the α/γ -tocopherol ratio, were also significantly associated with the number of diseased vessels. In all, more scientific evidence should be accumulated to establish clinical significance of the preventive role of antioxidant capacity against oxidative stress related to coronary disease.

5. Randomised Intervention Trials

5.1. Completed Randomised Trials of Antioxidants (Primary Prevention Studies)

From the positive results in the observational studies, there was a strong expectation that larger clinical trials and longer periods of follow-up could reveal the ultimate beneficial effects of antioxidants on cardiovascular prevention. Generally, randomised, double-blind, placebo-controlled trials are expected to

be unbiased and less affected by confounders than other epidemiological methods. Several large, randomised, controlled trials have attempted to unravel the antioxidant paradox (97–119). However, the actual effects of antioxidant vitamins in primary and secondary prevention have been rather disappointing.

In the case of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study in Finland, a total of 29,133 male smokers 50 to 69 years of age from southwestern Finland were randomly assigned to one of four regimens, α -tocopherol (50 mg/day, 75 IU) alone, β -carotene (20 mg/day) alone, both α -tocopherol and β -carotene, or a placebo in a 2×2 factorial design, and then followed for 5 to 8 years. α -tocopherol had no apparent effect on either total mortality or mortality from cardiovascular disease (98–100), but was associated with an increase in mortality from haemorrhagic stroke. The authors investigated the effects of α -tocopherol on stroke and found that systolic blood pressure was a possible effect modifier of vitamin E, and the further stratification analysis revealed that vitamin E increased the risk of subarachnoid haemorrhage and decreased the risk of cerebral infarction in hypertensive men. Of note, they suggested that vitamin E and aspirin are more effective in prevention of ischaemic stroke. The authors also studied the antioxidant effects on the incidence of angina pectoris in men (99) and found that β -carotene had no preventive effect, being associated instead with a slight increase in angina. The limitation of this study in terms of cardiovascular disease was that only 1% of the deaths in this population were due to cardiovascular disease, probably limiting statistical power. Secondly, the 50-mg vitamin E dose may be lower than the expected dosage needed to reduce the risk of cardiovascular disease.

The Beta Carotene and Retinol Efficacy Trial targeted heavy smokers and asbestos-exposed workers to examine the effects of a combination of 30 mg/day of β -carotene and 25,000 IU/day of retinol (vitamin A) in the form of retinyl palmitate (101, 102). However, because of the negative effects of carotene and vitamin A, that portion of the intervention trial was discontinued. The population studied, middle-aged male long term smokers, is not representative of general populations or their state of atherosclerosis.

The China study of Linxian adults found dietary vitamin and mineral supplementation, particularly in combination with β -carotene, vitamin E and selenium, to possibly reduce cancer risk. The study targeted 29,584 adults (40–69 years old), who received daily vitamin and mineral supplements in four communes in Linxian, an area with one of the world's highest rates of gastroesophageal cancer. They used a 2(4) factorial experimental design, testing for the effects of four nutrient combinations: retinol and zinc; riboflavin and niacin; vitamin C and molybdenum; and β -carotene, vitamin E and selenium. Significantly lower total mortality (RR, 0.91, 95% CI, 0.84 to 0.99) was documented among those receiving the β -carotene, vitamin E and selenium combination (97). However, the investigators did not calculate the specific cardiovascular disease relative risk; the target population had a relatively low risk of coronary events and is a poorly nourished population as compared to other European and American cohorts (97). These observed vitamin E benefit may have been due to pre-existing nutrient deficiencies.

5.2. Completed Randomised Trials of Antioxidants (Secondary Prevention Studies)

A few large randomised trials examining secondary prevention study that found antioxidants to have beneficial effects are listed in Table 5 (110–119). In the Cambridge Heart Antioxidant Study, 2002 patients with angiographically proven coronary atherosclerosis were followed for a median of 510 days, and assigned α -tocopherol capsules containing 800 IU daily (537 mg as free 2*R*,4',8'*R*- α -tocopherol) (112). α -tocopherol treatment significantly reduced the risk of the primary trial endpoints of cardiovascular death and non-fatal myocardial infarction. However, this decrease was mainly due to the decrease in myocardial infarction. On the other hand, other studies have found no beneficial effect of antioxidant supplementation on cardiovascular prevention. The Heart Outcomes Prevention Evaluation trial evaluated the effects of vitamin E and ramipril (angiotensin-converting enzyme) in patients at high risk for cardiovascular disease events, those 55 years of age or older with current cardiovascular disease, or those with diabetes and at least one additional coronary risk factor. A 2×2 factorial

design (400 IU vitamin E from natural sources (*RRR*- α -tocopherol) with 10 mg ramipril or their respective placebos) was used on the subjects, who were followed for an average of 4.5 years (114, 115). Daily administration of vitamin E to the study subjects was found to have no effect on cardiovascular outcomes or nephropathy, however, in the subgroup analysis, ramipril was associated with a significant reduction of cardiovascular mortality and morbidity. The large Alpha-Tocopherol, Beta-Carotene Cancer Prevention study and Linxian Province studies, both of which used a relatively small dose of vitamin E, and the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Prevenzione Trial showed slight or no benefits of antioxidants on cardiovascular disease. Also, the Beta Carotene and Retinol Efficacy Trial and the Physicians' Health Study, both of which focused on administering carotene supplement, found no beneficial effects on cardiovascular disease.

Some large randomised intervention trials (Tables 4 and 5) are currently ongoing. These ongoing studies should discuss the comparison of results from past randomised placebo trials, especially focusing on strategies of combining other medicine

Table 4 Randomised Controlled Trials (Primary Prevention Studies)

Study Country, Publication year	Study Population Age	Duration of Treatment (years)	Antioxidants	Endpoints	Results RRR or RR (95% CI)
1. Linxian Trial (97) China, 1993	29,584 subjects 40–69 years old	5.7	30 mg VE/day, 15 mg β -carotene/day Retinol, minerals (Zn, Mo, Se)	2,127 deaths	0.91 (0.84–0.99) [§] No significant effects [†]
2. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study [†] (99) Finland, 1996	22,269 male smokers 50–69 years old	4.7	50 mg VE/day or 20 mg β -carotene/day or both	1,983 Angina Pectoris	0.91 (0.83–0.99) VE No significant effects [‡]
3. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study [†] (100) Finland, 1998	27,271 male smokers 50–69 years old	6.1	50 mg VE or 20 mg β -carotene/day or both	1,204 nonfatal myocardial infarction and 907 CVD deaths	–4% (–12% to 4%) VE ^{††} 1% (–7% to 10%) β -Carotene
4. Beta-Carotene and Retinol Efficacy Trial [†] (101) USA, 1996	18,314 heavy smokers and asbestos workers 45–69 years old	4	30 mg β -carotene and 25,000 IU retinol/day	Total mortality CVD mortality	1.17 (1.03–1.33) 1.26 (0.99–1.61)
5. Physicians' Health Study (103, 104) USA, 1999	22,071 male physicians 40–84 years old	12	50 mg β -carotene ^{§§} , 325 mg Aspirin	CVD mortality	No significant effects
6. Physicians' Health Study (II) (105) USA, 2000	15,000 male physicians >55 years old	ongoing	β -carotene, VE, VC	Prostate cancer, CVD, eye diseases cataract and macular degeneration	No results yet
7. Supplementation en Vitamines et Mineraux Antioxydants (106) France, 1998	12,735 subjects male, 45–60 years old, female, 35–60 years old	ongoing (8 years)	120 mg VC, 30 mg VE/day 6 mg β -carotene/day, minerals (Se, Zn)	Total Mortality CVD and cancer	No results yet
8. Women's Health Study (107) [†] USA, 1999	39,876 female health professionals >45 years old	2.1	50 mg β -carotene (alternate day)	MI, CVD deaths	No significant effects
9. St. Francis Heart Study (108) USA, 2001	5,582 subjects 50–70 years old	ongoing (4 years)	Atorvastatin, VC and VE	Coronary events	No results yet
10. Antioxidant Supplementation in Atherosclerosis Prevention (109) Denmark, 2003	520 subjects cholesterol >5.0 mmol/L ⁻¹ 45–69 years old	3	136 IU VE/day or 250 mg VC	Carotid artery intima-media thickness	25% treatment effect (2 to 49%) ^{††}

IU=International unit; MI=Myocardial infarction; CVD=Cardiovascular disease; VC=Vitamin C; VE=Vitamin E; Se=Selenium; Zn=Zinc; Mo=Molybdenum.

[†] Terminated earlier than originally planned.

[§] Supplementation with β -carotene, VE, and Se.

[†] Supplementation with retinol and Zn, riboflavin and niacin, VC or Mo.

[‡] Supplementation with β -carotene.

^{††} RRR

^{§§} 325 mg aspirin alone, 50 mg β -carotene alone, both, or neither.

Table 5 Randomised Controlled Trials (Secondary Prevention Studies)

Study Country, Publication year	Study Population Age	Duration of Treatment (years)	Antioxidants	Endpoints	Results RR (95% CI)
1. Women's Antioxidant and Cardiovascular Study (110, 111) USA, 1995	8,000 CVD females >40 years old	ongoing	265 mg (400 IU) VE, 1,000 mg VC/day or β -carotene 20 mg/day	VE, nonfatal MI and nonfatal stroke coronary revascularisation	No results yet
2. Cambridge Heart Antioxidant Study (112) UK, 1996	2,002 CVD mean age, 62 years old	1.3	265 mg (400 IU) or 800 IU VE/day	CVD deaths and nonfatal MI	0.53 (0.34–0.83) 0.23 (0.11–0.47)
3. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (113) Finland, 1997	1,862 MI 50–69 years old	5.3	50 mg VE or 20 mg/day β -carotene both VE and β -carotene	Coronary events	1.75 (1.16–2.64) [†]
4. Heart Outcome Prevention Evaluation Study (114) Canada, 2000	9,297 high-risk subjects >55 years old	4.5	265 mg (400 IU) VE/day 10 mg ramipril/day	MI, stroke, CVD deaths	1.03 (0.88–1.21) [§]
5. Heart Outcome Prevention Evaluation Study (115) Canada, 2001	732 high-risk subjects >55 years old	4.5	265 mg (400 IU) VE/day 10 mg ramipril/day	Atherosclerosis progression [§]	No significant effects
6. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico-Prevention Trial (116, 117) Italy, 2001	11,323 MI (1,665 women) mean age, 59.3 years old	3.5	1 g PUFA, 300 mg VE/day	Total Mortality	0.59 (0.36 to 0.97) [†]
7. Women's Angiographic Vitamin and Estrogen Trial (118) USA, 2002	423 postmenopausal CVD patients	5	0.625 mg Equine estrogen/day 400 IU VE, 500 mg VC twice/day	Deaths, MI, stroke	No significant effects
8. MRC/BHF Heart Protection Study (119) UK, 2002	20,536 CVD high-risk subjects 40–80 years old	5	600 mg VE, 250 mg VC 20 mg β -carotene/day	Coronary events Fatal or non-fatal vascular events [‡]	No significant effects

IU=International unit; PUFA=Polyunsaturated fatty acids; MI=Myocardial infarction; VC=Vitamin C; VE=Vitamin E.

[†] RR for β -carotene and RR for vitamin E were not significantly different.

[§] RR for vitamin E and a similar RR were obtained for β -carotene supplementation.

[†] Only early effect of PUFA.

[‡] Subcategory analysis.

and vitamins versus single or fewer combinations of antioxidant administration. Other factors such as population age, nationality, gender, duration of study and pre-existing symptoms should also be discussed. Whether antioxidants combined with other drugs or vitamins for a long term are beneficial remains uncertain, and the ongoing trials are now under evaluation for further defining the antioxidant's role in cardiovascular prevention.

7. Research options for the 21st century

7.1. Issues in intervention studies

We need to take into account the possible problems of past clinical intervention trials, including limitations inherent to the studies, e.g., the different dosages of antioxidants used, the statistical power of the study and data validity, as well as differences among study populations. With respect to epidemiological and methodological issues, we depend to a great extent on the self-report or interview method for dietary assessment, which may lead to inaccurate assessment of dietary exposure or observer bias and missing information (120). As for study validity, not all investigations verified the reproducibility of their questionnaire results, and we should also be aware that self-reported assessments in observational studies may encounter systematic bias. Combined with random error in epidemiological research, these biases could influence the study results. In addition, the randomised trial study design may be affected by selection bias,

since subjects producing informed consent have a scientific interest beforehand, which limits the generalisability of the trial results. Past intervention studies were designed to target certain populations, such as the aged or those in late middle age, but atherosclerotic progression generally begins earlier in life, and vitamin intake at an early age is preferable. The duration of the follow-up term in randomised studies is relatively short for detecting nutrient effects, and is logistically difficult to continue. Longer cohort studies starting with younger subjects are anticipated to be less biased. In addition, methods of collecting and adjusting information for possible confounding factors may vary and thus produce inconsistent results. As for study endpoints, the differences in the ascertainment or progression of cardiovascular disease among studies must be carefully considered. In past intervention studies, the main likelihoods of coronary artery occlusion, myocardial infarction and death resulting from myocardial infarction were used, though other evaluating methods of the progression of atherosclerosis, including measurements of vascular function, carotid artery thickness and plaque size may yield other results (121, 122).

Furthermore, the optimal dosage for each individual should be taken into account, given differences in oxidative stress levels (123). High doses of antioxidants may have a pro-oxidant (e.g., ATBC study) rather than a beneficial effect (124). There is a relationship between nutrition and the immune system, as well as nutrient-nutrient interactions. The interaction between an an-

tioxidant supplement and other nutrients present at low levels, like selenium, may affect the immune system adversely, and be carcinogenic (125). The difference in absorption among isomers is an important issue. Natural *RRR*- α -tocopherol has twice the activity of synthetic *all-rac*- α -tocopherol in humans and it is estimated that 100 to 400 IU/day is needed to decrease the susceptibility of LDL to oxidation (126), therefore, supplementation of *RRR*- α -tocopherol may need to be discussed. Also, the relative availability of one nutrient may impair or enhance the action of another in the immune system. Examples include interactions between vitamin E and selenium, vitamin E and vitamin A, zinc and copper, and dietary fatty acids and vitamin A. Supplemental β -carotene may interfere with vitamin E absorption. Gey suggested the interesting concept that optimal health and self-administration rectification of vitamin C and vitamin E with doses of other micronutrients may reduce subsequent risk and randomized intervention with synchronous administration of vitamin A, vitamin C, vitamin E, vitamin B and minerals (127).

7.2. Beyond Antioxidants

The antioxidant hypothesis has been interpreted in several ways. The potential disease-preventive mechanisms of vegetables and fruits, such as antioxidant activity, modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, as well as antibacterial and antiviral effects, have been discussed (128, 129). The beneficial effects of vitamin C aside from antioxidant activity are the reduction of blood cholesterol levels by stimulating the conversion of cholesterol to bile acids, change in the lipoprotein profile, coagulation factors and vasodilation (130, 131). Antioxidants also play a pivotal role in reducing the adhesive interaction between immune and endothelial cells in the early stages of atherogenesis (132). Also, reactive oxygen species have recently been reported to be involved in the regulation of apoptosis and the process of T-lymphocyte maturation in the thymus, such that greater exposure to reactive oxygen species and diminished antioxidant protection may contribute to increased incidences of infection, neoplasia and autoimmune disorders in the elderly suffering from the age-related deregulation of the immune response (133). In addition, antioxidants exert an atherogenic inhibitory effect. Vitamin E and lycopene supplements reduce the cell-associated expression of adhesion molecules and adhesion to monocytes, when activated with the pro-inflammatory cytokine interleukin-1 β . Also, supplemental vitamin E produces the soluble form of intracellular adhesion molecule-1, decreasing excessive LDL cholesterol levels (134).

As for functional food, we may need to consider the multiple benefits of vegetables and fruits against cardiovascular disease due to nutrients such as folate, vitamin B6, and fiber, and minerals like potassium, which acts by reducing plasma homocysteine levels (135, 136).

As noted above, future epidemiological research should use more reliable oxidative markers with antioxidant measurements, and target high-risk groups for oxidative stress. The appearance of new or the progression of early lesions of atherosclerosis, which antioxidants have been shown to inhibit in animal models, should be studied. Since one reason for failures in intervention studies might be the target subjects having low peroxidation levels, more robust biomarkers of lipid peroxidation *in vivo* should be applied, including thiobarbituric acid-reactive substances, breath hydrocarbons, LDL resistant to oxidation and F₂ isoprostane. DNA damage, such as mitochondrial DNA damage, and primary DNA oxidation products, such as 8-Hydroxy-2'-deoxyguanosine, which may reflect individual oxidative stress, autoantibodies to oxidized DNA, and comet assay, respond to antioxidant therapy. Biomarkers of protein oxidation have been applied in studies of diseases such as Alzheimer's disease, Parkinson's disease, Duchenne muscular dystrophy, amyotrophic lateral sclerosis, rheumatoid arthritis and progeria. Some epidemiological studies focused on the antioxidant hypothesis have involved combining a good oxidative stress indicator and measuring antioxidants (137, 138), which can reduce the possible systematic bias. In future coronary risk assessment, molecular biology, genetics and cardiac physiology will contribute to epidemiological research. Howitz KT et al. reported on the association of polyphenol with calorie restriction and life span (139). Information of genetic susceptibility and its environmental interaction including dietary exposure will be a vital force of future cardiovascular epidemiology. In conclusion, antioxidant vitamin treatment as protection against cardiovascular disease should not be instituted, rather, a balanced diet with emphasis on antioxidant-rich fruits and vegetables and whole grains is recommended for the general population by the American Heart Association. In all, the treatment or prevention of atherosclerosis appears to require a multidisciplinary approach, encompassing lifestyle modification and pharmaceutical or nutritional therapies stressing the modification of the atherogenic potential of oxidized lipoproteins or the endothelium. The aforementioned clinical trial results for antioxidants reaffirm the importance of evidence-based medicine as the basis of disease prevention strategies. Public health practitioners must reinforce good nutrition in health promotion and be cognisant of new findings in antioxidant research.

References

- (1) Wagakuni no Hoken Tokei (H13). Tokyo: Health and Welfare Statistics Association, 2001 (Magazine in Japanese).
- (2) Weisburger JH. Lifestyle, health and disease prevention: the underlying mechanisms. *Eur J Cancer Prev.* 2002; 11: S1–7.
- (3) Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. *JAMA.* 2002; 288: 2569–2578.
- (4) National Research Council, USA, Committee on Diet and Health, Implication for reducing Chronic Disease risk. Washington, DC: National Academy of Sciences, 1989.
- (5) Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press, 2nd end, 1989.
- (6) Hirvonen T, Pietinen P, Virtanen M, et al. Intake of flavonols

- and flavones and risk of coronary heart disease in male smokers. *Epidemiology*. 2001; 12: 62–67.
- (7) De Bree A, Verschuren WM, Kromhout D, Kluijtmans LA, Blom HJ. Homocysteine determinants and the evidence to what extent homocysteine determines the risk of coronary heart disease. *Pharmacol Rev*. 2002; 54: 599–618.
- (8) Hinderliter AL, Caughey M. Assessing endothelial function as a risk factor for cardiovascular disease. *Curr Atheroscler Rep*. 2003; 5: 506–513.
- (9) Samman S, Sivarajah G, Man JC, Ahmad ZI, Petocz P, Caterson ID. A mixed fruit and vegetable concentrate increases plasma antioxidant vitamins and folate and lowers plasma homocysteine in men. *J Nutr*. 2003; 133: 2188–2193.
- (10) Gey KF. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Acta Cardiol*. 1989; 44: 493–494.
- (11) Gey KF. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. *Biochem Soc Trans*. 1990; 18: 1041–1045.
- (12) Gaziano JM, Manson JE, Ridker PM, Buring JE, Hennekens CH. beta carotene therapy for chronic stable angina. *Circulation*. 1990; 82: Abstract 0796.
- (13) Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. *Epidemiology*. 1992; 3: 194–202.
- (14) Gey KF, Stahelin HB, Eichholzer M. Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Basel Prospective study. *Clin Investig*. 1993; 71: 3–6.
- (15) Hennekens CH, Gaziano JM, Manson JE, Buring JE. Antioxidant vitamin-cardiovascular disease hypothesis is still promising, but still unproven: the need for randomized trials. *Am J Clin Nutr*. 1995; 62: 1377S–1380S.
- (16) Gaziano JM. Antioxidants in cardiovascular disease: randomized trials. *Nutrition*. 1996; 12: 583–588.
- (17) Rimm EB, Stampfer MJ. The role of antioxidants in preventive cardiology. *Curr Opin Cardiol*. 1997; 12: 188–194.
- (18) Steinbrecher UP. Dietary antioxidants and cardioprotection—fact or fallacy? *Can J Physiol Pharmacol*. 1997; 75: 228–233.
- (19) Manson JE, Bassuk SS, Stampfer MJ. Does vitamin E supplementation prevent cardiovascular events? *J Womens Health (Larchmt)*. 2003; 12: 123–136.
- (20) Davies KJ. Oxidative stress: the paradox of aerobic life. *Biochem Soc Symp*. 1995; 61: 1–31.
- (21) Griending KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation*. 2003; 108: 1912–1916.
- (22) Loscalzo J. Oxidant stress: a key determinant of atherothrombosis. *Biochem Soc Trans*. 2003; 31: 1059–1061.
- (23) Maxwell SR, Lip GY. Free radicals and antioxidants in cardiovascular disease. *Br J Clin Pharmacol*. 1997; 44: 307–317.
- (24) Chen K, Thomas SR, Keaney JF Jr. Beyond LDL oxidation: ROS in vascular signal transduction. *Free Radic Biol Med*. 2003; 35: 117–132.
- (25) Fri B. Cardiovascular disease and nutrient antioxidants: role of low-density lipoprotein oxidation. *Crit Rev Food Sci Nutr*. 1995; 35: 83–98.
- (26) Napoli C, de Nigris F, Palinski W. Multiple role of reactive oxygen species in the arterial wall. *J Cell Biochem*. 2001; 82: 674–682.
- (27) Ruef J, Peter K, Nordt TK, Runge MS, Kubler W, Bode C. Oxidative stress and atherosclerosis: its relationship to growth factors, thrombus formation and therapeutic approaches. *Thromb Haemost* 1999; 82: 32–37.
- (28) Keaney JF Jr, Vita JA. Atherosclerosis, oxidative stress, and antioxidant protection in endothelium-derived relaxing factor action. *Prog Cardiovasc Dis*. 1995; 38: 129–154.
- (29) Halliwell B. Albumin—an important extracellular antioxidant? *Biochem Pharmacol*. 1988; 15: 569–571.
- (30) Rush JW, Sandiford SD. Plasma glutathione peroxidase in healthy young adults: influence of gender and physical activity. *Clin Biochem*. 2003; 36: 345–351.
- (31) Martin A, Frei B. Both intracellular and extra cellular vitamin C inhibits atherogenic modification of LDL by human vascular endothelial cells. *Arterioscler Thromb Vasc Biol*. 1997; 17: 1583–1590.
- (32) May JM, Cobb CE, Mendiratta S, Hill KE, Burk RF. Reduction of the ascorbyl free radical to ascorbate by thioredoxin reductase. *J Biol Chem*. 1998; 273: 23039–23045.
- (33) Giugliano D. Dietary antioxidants for cardiovascular prevention. *Nutr Metab Cardiovasc Dis*. 2000; 10: 38–44.
- (34) Rice-Evans CA, Miller NJ, Bolwell PG, et al. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res*. 1995; 22: 375–383.
- (35) Sanz MJ, Ferrandiz ML, Cejudo M, et al. Influence of a series of natural flavonoids on free radical generating systems and oxidative stress. *Xenobiotica*. 1994; 24: 689–699.
- (36) Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J Pharm Pharmacol*. 2003; 55: 131–142.
- (37) Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*. 1992; 339: 1523–1526.
- (38) Cooke JP. Nutraceuticals for cardiovascular health. *Am J Cardiol*. 1998; 82: 43S–46S.
- (39) Basu TK, Dickerson JWT. In: *Vitamins and Human Health and Disease*. Oxford: Cab International, 1996.
- (40) Daily Reference Intakes for vitamin C, vitamin E, Selenium, and Carotenoids. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board. Washington DC: National Academy of Sciences, 2000.
- (41) Dietary Reference Values for food energy and nutrients for the United Kingdom, 41. London: HMSO, 1991.
- (42) COMA, Department of Health Report on Health and Social Subjects no 41. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on the Medical Aspects of Food Policy (COMA). Department of Health RHSS 41. London: HMSO, 1991.
- (43) Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation, Bangkok, Thailand. Geneva: World Health Organization Food and Agriculture Organization of the United Nations, 2002.
- (44) Recommended Daily Allowances for Japan. Tokyo: Ministry of Health and Welfare, 2000.
- (45) Gey KF, Puska P, Jordan P, Moser UK. Inverse correlation

- between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am J Clin Nutr.* 1991; 53: 326S–334S.
- (46) Hertog MG, Kromhout D, Aravanis C, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med.* 1995; 155: 381–386.
 - (47) Matilainen T, Vartiainen E, Puska P, et al. Plasma ascorbic acid concentrations in the Republic of Karelia, Russia and in North Karelia, Finland. *Eur J Clin Nutr.* 1996; 50: 115–120.
 - (48) Rimm EB, Stampfer MJ, Ascherio A, et al. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med.* 1993; 328: 1450–1456.
 - (49) Knekt P, Reunanen A, Jarvinen R, Seppanen R, Heliovaara M, Aromaa A. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol.* 1994; 139: 1180–1189.
 - (50) Morris DL, Kritchevsky SB, Davis CE. Serum carotenoids and coronary heart disease. The Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study. *JAMA.* 1994; 272: 1439–1441.
 - (51) Gaziano JM, Manson JE, Branch LG, Colditz GA, Willett WC, Buring JE. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol.* 1995; 5: 255–260.
 - (52) Pandey DK, Shekelle R, Selwyn BJ, et al. Dietary vitamin C and beta-carotene and risk of death in middle-aged men. The Western Electric Study. *Am J Epidemiol.* 1995; 142: 1269–1278.
 - (53) Klipstein-Grobusch K, Geleijnse JM, den Breeijen JH, et al. Dietary antioxidants and risk of myocardial infarction in the elderly: the Rotterdam Study. *Am J Clin Nutr.* 1999; 69: 261–266.
 - (54) Ford ES, Giles WH. Serum vitamins, carotenoids, and angina pectoris: findings from the National Health and Nutrition Examination Survey III. *Ann Epidemiol.* 2000; 10: 106–116.
 - (55) Osganian SK, Stampfer MJ, Rimm E, et al. Dietary carotenoids and risk of coronary artery disease in women. *Am J Clin Nutr.* 2003; 77: 1390–1399.
 - (56) Kritchevsky SB, Shimakawa T, Tell GS, et al. Dietary antioxidants and carotid artery wall thickness. Atherosclerosis Risk in Communities Study. The ARIC Study. *Circulation.* 1995; 92: 2142–2150.
 - (57) Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med.* 1996; 334: 1156–1162.
 - (58) Nyyssonen K, Parviainen MT, Salonen R, et al. Vitamin C deficiency and risk of myocardial infarction: prospective population study of men from eastern Finland. *BMJ.* 1997; 314: 634–638.
 - (59) Simon JA, Hudes ES. Serum ascorbic acid and cardiovascular disease prevalence in U.S. adults: the Third National Health and Nutrition Examination Survey (NHANES III). *Ann Epidemiol.* 1999; 9: 358–365.
 - (60) Simon JA, Hudes ES, Tice JA. Relation of serum ascorbic acid to mortality among US adults. *J Am Coll Nutr.* 2001; 20: 255–263.
 - (61) Muntwyler J, Hennekens CH, Manson JE, Buring JE, Gaziano JM. Vitamin supplement use in a low-risk population of US male physicians and subsequent cardiovascular mortality. *Arch Intern Med.* 2002; 162: 1472–1476.
 - (62) Osganian SK, Stampfer MJ, Rimm E, et al. Vitamin C and risk of coronary heart disease in women. *J Am Coll Cardiol.* 2003; 42: 246–252.
 - (63) Stampfer MJ, Hennekens CH, Manson JE, et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med.* 1993; 328: 1444–1449.
 - (64) Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. *Am J Clin Nutr.* 1996; 64: 190–196.
 - (65) Evans RW, Shaten BJ, Day BW, Kuller LH. Prospective association between lipid soluble antioxidants and coronary heart disease in men. The Multiple Risk Factor Intervention Trial. *Am J Epidemiol.* 1998; 147: 180–186.
 - (66) Brubacher D, Moser U, Jordan P. Vitamin C concentrations in plasma as a function of intake: a meta-analysis. *Int J Vitam Nutr Res.* 2000; 70: 226–237.
 - (67) Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet.* 2003; 361: 2017–2023.
 - (68) van't Veer P, Jansen MC, Klerk M, Kok FJ. Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutr.* 2000; 3: 103–107.
 - (69) Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet.* 1993; 342: 1007–1011.
 - (70) Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ.* 1996; 312: 478–481.
 - (71) Jarvinen R, Seppanen R, Knekt P. Short-term and long-term reproducibility of dietary history interview data. *Int J Epidemiol.* 1993; 22: 520–517.
 - (72) Josphipura KJ, Hu FB, Manson JE, et al. The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann Intern Med.* 2001; 134: 1106–1114.
 - (73) Hertog MG, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am J Clin Nutr.* 1997; 65: 1489–1494.
 - (74) Fraser GE, Shavlik DJ. Risk factors for all-cause and coronary heart disease mortality in the oldest-old. The Adventist Health Study. *Arch Intern Med.* 1997; 157: 2249–2258.
 - (75) Sesso HD, Gaziano JM, Liu S, et al. Flavonoid intake and the risk of cardiovascular disease in women. *Am J Clin Nutr.* 2003; 77: 1400–1408.
 - (76) Kritchevsky SB. beta-Carotene, carotenoids and the prevention of coronary heart disease. *J Nutr.* 1999; 129: 5–8.
 - (77) Rissanen TH, Voutilainen S, Nyyssonen K, Salonen R, Kaplan GA, Salonen JT. Serum lycopene concentrations and carotid atherosclerosis: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am J Clin Nutr.* 2003; 77: 133–138.
 - (78) Rissanen T, Voutilainen S, Nyyssonen K, Salonen JT. Lycopene, atherosclerosis, and coronary heart disease. *Exp Biol Med (Maywood).* 2002; 227: 900–907.
 - (79) Meydani M. Nutrition interventions in aging and age-associ-

- ated disease. *Ann NY Acad Sci.* 2001; 928: 226–235.
- (80) McKay DL, Blumberg JB. The role of tea in human health: an update. *J Am Coll Nutr.* 2002; 21: 1–13.
 - (81) Rietveld A, Wiseman S. Antioxidant effects of tea: evidence from human clinical trials. *J Nutr.* 2003; 133: 3285S–3292S.
 - (82) Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semi-quantitative food frequency questionnaire among male health professionals. *Am J Epidemiol.* 1992; 135: 1114–1126.
 - (83) Heitmann BL, Lissner L, Osler M. Do we eat less fat, or just report so? *Int J Obes Relat Metab Disord.* 2000; 24: 435–442.
 - (84) Yokoi H, Daida H, Kuwabara Y, et al. Effectiveness of an antioxidant in preventing restenosis after percutaneous transluminal coronary angioplasty: the ProbucoL Angioplasty Restenosis Trial. *J Am Coll Cardiol.* 1997; 30: 855–862.
 - (85) Daida H, Kuwabara Y, Yokoi H, et al. Effect of probucol on repeat revascularization rate after percutaneous transluminal coronary angioplasty (from the ProbucoL Angioplasty Restenosis Trial [PART]). *Am J Cardiol.* 2000; 86: 550–552.
 - (86) Tardif JC, Cote G, Lesperance J, et al. ProbucoL and multivitamins in the prevention of restenosis after coronary angioplasty. Multivitamins and ProbucoL Study Group. *N Engl J Med.* 1997; 337: 365–372.
 - (87) Tardif JC, Cote G, Lesperance J, Gosselin G, et al. Impact of residual plaque burden after balloon angioplasty in the Multi-Vitamins and ProbucoL (MVP) trial. *Can J Cardiol.* 2001; 17: 49–55.
 - (88) Wayner DD, Burton GW, Ingold KU, Locke S. Quantitative measurement of the total, peroxy radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. The important contribution made by plasma proteins. *FEBS Lett.* 1985; 187: 33–37.
 - (89) Cao G, Alessio HM, Cutler RG. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radic Biol Med.* 1993; 14: 303–311.
 - (90) Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. *Methods Enzymol.* 1994; 234: 279–293.
 - (91) Ghiselli A, Serafini M, Ferro-Luzzi A. New approaches for measuring plasma or serum antioxidant capacity: a methodological note. *Free Radic Biol Med.* 1994; 16: 135–137.
 - (92) Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal Biochem.* 1996; 239: 70–76.
 - (93) Rigatieri S, Bufon A, Ramazotti V, et al. Oxidative stress in ischemia-reperfusion injury: assessment by three independent biochemical markers. *Ital Heart J.* 2000; 1: 68–72.
 - (94) Tomasetti M, Allegra R, Piva R, et al. Evaluation of ischemia-reperfusion damage during coronary angioplasty. Electrocardiographic assessment and biochemical modifications in blood from the coronary sinus. *Ital Heart J.* 2000; 1: 216–220.
 - (95) Kummerow FA, Olinescu RM, Fleischer L, Handler B, Shinkareva SV. The relationship of oxidized lipids to coronary artery stenosis, Atherosclerosis. 2000; 149: 181–190.
 - (96) Nojiri S, Daida H, Mokuno H, et al. Association of serum antioxidant capacity with coronary artery disease in middle-aged men. *Jap Heart J.* 2001; 42: 677–690.
 - (97) Blot WJ, Li JY, Taylor PR, Guo W, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst.* 1993; 85: 1483–1492.
 - (98) The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med.* 1994; 330: 1029–1035.
 - (99) Rapola JM, Virtamo J, Haukka JK, et al. Effect of vitamin E and beta carotene on the incidence of angina pectoris. A randomized, double-blind, controlled trial. *JAMA.* 1996; 275: 693–698.
 - (100) Virtamo J, Rapola JM, Ripatti S, et al. Effect of vitamin E and beta carotene on the incidence of primary nonfatal myocardial infarction and fatal coronary heart disease. *Arch Intern Med.* 1998; 158: 668–675.
 - (101) Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med.* 1996; 334: 1150–1155.
 - (102) Redlich CA, Chung JS, Cullen MR, Blaner WS, Van Bennekum AM, Berglund L. Effect of long-term beta-carotene and vitamin A on serum cholesterol and triglyceride levels among participants in the Carotene and Retinol Efficacy Trial (CARET). *Atherosclerosis.* 1999; 145: 425–432.
 - (103) Manson JE, Buring JE, Satterfield S, Hennekens CH. Baseline characteristics of participants in the Physicians’ Health Study: a randomized trial of aspirin and beta-carotene in U.S. physicians. *Am J Prev Med.* 1991; 7: 150–154.
 - (104) No author, Final report on the aspirin component of the ongoing Physicians’ Health Study. Steering Committee of the Physicians’ Health Study Research Group. *N Engl J Med.* 1999; 321: 129–135.
 - (105) Christen WG, Gaziano JM, Hennekens CH. Design of Physicians’ Health Study II—a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. *Ann Epidemiol.* 2000; 10: 125–134.
 - (106) Hercberg S, Preziosi P, Briancon S, et al. A primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers in a general population: the SU.VI.MAX study—design, methods, and participant characteristics. *Supplementation en Vitamines et Mineraux Antioxydants. Control Clin Trials.* 1998; 19: 336–351.
 - (107) Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women’s Health Study. *J Natl Cancer Inst.* 1999; 91: 2102–2106.
 - (108) Arad Y, Newstein D, Roth M, Guerci AD. Rationale and design of the St. Francis Heart Study: a randomized clinical trial of atorvastatin plus antioxidants in asymptomatic persons with elevated coronary calcification. *Control Clin Trials.* 2001; 22: 553–572.
 - (109) Salonen RM, Nyyssonen K, Kaikkonen J, et al. Six-year effect of combined vitamin C and E supplementation on atherosclerotic progression: the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study. *Circulation.* 2003; 107: 947–953.
 - (110) The WACS Research Group. A secondary prevention trial of antioxidant vitamins and cardiovascular disease in women. Rationale, design, and methods. *Ann Epidemiol.* 1995; 5:

- 261–269.
- (111) Mason PJ, Manson JE, Sesso HD, et al. Blood pressure and risk of secondary cardiovascular events in women: the Women's Antioxidant Cardiovascular Study (WACS). *Circulation*. 2004; 109: 1623–1629.
 - (112) Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet*. 1996; 347: 781–786.
 - (113) Rapola JM, Virtamo J, Ripatti S, et al. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet*. 1997; 349: 1715–1720.
 - (114) Angiotensin-converting enzyme (ACE) inhibition in the secondary prevention of vascular disease: the Heart Outcomes Prevention Evaluation (HOPE) Trial and its substudies. *Curr Atheroscler Rep*. 2000; 2: 361–362.
 - (115) Lonn E, Yusuf S, Dzavik V, et al. Effects of ramipril and vitamin E on atherosclerosis: the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). *Circulation*. 2001; 103: 919–925.
 - (116) Marchioli R, Schweiger C, Tavazzi L, Valagussa F. Efficacy of n-3 polyunsaturated fatty acids after myocardial infarction: results of GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico. *Lipids*. 2001; 36: S119–S116.
 - (117) Marchioli R, Barzi F, Bomba E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation*. 2002; 105: 1897–1903.
 - (118) Hsia J, Alderman EL, Verter JJ, et al. Women's angiographic vitamin and estrogen trial: design and methods. *Control Clin Trials*. 2002; 23: 708–727.
 - (119) Heart Protection Collaborative Group, MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002; 360: 23–33.
 - (120) Hennekens CH, Buring JE. *Epidemiology in medicine*. Philadelphia: Lippincott Williams & Wilkins, 1987.
 - (121) Hodis HN, Mack WJ, LaBree L, et al. VEAPS Research Group, Alpha-tocopherol supplementation in healthy individuals reduces low-density lipoprotein oxidation but not atherosclerosis: the Vitamin E Atherosclerosis Prevention Study (VEAPS). *Circulation*. 2002; 106: 1453–1459.
 - (122) Leeson CP, Mann A, Kattenhorn M, Deanfield JE, Lucas A, Muller DP. Plasma vitamin E, total antioxidant status and vascular function in young adults. *Eur J Clin Invest*. 2002; 32: 889–894.
 - (123) Halliwell B. Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward? *Cardiovasc Res*. 2000; 47: 410–418.
 - (124) Eastwood MA. Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? *QJM*. 1999; 92: 527–530.
 - (125) Patrick L. Beta-carotene: the controversy continues. *Altern Med Rev*. 2000; 5: 530–545.
 - (126) Jialal I, Fuller CJ, Huet BA. The effect of α -tocopherol supplementation on LDL oxidation: a dose-response study. *Arterioscler Thromb Vasc Biol*. 1995; 15: 190–198.
 - (127) Gey KF. Vitamins E plus C and interacting nutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *Biofactors*. 1998; 7: 113–174.
 - (128) Parthasarathy S, Khan-Merchant N, Penumetcha M, Khan BV, Santanam N. Did the antioxidant trials fail to validate the oxidation hypothesis? *Curr Atheroscler Rep*. 2001; 3: 392–398.
 - (129) Heinecke JW. Clinical trials of vitamin E in coronary artery disease: is it time to reconsider the low-density lipoprotein oxidation hypothesis? *Curr Atheroscler Rep*. 2003; 5: 83–87.
 - (130) Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am J Clin Nutr*. 1999; 70: 475S–490S.
 - (131) Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am J Clin Nutr*. 1999; 69: 1086–1107.
 - (132) Bakalova RA, Hadzhimitova V, Ribarov S. Relationships between serum levels of autoantibodies against oxidized low density lipoproteins, lipid-soluble antioxidants and apolipoprotein B in patients with coronary heart disease. *Gen Physiol Biophys*. 2000; 19: 103–113.
 - (133) Ottonello L, Frumento G, Arduino N, Dapino P, Tortolina G, Dallegri F. Immune complex stimulation of neutrophil apoptosis: investigating the involvement of oxidative and nonoxidative pathways. *Free Radic Biol Med*. 2001; 30: 161–169.
 - (134) Meydani M. Soluble adhesion molecules: surrogate markers of cardiovascular disease? *Nutr Rev*. 2003; 61: 63–68.
 - (135) Calder PC, Kew S. The immune system: a target for functional foods? *Br J Nutr*. 2002; 88: S165–S177.
 - (136) Hasler CM, Kundrat S, Wool D. Functional foods and cardiovascular disease. *Curr Atheroscler Rep*. 2000; 2: 467–475.
 - (137) Meraji S, Abuja PM, Hayn M, et al. Relationship between classic risk factors, plasma antioxidants and indicators of oxidant stress in angina pectoris (AP) in Tehran. *Atherosclerosis*. 2000; 150: 403–412.
 - (138) Kristenson M, Kucinskiene Z, Schafer-Elinder L, Leanderson P, Tagesson C. Lower serum levels of beta-carotene in Lithuanian men are accompanied by higher urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine. The LiVicia study. *Nutrition*. 2003; 19: 11–15.
 - (139) Howitz KT, Bitterman KJ, Cohen HY, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*. 2003; 425: 191–196.