BUTTERWORTH HEINEMANN

Ten-year retrospective on the antioxidant hypothesis of arteriosclerosis: Threshold plasma levels of antioxidant micronutrients related to minimum cardiovascular risk

K. Fred Gey

Vitamin Unit, Institute of Biochemistry and Molecular Biology, University of Berne, Switzerland

The antioxidant hypothesis postulates that suboptimal levels of principal antioxidant micronutrients are hitherto underrated risk factors for cardiovascular diseases. Complementary observational data consistently suggest optimal, i.e., potentially protective plasma levels of approximately >50 μmol/L of vitamin C, >30 μmol/L of lipid-standardized vitamin E (α-tocopherol/cholesterol ratio >5.2 μmol/mmol), and >0.4 μmol/L β (>0.5 μmol/L total)-carotene. Relative risks are doubled at >25 to 50% lower values. Suboptimal levels of each factor increase the risk singly, or in combination risk increases multiplicatively. They can be stronger predictors of coronary heart disease than classical risk factors such as hypercholesterolemia and hypertension, at least in Northern Europe. In male Americans, the relative risk of cardiovascular diseases was substantially reduced by daily intake of >130 mg of vitamin C, >100 IU of vitamin E (100 mg of d,l- or 74 mg of d-α-acetyl-tocopherol) in all subjects, and by >9 mg of β-carotene, but only in smokers—in comparison with a suboptimal intake that very probably permits only suboptimal plasma levels. Antioxidant deficits can be avoided by "prudent diets" rich in fruits/vegetables, and net vitamin E (high vitamin E/polyunsaturated fatty acids ratio) as is common in European communities where premature cardiovascular death is low. These essential antioxidants may be crucial components of such protective diets but other, presumably synergistic constituents await evaluation, e.g., carotenoids other than β-carotene, phenols/bioflavonoids, minerals such as potassium and selenium, fibers, mono- and n-3 polyenic fatty acids, and oxygen-sensitive B vitamins such as folate. (J. Nutr. Biochem. 6:206–236, 1995.)

Keywords: antioxidants; arteriosclerosis (atherosclerosis); coronary heart disease; stroke; carotene; vitamins A, C, E; optimum plasma levels of antioxidants (recommended optimum intake)

Introduction

The antioxidant hypothesis of arteriosclerosis as proposed in 1984[1] postulates that a poor overall status of essential antioxidants ("poor antioxidant potential") is a major, but hitherto underrated risk factor of coronary heart disease (CHD), and that CHD is preventable by the optimization of plasma antioxidant levels. Principal essential antioxidant are vitamin C (ascorbic acid), vitamin E (RRR-α-tocopherol), and β-carotene (a typical and major carotenoid, as well as a potential vitamin A precursor). The antioxidant hypothesis requires an optimal antioxidant counterbalance against reactive oxygen species (ROS) wherever and however the latter could initiate or promote

Address reprint requests to Prof. K.F. Gey, Institute of Biochemistry and Molecular Biology, University of Berne, Bühlstrasse 28, CH-3012 Berne, Switzerland.
Received June 2, 1994; accepted July 15, 1994.

Nutritional Biochemistry 6:206–236, 1995

655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
0955-2863(95)00032-U

furtive multifactorial multistaged diseases such as CVD (cardiovascular diseases)[1–4] and cancer.[4,5] "Oxidative stress," i.e., any imbalance of pro-oxidants over antioxidant defense potentials[6–10] can be mediated by regular metabolites, e.g., superoxide anion, peroxy and alkoxy radicals, singlet oxygen, hydrogen peroxide, nitric oxide, peroxynitrite, hypochlorite, or by exogenous radicals, e.g., from tobacco smoke, ozone. A poor or suboptimal antioxidant status refers to metabolic situations "beyond" marginal or overt deficiency of antioxidant vitamins (e.g., scurvy), but insufficient to prevent diseases that restrict optimum health as defined by WHO/FAO. The antioxidant hypothesis of arteriosclerosis was initially[1] based on defensive properties of antioxidants against ROS as demonstrated in vitro and in vivo, on arteriosclerosis-like lesions in animals with chronic deficiency of vitamins C and E, and on the first plasma measurements of essential antioxidants in cross-cultural epidemiology. Much more information has been accumulated since, e.g., on radical biochemistry, foam cell formation of cultured macrophages, cell–cell interactions, animal experiments on reperfusion injury, dietary antioxidant intake by the human, case-control and prospective studies, as well as antioxidant supplementation in large study cohorts. The presently available human data provide compelling evidence that the relative risk of CHD is significantly greater at suboptimal plasma levels of any single principal essential antioxidant, and that the risk increases additively/multiplicatively at suboptimal levels of several antioxidants. First evaluations of antioxidant supplements, presumably as important part of a health-oriented life style, indicate that this risk is preventable. Preliminary data suggest that the relative risk of cerebrovascular disease (stroke) is also inversely related at least to vitamin C and carotene. Therefore, a 10-year retrospective survey may be timely.

In the last decade the importance of well established classical risk factors of CVD, i.e., of hypercholesterolemia and -triglyceridemia, low ratio of high/low-density lipoproteins, hypertension, smoking, diabetes, fibrinogen, etc., was further strengthened and in part specified, e.g., for genetic isoforms of apolipoproteins, for apolipoprotein(a) and for more atherogenic dense subfractions of LDL (low density lipoproteins). Additional independent strong risk factors also became evident, e.g., nongenetic hyperhomocysteinemia. Genetic polymorphisms may be able to explain many aspects in the future, but will hardly solve all riddles of CVD. New data reconfirmed that clinical events are not arithmetic functions of obstructive plaques, but require specific local factors in triggering thromboembolic complications and/or severe vasoconstriction, e.g., endothelial dysfunctions or denudation and plaque fissures. Such data reinforced the interest in platelet adhesion and aggregation, "cross-talking" eicosanoids and cytokines, as well as in the inability of arteriosclerotic vessels to relax (involving plasminogen-activator inhibitor complex, angiotensin-converting enzyme, endothelin, nitric oxide–derived endothelial relaxing factors, etc.), many of which could potentially be modulated by antioxidants. But despite a large accumulation of data, very little has emerged that resembles a new concept of disease. A fairly comprehensive prediction of CHD at the epidemiological and individual level still requires a better knowledge of additional, previously unrecognized risk factors. At that, antioxidant micronutrients seem highly intriguing since they can modulate multiple crucial pathogenetic mechanisms, and thus they could stand for a "unifying hypothesis." They deserve the interest of public health medicine as well because of their preventive potentials in nutritive doses, which are free of health hazards.

Mainly four reasons have stimulated antioxidant research.

1. Attraction of early "natural" prevention. CVD and cancer, as major causes of premature death, are a heavy community burden. Prevention should have greater priority than treatment since the latter is still limited, in spite of its excellent progress. Furthermore, the use of treatment is restricted by escalating costs. Since prevention should ideally begin in childhood and early adulthood, it must focus on improvements of life style. Improving the diet and not smoking may be the easiest practicable life style measures. Nutritional improvements may also have a more positive appeal in contrast to prohibition particularly to the youth, e.g., not to be sedentary, not to smoke, not to consume sumptuous fatty meals, etc. There is a consensus that certain special diets are inversely associated with a lower risk of premature death, and thus increase life expectancy, e.g., diets rich in fruits/vegetables and the Mediterranean diet, both of which are rich in essential antioxidants. Typical advice to the general population is that given by the National Cancer Institute and the United States National Research Council–National Academy of Science, i.e., to consume at least five servings of fruits and vegetables daily, particularly citrus fruits, and green and yellow vegetables, as well as whole grain cereals and breads.[5] Special diets, mainly that of vegetarian and thus mostly rather low in fat, have been shown to lower atherogenic plasma lipoproteins, and even to be effective in secondary prevention and regression of CHD.[11–13] Therefore, three questions have become crucial, i.e., on the pivotal constituents of health-promoting diets, their effective plasma levels and corresponding optimum intake. The latter can be a problem for special micronutrients, e.g., an optimal supply of liposoluble vitamin E may become difficult within low fat diets and processed food.

2. Availability of antioxidant analysis. The (calculated weighted) dietary intake of antioxidant micronutrients and corresponding plasma levels revealed inverse correlations to both major causes of premature death.[1,4,5] Specific routine methods for measuring antioxidant plasma levels were a great step forward since they assess the actual antioxidant status in the body, and thus the ratio of antioxidant intake to the individual requirement, in other words to "oxidative stress" (as discussed below). The assay of antioxidants proceeded at the expense of other intriguing vegetable components all of which are hard to analyze, i.e., the bulk of the chemically heterogeneous plant phenols/bioflavononoids, fibers, potassium, etc. The research impetus of reliable methods for the analysis of plasma antioxidant micronutrients in the last 20 years is perhaps comparable to that of the technology of lipoprotein fractionation 4 decades ago.

3. Awareness of ROS as health hazards. When it was generally accepted that the human body regularly produces

considerable amounts of ROS,[1–4,6–10] an exponential growth of free radical biological research resulted. In fact, highly aggressive and thus very short-lived ROS are immediately able to destroy all neighboring organic material, but less aggressive ones, or their still potentially dangerous reaction products, can travel and act "at a distance," e.g., aldehydes, or transiently inactive hydroperoxides which can be reconverted by free transition metals into destructive oxygen radicals.[1–4,6–10] In consequence, numerous pathological phenomena have been implicated with an imbalance of pro-oxidants/antioxidants to the disfavor of the latter.[6–10] The status of essential antioxidants seems to be particularly informative since at any oxidative overcharge the essential antioxidants, once exhausted, can neither be replaced (such as the endogenous antioxidant glutathione) nor upregulated (such as the antioxidative defense enzymes superoxide dismutase, glutathione peroxidase, etc.). Suboptimal antioxidative protection could contribute to acute shock-related organ failure involving the steps of ischemia-reoxygenation ("reperfusion injury") and inflammatory reactions.[6–9] The "oxidative stress" of coronary by-pass surgery exhausts preferentially and almost completely the vitamin C stores of the body.[14] Examples of semiacute pathological situations are hemosiderosis/heavy iron overload or exposure to environmental[6] or iatrogenic radical sources, e.g., radiochemotherapy.[6,15] ROSs, however, are not only able to damage various organic materials and to deplete both the endogenous antioxidants (primarily glutathione, but in addition ubiquinol-10, dehydrolipoic acid, etc.), and the antioxidant micronutrients. Smaller quantities of ROS can also subtly, perhaps even physiologically, modulate intracellular signal transduction (e.g., by raising intracellular calcium and/or activating protein kinase C or other phosphorylating enzymes), as well as "cross-talking" cell–cell interactions (by cytokines and eicosanoids), and gene expression at various levels.[3,4,6–10,16–18] Since many of these modulations by ROS have been reported to be counteracted by antioxidants, the potential impact of antioxidants is presently still beyond our comprehension.

4. Steinberg's LDL oxidation hypothesis. This hypothesis,[19,20] also proposed around 1984,[21] very elegantly merged the well known, but mechanistically unclarified CHD risk of hypercholesterolemia and/or arterial lipid imbibition, with the fact that oxidative LDL modification can provoke the formation of atherogenic foam cells. Hence, the unregulated increase uptake of oxidized LDL by scavenger receptors of monocyte-derived macrophages (in contrast to the regulated uptake of native LDL via the regular LDL receptor) can result in intracellular lipid accumulation. Lipid-laden foam cells are the first qualitatively and quantitatively abnormal cells in the subintimal space, and if clustered they form as fatty streaks the initial stage of the atherosclerotic plaque.[19–23] The LDL oxidation hypothesis itemizes on one hand the lipid peroxidation theory[24,25] and bridges on the other over to the fundamental level of inflammatory cell–cell interactions proposed in Ross' response-to-injury hypothesis.[26] The idea of oxidative LDL modification in the subendothelial environment concurs with many data, e.g., with the demonstration of oxidative LDL modification in arterial lesions in experimental animals and in human plaques,[19,20,22,23] with the improvement in oxidation resistance of isolated LDL by the antioxidant vitamins C and/or E,[27] and with some effects of probucol, a drug resembling vitamin E in its LDL protection and some cellular benefits.[3,19–23,28] All available data strongly support the assumption that LDL oxidation may be relevant to atherosclerosis, but any causal role for oxidized LDL in CHD remains to be proven.[22,23,29] Clearly, antioxidants have numerous beneficial effects beyond the attenuation in LDL oxidizability (see below), i.e., in maintaining the physiology of almost all blood vessel cells, in improving immunoresponses including cytokine balance, and on hemostatic factors.[1–3,29] Therefore, numerous mechanisms as conceivable within an "universal" antioxidant hypothesis of arteriosclerosis[1–3] may complement specific versions, such as the LDL oxidation hypothesis[19,20] and the cytokine hypothesis.[30]

Recent evidence from animal experiments

Arteriosclerosis-like lesions, associated with chronic marginal deficiency of vitamin C or E in rodents, hens, piglets, and baboons[1–3,29] were confirmed in the guinea pig, together with the accumulation of the atherogenic lipoprotein(a).[31] Vitamin E supplements (in comparison to very low vitamin E levels in the feed) attenuated aortic lesions in the cholesterol-fed rabbit, partly by a species-dependent hypocholesterolemic effect of vitamin E.[32] This was also true for the spontaneous atheromatosis of the Watanabe Heritable Hyper-Lipemic (WHHL) rabbit, a model for familial hypercholesterolemia.[33] But in the WHHL rabbit, the plaque surface of the aorta can also be significantly reduced by moderate doses of vitamin E, which lack hypolipidemic effects.* In cholesterol-fed primates (*Macaca fascicularis*), an increase of plasma vitamin E to about 40 μmol/L, i.e., a level near the optimum range of the human (below), decreased carotid stenosis significantly.[34] Correspondingly, in (nonlaying) ovulatory chickens, vitamin E supplements normalized plasma thiobarbituric acid-reactive materials (TBARs), a marker of increased susceptibility toward lipid peroxidation, and intimal thickening, without affecting hyperlipidemia.[35] Feeding synthetic antioxidants to rabbits has yielded equivocal results. Several laboratories showed that probucol, a cholesterol-lowering drug with a radical scavenging bis-phenol structure, increases oxidative resistance of isolated LDL and reduces (probably independent of its hypocholesterolemic potential[36,37]) the degree of LDL modification and lipid accumulation in macrophages, as well as the atheromatosis in the aorta of the WHHL rabbit.[3,19,20,22,23] But it is questionable whether the undoubted antiatherosclerotic effect of probucol is exclusively or primarily due to an increased resistance of LDL to oxidation or to a multipronged mode of action which includes antioxidant effects at the cellular level,[23,38–41] as it has been proposed for the essential antioxidants.[1–4,29] Data on the antiatherosclerotic action of probucol in the cholesterol-fed rabbit are equivocal.[37,42] Butylated hydroxy-toluene (BHT, a

* U. Moser, H. Georgi, P. Jordan, and K.F. Gey. Pilot experiments 1988–1992; in preparation 1995.

common non-natural antioxidant preservative of food as well as the structural unit of probucol) counteracts the atheromatosis of cholesterol-fed rabbits, as well as the accumulation of cholesterol 5α,6α-epoxide in plasma,[43] although both effects could, at least in part, be due to the prevention of cholesterol oxidation in feed. Oxidized cholesterol is more atherogenic than native cholesterol in animals, perhaps even in the human, and peroxidized diets are also known to damage endothelium, heart muscle, and to cause smooth muscle proliferation.[1,3,44–48]

Present evidence from observational studies on plasma levels in man

European cross-cultural epidemiology

Cross-sectional comparisons take advantage of large differences in CHD between communities and can inform on differences in life style, provided that they remain similar from early adulthood through the period of accumulating clinical events. Since this continuance may exist in many middle-aged European men, cross-cultural comparisons can uncover risk factors that might have persisted since early stages of the disease. Of course, any differences between communities could also be due to genetic and other geographic factors.

The initial International Collaborative Study on the Antioxidant-Fatty Acid Hypothesis of Arteriosclerosis[1] became in 1985 the basis of the Optional Study of Vitamin Antioxidants and PUFAs of the WHO/MONICA Project (Vitamin Substudy) within the world's largest survey *mon*itoring determinants and trends of *ca*rdiovascular diseases. The Vitamin Substudy is going to compare the age-standardized mortality from CVD with the plasma antioxidant status of 23 study populations represented by randomly selected, apparently healthy middle-aged males (40 to 49 years old; *n* mostly 100). To date 16 European study populations have been evaluated regarding the age-specific CHD mortality (ICD 410 to 414), which varies in Europe up to 6 fold, whereas the plasma levels of most antioxidants show an approximately 2 fold variation. In the majority, i.e., in 12 study populations, the medians of the classical risk factors, plasma cholesterol, blood pressure, and smoking, did not differ significantly and thus cannot adequately explain the 6 fold differences in CHD mortality.[49–51] The latter could, however, widely be predicted by the plasma status of essential antioxidants. Most impressively and consistent in examining from five study populations on,[1–3,49–55] was the strong inverse correlation of vitamin E medians ($r^2 > 0.6$; $P < 0.0003$) to CHD mortality; this was found in populations with similar plasma lipoproteins as well as for lipid-standardized vitamin E in all study populations which included hypo- and hypercholesterolemic cohorts. Adjustment on the amount of circulating and potentially vitamin E transporting lipoproteins (primarily VLDL and LDL) is mandatory in case of different plasma lipids since the biological vitamin E status cannot be appreciated from absolute vitamin E levels.[1–3,49,54,56] Lipid standardization can most simply be done using the vitamin E/cholesterol ratio,[1,54] or by adjusting vitamin E to the common European level of 220 mg/dL of cholesterol,[49,52,53] or more precisely by adjusting to 220 mg/dL of cholesterol plus 110 mg/dL of triglycerides.[50,51] The vitamin E concentration in LDL has to be measured only for studies on LDL oxidation but not for the general vitamin E status under steady state conditions because of a thermodynamic partitioning of vitamin E between all lipid compartments.[57] The necessity for lipid-adjusted vitamin E in comparison with absolute vitamin E concentration in cross-cultural epidemiology is exemplified by the fact that in China, which has a typically low CHD prevalence, a high α-tocopherol/cholesterol ratio of 5.35 ± 0.07 μmol/mmol has been found (as required to satisfy the antioxidant hypothesis) in spite of absolute plasma α-tocopherol levels as low as 17.5 ± 0.3 μmol/l.[58]

In univariate analysis of European study populations of the Vitamin Substudy, the vitamin E median has been a stronger predictor of CHD mortality (with $r^2 > 0.6$; $P < 0.005$) than the medians of the classical risk factors of total plasma cholesterol ($r^2 = 0.29$; $P = 0.03$), diastolic blood pressure ($r^2 = 0.25$; $P = 0.05$), and smoking ($r^2 < 0.01$), and this is still true after their combination in multiple regression analysis ($r^2 = 0.44$; $P = 0.02$).[3,50,51] There was practically no overlap in the distribution of lipid-standardized vitamin E when study populations with high and low risk of CHD were compared.[3] Essential antioxidants other than vitamin E made relatively smaller contributions ($r^2 < 0.4$–0.5), the rank order of which varied in the sequential evaluation of the available study populations.[1–3,49–54] Finnish study populations were obvious outlyers for almost all variables examined, except vitamin E.[51] When a special, possibly in part genetic "Finland factor"[4,51] was tentatively considered, the medians of vitamin C, as well as of carotene (β- together with minor amounts of α-carotene), in univariate analysis came very close ($r^2 = 0.56$ and $r^2 = 0.50$, respectively) to vitamin E.

Multivariate stepwise regression analysis of the latest report on 16 study populations,[51] either with or without complementary data from seven study populations,† broadly suggests the same as observed initially in the five study populations without the "Finland factor,"[1] the tentative rank order for CHD: vitamin E > cholesterol and/or a "genetic Finland factor" > carotene ≈ vitamin C > diastolic blood pressure > vitamin A.

The four communities with lowest CHD mortality (Spain, France, Italy, and Switzerland with <115 CHD deaths/100,000 males 40 to 49 years of age) had an "optimal" vitamin status of both vitamin C and E, and three out of four communities revealed concurrently very high carotene levels.[4,51] The highest carotene status occurred in France where it conceivably "compensated" for an almost moderate status of vitamin E, as conceivable from known interactions. In Spain a particularly high vitamin C status was associated with critically low carotene levels (being as modest as in two Scottish study populations).[51] This might suggest that either vitamin C can "compensate" for a poor

† K.F. Gey, A. Evans, P. Jordan, et al., unpublished evaluations, 1992–1994.

carotene or that low carotene becomes a risk factor only in case of concurrently suboptimal vitamin C. The latter would concur with an overmultiplicative interaction of both antioxidants in the prospective Basel Study (below). In the six communities with at least 2 fold higher CHD mortality (>260/100,000 in two areas of North Karelia/Finland, in Southwest Finland, and all Scottish study sites, i.e. Glasgow, Aberdeen, and Edinburgh), the vitamin E status was generally low but vitamin C and carotene, respectively, were strikingly low only in the Scottish study populations. Presumably in Finland the special "Finland factor" replaced the great risk exerted by suboptimal levels of both vitamin C and carotene in Great Britain. In 5 out of 6 communities with medium CHD mortality (suburban East Belfast/Northern Ireland, Copenhagen/Denmark, three areas in former Eastern Germany, and Tel Aviv/Israel) either vitamin C or carotene was suboptimal,[4,51] The cross-cultural data taken together suggest that a suboptimal level of any single antioxidant other than vitamin E will increase the CHD risk as well. The assumption that in some communities a particularly high level of any single antioxidant (e.g., of carotene in France, and of vitamin C in Spain similar to Israel) partially "compensates" for a medium-high status of vitamin E and carotene, respectively, is based on interdependencies of all principal antioxidant micronutrients in vitro and concurs with corresponding mutual sparing effects in animals and in man. This has been demonstrated for various combinations, e.g., for vitamins C and E,[1,3,7,8,59–71] for vitamin E and carotene,[72–75] for vitamin C and carotene,[66] as well as for glutathione[64,76–78] and folate, respectively, for vitamin E and glutathione,[79] etc. Correspondingly, the oxidation resistance of liver tissue is greater in rats fed the combination of vitamin E, selenium, and β-carotene in comparison with the single compounds,[69,74] The interaction between vitamin C and E may not only be restricted to the vital recycling of vitamin E by vitamin C, but also to complementary antioxidant actions in different compartments; vitamin C is the principal antioxidant in aqueous compartments,[70,71] but vitamin E occurs exclusively in lipid phases.[71,80,81] In any case, the cross-cultural data suggest that a suboptimal level of any single antioxidant can increase the CHD risk, but the latter is lowest only at concurrently optimal levels of vitamin E, vitamin C, and carotene. In consequence, there is little hope of any "bullet antioxidant" to prevent CHD (or cancer),[4] but there is hope for the simultaneous optimization of all antioxidant micronutrients, in other words for an optimized overall antioxidant defense potential.[1]

The multivariate combination of CHD risk factors emerging from stepwise regression analysis[4,51] indicates that lipid-standardized plasma vitamin E when complemented by total plasma cholesterol, carotene, vitamin C, and diastolic blood pressure can predict 81% of the existing cross-sectional differences of CHD mortality in 16 European study sites, and 90% when the hypothetical but likely "Finland factor" is also admitted. With that, the great cross-cultural differences of CHD mortality can be predicted to a previously unrivaled degree. In the subgroup of 13 study populations outside Finland (in which differences in CHD mortality were not significantly correlated to plasma cholesterol, blood pressure, and smoking habits) the actual CHD mortality was predictable to 88% by lipid-standardized vitamin E (again mainly, i.e., by 68% in univariate analysis), combined with carotene and vitamin C.[51]

The plasma antioxidant turn-off points to minimal risk, as tentatively deduced from the observed European plasma antioxidant medians *(Table 1)*, may require some final upgrading to optimum levels in order to account for inherent weaknesses, e.g., in the randomization of study subjects and in their mortality data, respectively, in small seasonal and analytical variations, skewed distribution pattern, etc. With that the threshold levels of CHD risk may slightly move upwards toward tissue saturation levels, e.g., for vitamin C from about 50 to 55 μmol/L to roughly 60 μmol/L (see below).

In populations with an approximately 2 fold risk of CHD mortality, the relative deficit of vitamins C and E and carotene varied between −15 and −45%, as compared with study sites with minimal risk. This difference corresponds roughly to the percent increase of classical CHD risk factors that will at least double the relative risk of CHD, e.g., cholesterolemia from 5.7 to 6.6–8.3 mmol/L (or from 5.2 to 7.54 mmol/L), homocysteinemia from 10 to 14 μmol/L,[82] and diastolic blood pressure from 90 to 104–131 mm Hg. Apparently health is restricted to a relatively narrow band of physiological variations for both classical CHD risk factors and plasma antioxidants.

The cross-cultural differences of plasma antioxidant levels reflected great differences in the consumption of vegetable items. When calculated with comparable methodology, the intake of vegetables/fruits is several-fold higher in France[83] than in Scotland[84] and Northern Ireland, respectively.[85]

Of course, cross-cultural differences can result from many factors other than nutritional, ranging from non-nutritional variations of the life style over geophysical influences (yearly sunshine, electromagnetic gravity field,

Table 1 Differences in relative CHD risk in cross-cultural comparisons at different ranges of plasma antioxidant micronutrients in the Vitamin Substudy, WHO/MONICA Project[51]

Moderately increased risk of CHD mortality (>250 deaths/100,000)	Low risk of CHD mortality (<130 deaths/100,000)
• Carotene (β-with about 15 to 25% α-carotene)	
<0.3 μmol/L	>0.4 to 0.6 μmol/L
• Retinol (lipid-standardized to 210 mg/dl cholesterol & 110 mg/dl triglycerides)	
<2.1 μmol/L	>2.2 μmol/L
• α-tocopherol, lipid-standardized (as above)	
<24 μmol/L	>27 to 28 μmol/L
• α-tocopherol/cholesterol ratio (the minimal lipid adjustment of vitamin E)	
<4.1 μmol/mmol	>4.8 to 5.6 μmol/mmol
• Vitamin C	
<24 μmol/L	~35 to 55 μmol/L

hardness of drinking water, etc.) to genetic differences. If the latter played a role among the European Caucasians, the great CHD risk on the British Isles could possibly be related to "Gaelic-Celtic-Germanic" genes (which could then, in theory, also be related to the fairly high CHD incidence in Australia, New Zealand, and South Africa), the high Finnish risk to "Fen-ugric" genes including "Finland factor," the medium risk of Germans to rather neutral "Germanic" genes, whereas the mix of "Celtic-Roman-Germanic" genes in Switzerland and "Greek-Roman-Celtic" genes in the Mediterranean countries might be CHD-protective. Although racial differences can play a role in the fractional turnover of atherogenic lipoproteins and other CHD risk factors, no data have suggested strong ethnic differences in the antioxidant metabolism yet. It has also to be noted that genetically homogenous populations are scarce in Europe. Regardless of whether the CHD risk is affected by racial or geophysical factors aside from antioxidant status, the cross-cultural data of MONICA's Vitamin Substudy clearly indicate that Northern regions of Europe are prone to CHD at concurrently suboptimal antioxidant levels, whereas low CHD in Mediterranean areas and Switzerland is associated with a higher overall status of antioxidant micronutrients.

Case-control studies

All epidemiological approaches require reconfirmation and/or extension by complementary study types. Cross-cultural findings require particularly reconfirmation in individuals who do not grossly differ in location, culture, and genetics. Common case-control studies on patients with arteriographically established, i.e., advanced coronary obstruction or even with acute infarction may be interesting, but not conclusive since they cannot differentiate between cause and result of the disease. Furthermore, acute phase responses to the coronary infarct may involve strong immunological, hormonal, and metabolic disturbances, and for rehabilitation, life style changes are generally recommended. Medical treatment of established CHD can potentially become a substantial bias since all kinds of cardiaca have been said to affect the antioxidant status,[86–95] some even to increase oxidation resistance of LDL[4,94] and/or the vitamin E content in LDL.[93] LDL oxidizability, which recently got a lot of attention, exemplifies the difficulties of interpretation in developed disease and/or under therapy. On the one hand, LDL oxidation resistance was reported to be increased in hyperlipemia (with an inverse correlation to the vitamin E and carotenoids in LDL),[96] but on the other hand, arteriographically established coronary lesions were only weakly inversely correlated to LDL oxidation resistance (explaining 20% of the variation).[97] Such inherent problems are merely absent in subjects whose first CHD symptoms have previously been disregarded, not yet been diagnosed, and thus resulted in no life style changes and/or treatment.

The Edinburgh Case-Control Study on Previously Undiagnosed Angina Pectoris[98] deals with middle-aged men from a population of very high CHD morbidity, but total plasma cholesterol and blood pressure of common European order. The population reveals, however, a great variation of plasma antioxidant levels, i.e., from sufficient/fair to very poor, the latter as a consequence of a notoriously rare consumption of fresh fruits and vegetables,[84,99,100] which is greatly related to differences of social classes.[101] Inhabitants of Edinburgh were screened for cases with previously undiagnosed angina pectoris for comparison with apparently healthy matched controls. Low plasma levels (quintile 1) of vitamin E, of vitamin C, and of carotene were associated with an up to 2.6 fold higher risk of this early stage of CHD as compared with high antioxidant levels. The statistically significant, linear risk increase at decreasing vitamin E levels was independent of classical risk factors.[98] By contrast, the increased risk of low vitamin C and carotene was strongly confounded by cigarette smoking (other classical risk factors being of secondary importance). But since the low levels of vitamin C and β-carotene of Scotsmen are primarily due to smoking rather than only to a frequently lower antioxidant intake,[84,100] the CHD risk of smoking may, at least in part, be mediated by the smoking-induced decrease of plasma vitamin C and carotene, mainly caused by antioxidant-consuming radicals in tobacco smoke.[70,102–105] This concurs with the cross-cultural Scottish Heart Health Study (comparing 22 districts throughout mainland Scotland).[99] The latter showed in univariate analysis with $r_s = -0.73$ the highest inverse association between CHD mortality and vitamin C intake (vitamin E and carotene unfortunately not reported), and a high dependence on current smoking ($r_s = 0.66$) with a high correlation of both variables ($r_s = -0.78$). On the assumption that in the Edinburgh Study on Angina[98] critically low levels of vitamin C and carotene mediated at least in part the special risk of smoking, it may be permitted to consider the following rank order of essential antioxidants for the risk of angina pectoris: vitamin E > carotene ≈ vitamin C > vitamin A. This rank order is similar to that in the above reviewed cross-cultural MONICA Vitamin Substudy.

In the Edinburgh Study on Angina,[98] the plasma levels associated with the greatest relative risk (without adjustment for smoking) were lower *(Table 2)* than those of moderate CHD risk in the cross-cultural comparisons *(Table 1)*, whereas the threshold levels to the optimum range was similar in both epidemiological approaches *(Tables 1 and 2)*. In the Edinburgh Study the plasma values of vitamins A and E, and of carotene in the lowest quintile *(Table 2)* differed percentagewise between −29 to −50% from the values in the highest quintile, i.e., similar to the cross-cultural comparisons. The relative deficit of vitamin C in the lowest

Table 2 Relation of plasma levels and relative risk of angina morbidity in the Edinburgh case-control study on previously undiagnosed angina pectoris[98]

Antioxidant	Greatest relative risk (first quintile with risk 1.4 to 2.7)	Lowest relative risk (fifth quintile with risk <1.2)
β-(with 15 to 25% α-) carotene	<0.26 μmol/L	>0.50 μmol/L
Retinol	<1.93 μmol/L	>2.69 μmol/L
α-tocopherol, abolute	<18.9 μmol/L	>28.2 μmol/L
α-tocopherol/cholesterol	<3.1 μmol/mmol	>4.6 μmol/mmol
Vitamin C	<13.0 μmol/L	>41.5 μmol/L

quintile was with −79% somewhat greater. Thus deficits of these antioxidants seem to be more frequent than corresponding increases of classical CHD risk factors, such as a rise of plasma cholesterol from 5.7 to 10.2 mmol/L and 5.2 to 9.3 mmol/L, respectively or of diastolic blood pressure from 90 to 161 mm Hg. In fact, in Scottish males of corresponding age, cholesterol levels >8 mmol/L occur only in 1 out of 10 subjects, and values ≥10 mmol/L exist in 1 out of 100.[106] This suggests again that in Scotland the relative risk of suboptimal antioxidant micronutrients is relatively more prominent than that of classical CHD risk factors.

The occurrence of poor antioxidant levels as found in every fifth of the cohort of the Edinburgh Study on Angina (Table 2) may be typical for Scotland where CHD morbidity and mortality is high. Thus, the mean plasma values of the Edinburgh cohort[84] correspond to those of a dietary survey in randomized middle aged-males of the Scottish MONICA populations of Glasgow and Aberdeen[84] and an independent study cohort in Aberdeen[100] *(Table 3)*. These surveys suggest a weaker plasma/intake response of vitamin C and β-carotene in Scottish smokers as compared with nonsmokers *(Table 3)*. The consistency of the data suggest that they are not chance findings related to well-known inherent weaknesses of the calculation of small amounts of dietary antioxidants (below). Hence, the estimated mean intake of approximately 3 mg of β-carotene daily results in nonsmokers in a presumably "safe" plasma level (>0.42 μmol/L) whereas in smokers the plasma levels are significantly, actually about 30%, lower and near to the band of high CHD mortality *(Table 1)*. At any given β-carotene intake, the Scottish smoker's plasma equivalent was clearly much poorer than in nonsmokers, with an extremely low correlation coefficient,[84] which corresponded in principal to the behavior of smokers studied in Massachusetts.[107] Plasma vitamin C of nonsmoking Scotsmen corresponded to their daily intake of approximately 61 mg with plasma levels of 37 to 45 μmol/L, i.e., levels somewhat below the "safe" range *(Table 1)*. In contrast, Scottish smokers, when also consuming ≥61 mg daily, revealed in plasma only 27 μmol/L of vitamin C, i.e., an approximately 35% lower plasma value being associated with an approximately 2 fold CHD mortality; and in the case of a 20% smaller vitamin C intake (49 mg) by Scottish smokers, the mean plasma vitamin C was as poor as 18 μmol/L *(Table 1)*, i.e., at a level of accepted marginal vitamin C deficiency.[101,108–110] The parallel rather than divergent regression lines of plasma vitamin C in Scottish smokers and nonsmokers,[84] which fit the parallel regression lines in American smokers and nonsmokers of NHANES II (Second US National Health and Nutrition Examination Survey),[111] are in line with the general belief that smokers need an extra ~40 to 60 mg of Vitamin C daily to achieve the corresponding plasma level, as shown for subjects in the USA and Sweden.[108,109,111,112] The estimated Scottish vitamin E intake on the order of 6 to 8 mg daily yielded a materially smoking-unrelated status, but with <25 μmol/L a very poor plasma vitamin E status *(Table 1)*, which may be associated with an at least 2 fold CHD mortality.

The cross-cultural Scottish Heart Health Study[99] confirmed, in further agreement with the Edinburgh Case-Control Study on Angina, only moderate correlations between CHD and classical risk factor such as total plasma cholesterol >6.5 mmol/L ($r_s = -0.25$) or mean diastolic blood pressure ($r_s = 0.40$), but revealed the expected very strong inverse correlation of CHD mortality with vitamin C as dietary marker antioxidant of this study ($r_s = -0.73$). Since CHD data from Scotland are typical for Britain as a whole[106] the nutritional pattern of other parts of the British Isles deserves interest as well. In Northern Ireland, another area with fairly frequent CHD, not only are the calculated intake of vitamin C (mean ± SD, 60 ± 47 mg daily) and β-carotene (2.06 ± 2.09 mg)[85] similar to Scotland, but also the population fraction that consumes practically no fruits and vegetables. Hence, the 5th percentiles of randomized middle-aged Ulster men consumed as little as 17 mg of vitamin C (just preventing overt scurvy, with an expected plasma equivalent of 12 μmol/L at borderline of scurvy, and in the range of several-fold CVD risk), and 0.2 mg of β-carotene (presumably with a plasma equivalent of <0.2 μmol/L, i.e., the range of several-fold CVD risk). The

Table 3 Relation between estimated dietary intake and plasma levels of micronutrients (means ± SD) in nonsmoking and smoking Scotsmen

Study	β-Carotene*		Vitamin C		Vitamin E		
	Intake‖ (mg)	Plasma (μmol/L)	Intake‡ (mg)	Plasma (μmol/L)	Intake‡ (mg)	Plasma (μmol α-tocoph/ mmol cholester)	Plasma (μmol absol.)
Edinburgh Angina Case-Control Study[98]							
Nonsmokers	—	0.42 ± 0.03	—	41 ± 2	—	3.88 ± 0.06	26 ± 1†
Smokers	—	0.34 ± 0.02‡	—	24 ± 2‡	—	3.63 ± 0.07	24 ± 1†
Healthy random sample of clinically healthy middle-aged Scotsmen in Glasgow/Aberdeen[84]							
Nonsmokers	3.5 ± 0.2	0.44 ± 0.03	61 ± 2	37 ± 3	7.6 ± 1	1.80 ± 0.1	25 ± 1†
Smokers	2.9 ± 0.2§	0.31 ± 0.02	49 ± 2‡	18 ± 2‡	7.6 ± 1	1.60 ± 1	23 ± 1§†
Dietary survey in Aberdeen[100]							
Nonsmokers	2.5 ± 0.2	—	61 ± 6	45 ± 3	5.7 ± 0.4	2.75 ± 0.13	15 ± 1
Smokers	2.5 ± 0.2	—	63 ± 6	27 ± 3‡	6.0 ± 0.4	2.62 ± 0.13	15 ± 1

*Assumption 80% of total carotene; †identical analytical laboratory; ‡$P < 0.01$, §$P < 0.05$, as compared with nonsmokers. ‖Food-frequency questionnaires assigning >70% of subjects in the corresponding or adjacent tertile of plasma levels.

Dietary and Nutritional Survey of British Adults[113] comprising mainly London/South East England, Wales, Northern England, and only a small fraction (9%) from Scotland in 1986–1987 (n = 1,087 for males) reported a daily mean intake ± SE of 2.4 ± 0.1 mg of β-carotene, 67 ± 1 mg of vitamin C from diet and 75 ± 2 mg from diet plus supplements, and 12 ± 1 mg of vitamin E. In general, southern areas of Britain tended to have a slightly greater intake, e.g., 12 and 23% for vitamin E and C, respectively. The all-over-Britain median and SE of mean for plasma β-carotene appears with 0.24 ± 0.01 μmol/L to be critically low, the vitamin E status with a median ± SE of 25.5 ± 0.3 μmol/L (α-tocopherol/cholesterol ratio of 4.54 ± 0.04) fails to approach the threshold of minimal CHD risk at >28 to 30 μmol/L (or α-tocopherol/cholesterol ratio of >5.2 ± 0.4).[113] More important, large fractions of the British males revealed plasma levels associated with a several-fold increased CVD risk, e.g., 47% of male adults had a plasma vitamin E level <25 μmol/L (36% with an α-tocopherol/cholesterol ratio <4.2), 52% with β-carotene <0.25 μmol/L. Taken together, all British data agree with the conclusion of the cross-cultural vitamin comparisons of the *WHO/MONICA Project*, i.e., that a suboptimal antioxidant status in Britain may be an important factor of the nations's relatively high incidence of CVD.

The Edinburgh Artery Study[114] extended the Edinburgh Case-Control Study on Angina toward peripheral arterial disease. Hence, in the former the calculated dietary vitamin E intake was inversely correlated to the risk of peripheral arterial disease independent of smoking and other risk factors. Low vitamin C intake increased the risk only in subjects who smoked, and high intake of β-carotene and vegetable fibers was associated with less peripheral arterial disease although without statistical significance. A joint study on hemostatic parameters[115] revealed the following rank order of statistically significant risk factors of peripheral arterial disease: relative risk of endothelial disturbance 3.3 ⩾ cross-linked fibrin degradation products 2.8 ⩾ fibrinogen 1.82 to 2.22 = platelet activation by plasma β-thromboglobulin 2.0 ⩾ plasminogen activator inhibitor 1.4 > lipid peroxides 1.2. This might suggest associations of a poor antioxidant status and dysfunctions of the endothelium and/or coagulation.

Two case-control studies outside Britain failed to reveal the expected association with antioxidants, very likely because of a lacking between-individual variation. First, the Study on Middle-Aged Men in Eastern Finland,[116] which compared in 1986–1987 patients with clinical symptoms and/or electrographical abnormalities to controls, revealed in all subjects a very similar poor vitamin E status (α-tocopherol/cholesterol ratio 3.62 ± 0.04 in cases versus 3.39 ± 0.03 in controls). In contrast, vitamin C values were in a moderately high although not optimal range but again they lacked variations (40.7 ± 1.2 μmol/L in cases versus 46.7 ± 1.2 μmol/L in controls). Previous public education campaigns promoting the consumption of berries and other fruits had raised plasma vitamin C status in the area between 1983 and 1987 from critically low to optimum levels (from 26 to 46 μmol/L) as evident from two subsequent *MONICA* samples of this county.[51] In consequence, the typically high CHD risk of man in Eastern Finland may have potentially been related only to poor vitamin E levels but without marked between-individual differences. Second, the Dutch study on heavily progressed CHD[117] compared hypercholesterolemic CHD patients with the severest coronary obstructions (>85% in at least one artery) and those of moderate stenosis (<50% in all three vessels). But the mean ± SD of plasma vitamin E status of all Dutch patients was even higher (α-tocopherol/cholesterol ratio 4.83 ± 1.26 and 5.12 ± 1.12, respectively) than that of males in Edinburgh with minimal risk of angina.[98] The Dutchmen[117] are fully comparable to Germans or Swiss whose high vitamin E status (α-tocopherol/cholesterol ratio about 5.2 ± 0.4; see below) lacks any material CHD risk. The two negative studies taken together, the inverse correlation of CHD risk with the antioxidant status (as that of any other risk factor) becomes detectable in individuals of a given study cohort only in case of a sufficient variation between high- and low-risk levels. The latter occurred in the cross-cultural comparisons of the MONICA Project and between individuals in the Edinburgh Study respectively. But at an inadequate between-individual variation in eastern Finland and the Netherlands, the results are rather more inconclusive than contradictive.

The EURAMIC Study[118] measured in 10 European areas the amount of α-tocopherol and β-carotene stored in adipose tissue lipids. The comparison of fresh cases of coronary infarcts cases (n = 683) versus controls (n = 727) revealed a statistically significant increase of relative risk (2.39, CI 1.35 to 4.25) at the lowest quintile of β-carotene in smokers only, whereas α-tocopherol lacked any correlation. The interpretation is intricated by lacking direct information on differences of both antioxidants regarding the physiological deposition in adipocytes and the remobilization by lipolysis, respectively, as well as on metabolic and hormonal effects on adipocytes in the acute phase syndrome after myocardial infarct. With that it is difficult to understand why the well known cross-cultural differences in the intake of β-carotene and vitamin E are reflected in plasma (details below), but not in adipose tissue levels. Since it has long been assumed that β-carotene, in contrast to α-tocopherol, is practically sequestered in adipocytes,[119] the β-carotene content of adipose tissue could be a life-long integral marker of β-carotene plasma levels. If this is true, the β-carotene findings of the EURAMIC Study would support the inverse correlations of plasma β-carotene with CHD mortality in European populations,[51] with early angina in smoking Scotsmen,[98] with individual CVD mortality in the prospective Basel Study (below), and be in line with the CHD protection by substantial β-carotene supplements in smoking American Health Professionals. The β-carotene data in adipose tissue point again to a crucial interaction between smoking and β-carotene, as is also obvious from the insufficient plasma/intake response of smokers in Scotland and in the US.

Prospective epidemiology

Longitudinal follow-ups of initially healthy subjects until occurrence of clinical events provide conclusive individual

Table 4 Differences in relative CVD risk at different orders of plasma antioxidant micronutrients in the Basel Prospective Study

Antioxidant	First quartile (associated with increased risk)	Higher orders (no or minimal risk)
β-(with 15 to 25% α-)carotene	<0.23 μmol/L	>0.4 to 0.5 μmol/L
Vitamin C, related to CHD	<37.5 μmol/L	>50 μmol/L
related to stroke	<22.3 μmol/L	>50 μmol/L
Vitamin E, lipid standardized	[~30 μmol/L no risk]	>31 μmol/L
α-Tocopherol/cholesterol	—	>5.2 μmol/mmol

information on the prevalent risk factors of the special study cohort provided that the life style remains fairly constant over time. The prospective Basel Study comprised 2,974 initially healthy males with a mean age of 51 years (range 25 to 75), similar genetic mix-up and relatively homogenous fair socioeconomic standard.[120,121] All principal plasma antioxidants were measured prior to the decay of plasma carotenoids and vitamin E by longer storage in the deep-freeze. Hence the common storage problem of prospective cohort studies of the "blood-bank" design[122] was avoided. A single antioxidant measurement at base-line indicated the order of the individual plasma status of most antioxidants with sufficient accuracy for several years. Hence 6 years later a 17% subsample[123] revealed fair correlations at least for liposoluble plasma antioxidants, i.e., with r_P = 0.45 for carotene, 0.46 for vitamin A, 0.65 for vitamin E, and 0.28 for vitamin C. The fair validity of a single plasma assay may be related to two facts. First, any changes of individual dietary habits are not drastic as obvious from sequential calculations of nutrient intake.[122,124] Second, plasma levels of liposoluble antioxidants are relatively stable since they equilibrate only slowly between body compartments, i.e., within weeks to months. In the 12-year mortality follow-up of the Basel Study[51,123,125] the lowest percentile of lipid-standardized plasma carotene or of vitamin C showed (in Cox's model of proportional hazards adjusting for age, cholesterol, blood pressure, smoking, and all other variables) statistically significant associations with an increased CHD mortality: relative risk 1.53 (95% CI 1.07 to 2.20; P = 0.02) at <0.23 μmol/L of carotene; relative risk 1.25 (CI 0.77 to 2.01) at <22.7 μmol/L of vitamin C, and a relative risk of 1.96 (CI 1.10 to 3.50; P = 0.02) at the combination of both. Interestingly, death by stroke behaved correspondingly: relative risk of 2.07 at low carotene, of 1.28 at low vitamin C, and of 4.17 (CI 1.68 to 10.33; $P < 0.01$ for overmultiplicative interaction) at low levels of both. The present evaluation of the 17-year mortality‡ reveals even stronger correlations for both CHD mortality: relative risk 1.09 at low carotene; of 2.03 ($P < 0.03$) at <38 μmol/L of vitamin C, and of 3.32 ($P < 0.02$ for overmultiplicative interaction) at the combination of both. The relative risk of death by stroke increased correspondingly: relative risk of 2.93 ($P < 0.04$) at low carotene, of 0.97 at <22.7 μmol/L of vitamin C, and of 3.69 at the combination of both. Hence, vitamin C might be relatively more important for CHD and carotene for stroke. The risk of smoking for CHD mortality could mainly be explained by low plasma levels of vitamin C and carotene, but plasma carotene was also significantly lower in all subjects >60 years of age,[126] similar to a Finnish cohort.[127] Optimal vitamin A levels were reported to reduce mortality and improve neurological recovery after stroke.[128]

The carotene levels associated in the Basel Prospective Study, with an increased CVD mortality, respectively, are comparable with those for increased angina risk mentioned earlier,[98] whereas in the 17-year evaluation of the Basel Study the CHD risk was already significantly increased at <37.5 μmol/L of vitamin C *(Table 4)*, i.e., at a level hitherto considered safe.[49,108–110] The optimum range was comparable with that of the cross-cultural data of the WHO/MONICA Project, as well as on the Edinburgh Study on Angina. Percentagewise the vitamin C and carotene of the lowest percentile of the Basel subjects indicated again a deficit of about 25 to 50%, as compared with levels above the risk threshold.

In the Basel Prospective Study the relative risk of CVD was significantly related to suboptimal levels of vitamin C and/or carotene despite concurrent abundance of vitamin E.[51,125] Thus, in this cohort lipid-standardized vitamin E had a mean of 35 μmol/L, with practically no values below the critical threshold of 30 μmol/L, and correspondingly the mean α-tocopherol/cholesterol ratio was 6.1 μmol/mmol, with a ratio mostly above 5.2. Correspondingly, other studies found also no correlation when high plasma vitamin E varied in the band of optimum levels (lipid-standardized >30 μmol/L or α-tocopherol/cholesterol ratio >4.8 to 5.5 μmol/mmol). This was true for a prospective study on CHD events in Germany (mean vitamin E 33 ± 1 μmol/l; α-tocopherol/cholesterol ratio of 4.8 ± 0.1),[129] as well as for the survival rate after stroke (33 ± 11 μmol/l).[128] This is also likely for the Dutch EPOZ Study in which vitamin E of plasma samples stored for 9 years lacked any correlation to CVD mortality,[130] but α-tocopherol levels were at base line (prior to losses by storage) presumably very similarly high as in the plasma of other Dutchmen subsequently assayed in the same laboratory (α-tocopherol/cholesterol ratio 4.8 through 5.1).[117]

An abstract on a nested case-control study in Washington County, MD,[131] reported a protective association of plasma β-carotene, whereas the association with plasma vitamin E had not yet been entangled from its correlation to plasma lipids.

‡ K.F. Gey, H.B. Stähelin, M. Eichholzer, et al., in preparation, 1995.

In the Eastern Finland Heart Survey,[132] a nested case-control study, the plasma vitamin E levels were during 5 years unrelated to death from CHD, but the follow-up was relatively short, and vitamin E varied presumably only within a band of notoriously poor vitamin E levels, i.e., about 20 to 23 μmol/L (and an α-tocopherol/cholesterol ratio ~3.5 to 3.9)[3,51,54,133] associated with highest CHD risk. Again it has to be noted that the association of CVD with any risk factors can epidemiologically only be detected in the case of adequate between-individual variation from the high risk to low risk range.

Taking together all prospective data on CVD, they contribute substantially to the compelling evidence that the prevention of suboptimal antioxidant plasma levels as listed earlier minimizes the relative risk of CVD, as far as antioxidant micronutrients are concerned as permissive risk factors. The rank order of relevant risk factors may be expected to be revealed in more detail by the current PRIME Study,[134] which is going to compare all presently conceivable nutritional and genetic CHD risk factors in middle-aged men in Northern Ireland (with meager consumption of fruits and vegetables[85] and a correspondingly poor antioxidant status, but high CHD risk) and France (with high fruit/vegetable consumption,[37] fair antioxidant status, but low CHD risk).

The optimum levels of plasma antioxidant micronutrients, as deduced from cross-cultural comparisons and complemented by data on individuals regarding early angina pectoris in Edinburgh and the prospective CVD mortality in Basel, are of similar order. Although the data are still incomplete they may be consistent enough to condense all presently available observational data, rounding up with an adequate safety margin. Summary of the plasma levels of antioxidant micronutrients which should reduce antioxidant-related CVD risks: >50 (−60) μmol/L of vitamin C; >30 μmol/L of lipid-standardized vitamin E or α-tocopherol/cholesterol ratio >5.2 (4.8 to 5.6); >0.5 μmol/L of total carotene and >0.4 μmol/L of β-carotene, respectively. These levels may serve as a basis for the formulation of a recommended optimum intake (ROI; below) and also as tentative guidelines for further research studies including intervention trials.

Recent prospective studies of the calculated intake of antioxidant vitamins in the diet and from supplements, and the estimated plasma levels in these studies

Observational data are well suited to reveal hitherto unrecognized correlations, but they cannot prove a causal relationship. According to a recent consensus, the still incomplete but consistent epidemiological data justify the great effort in mounting intervention trials, probably with combinations of vitamins C, E, and β-carotene,[135] although other synergistic nutrients remain to be considered. Primary prevention by specific micronutrients can only be tested by primary intervention trials in sufficiently large groups of subjects of randomized compliant, and still healthy subjects, but prone to the disease and with suboptimal antioxidant status. Testing secondary prevention, although technically easier, is less justifiable because of serious problems of interpretation of the results. First, any benefits from antioxidant micronutrients on overt disease cannot be easily differentiated from the known antioxidant effects of concurrent drug therapy.[86–96] Second, the outcome may be complicated by aging and the multimorbidity of older age. Other diseases, undiagnosed at baseline, might even cause concerns about adverse effects. Finally, and perhaps most importantly, antioxidant intervention is likely to be too late to improve complicated advanced lesions that have already provoked clinical events. Neither observational nor experimental data have so far provided conclusive evidence for regression in advanced lesions by antioxidants, as has been reported for cholesterol lowering. Any convincing demonstration of the potential of antioxidant micronutrients will only come from primary CVD prevention trials which show that raising poor plasma antioxidant levels reduces clinical endpoints. So far the available data do not yet suggest benefit from high antioxidant intake per se, but only after the correction of suboptimal levels. Unfortunately, in the harsh reality of a money-conscious world, compromises and/or combinations of drugs are likely to be used, and supplements might be tested regardless of any justification; all of which could seriously discredit the antioxidant hypothesis.

Large cohort studies in the USA have evaluated dietary etiologies of CVD with the assessment of selfascribed multivitamin supplements. Their regular consumption was associated with a reduced risk of CVD. Polyvitamin supplements, however, might only be one, although presumably an important part of a health-oriented life style, which may frequently include physical exercise, nonsmoking, low-fat diets, etc. Therefore, the contributions of the key constituents of polyvitamin preparations can at present only be interpreted in the context of corresponding observational data and of the response of plasma levels to a given antioxidant intake. In these US cohort studies, the essential antioxidants in univariate analysis have been the most impressive constituents of multivitamin supplements, suggesting a significative potential for preventing CVD. Synergistic interactions of antioxidants with other constituents of multivitamin supplements remain to be elucidated. Although dietary studies have weaknesses in assessing antioxidant intake (related to the inherent inadequacy of food tables and to the limitations of nutritional questionnaires) they still allow crude interindividual comparisons, are reasonably reproducible,[124] and their validity is reasonable where the variation in supplement is large.[107,124,136–145] Plasma levels equate fairly well with the calculated antioxidant intake, although, of course, other determinants can play a role, e.g., age, gender, body mass, homeostatic factors, smoking, alcohol, hormones, etc.[113,122,145] The between-community comparison of antioxidant intake is hampered by differences in survey methods and questionnaires as well as by the limitation of food table adequacy. Nevertheless, the available dietary surveys of large numbers of participants provide a broad picture of antioxidant consumption. They may be sufficient for the tentative comparison of intake with optimum plasma levels of antioxidant micronutrients.

The First US National Health and Nutrition Examination Survey (NHANES I).

A 10-year follow-up of mortality[146] examined a large representative sample of noninstitutionalized US adults (n = 4,479 males and 6,869 females) of 25 to 74 years of age, 29 and 26% of which, respectively, were smokers. In males with a calculated regular vitamin C consumption of ≥130 mg (dietary vitamin C plus that from polyvitamin supplements) the relative risk for all causes of death was reduced to 0.65 (95% CI 0.57 to 0.80), and of all CVD to 0.58 (CI 0.41 to 0.78), as compared with supplement nonusers with a dietary vitamin C intake of <50 mg (mean 22 mg) and a relative risk of 1.0. An even lower relative risk for CVD was found in supplementing women: 0.44 (CI 0.27 to 0.66).

But in subjects who regularly consumed >130 mg of vitamin C solely from the diet, the CVD mortality was 0.94 (CI 0.80 to 1.09), i.e., not significantly reduced. Overcalculation of dietary vitamin C is unlikely since higher amounts of vitamin C only occur in a few frequently consumed items, such as oranges, broccoli, etc. Therefore, with a high dietary vitamin C intake, the inherent imprecision of food tables[147] (only in part accounting for variation due to harvesting, processing, and cooking) produced less bias than for the calculation of a low dietary intake of vitamin C. In conclusion, an intake of >130 mg of vitamin C requires the consumption of other micronutrients to exert preventive effect. Dietary sources very rich in vitamin C are also rich in antioxidative bioflavonoids (e.g., "vitamin P" occurs in substantial quantities in oranges, grapes, berries, etc.) or certain carotenoids such as lutein (e.g., in broccoli) or β-cryptoxanthin (in oranges). It is plain that such antioxidant "fellow-travelers" did not promote vitamin C in NHANES I. In contrast, and as already mentioned, vitamin C showed significant CVD protective properties when consumed in polyvitamin preparations. The latter differ from food items rich in vitamin C mainly in the content of liposoluble vitamins A and E and possibly some vitamins of the B complex.

Unfortunately, all the vitamins in multivitamin preparations could not be evaluated in the multivariate analysis of the NHANES I mortality data. But at the time of the survey (1971–4 through 1982–4), liposoluble antioxidant vitamins could have been consumed in appreciable doses: the RDA for 1968 for adult males was 5,000 IU vitamin A = 1.5 mg of retinol (presently 1.0 mg) and 30 IU of vitamin E = 20 mg of α-tocopherol (twice the present RDA of 10 mg).[148] The impressive reduction of CHD risk in US health professionals after correcting a suboptimal vitamin E intake with multivitamin preparations (below), if taken in the context of the observational data cited above, strongly suggests an important role for concurrent vitamin E supplements and echoes the previously mentioned interaction of vitamins C and E in vitro and in vivo in animals and men.

In NHANES I, the vitamin C consumption in US males was undesirably low since as many as 46% consumed <50 mg of vitamin C daily with a mean of 22 mg,[146] i.e., less than half the RDA of 60 mg. Similarly, in the Continuing Survey of Food Intake (CSF II) 25% of adult Americans consumed only 39 mg of vitamin C daily,[149] and thus it had to be concluded that substantial segments of the US population routinely consumed considerably less than the RDA.[150,151] Similarly, 50 μmol/L of plasma vitamin C, i.e., the optimum value, is roughly equal to plasma levels found in only 40% of white and 25% of black adults in the USA.[152] In supplement nonusers of NHANES II, a vitamin C plasma median of 41 μmol/L was found, i.e., a suboptimal level, and only 25% of nonsupplementing Americans revealed the desirable level of >50 μmol/L, whereas all supplement users had plasma vitamin C values above this risk threshold.[153]

Unfortunately the mortality follow-up of NHANES I did not differentiate between nonsmokers and smokers in view of the generally accepted deleterious effect of cigarette smoking on antioxidant balance. But such a differentiation was made for the plasma/intake response in the NHANES II (n = 15,795 persons with serum vitamin C analysis, 3 to 74 years of age, from 64 geographical locations in the USA, carried out from 1976 to 1980). Among 45- to 74-year-old male supplement nonusers of NHANES II the mean consumption of vitamin C was similar to that of NHANES I, i.e., in nonsmokers 80 mg and in cigarette smokers 53 mg daily and thus slightly less than the RDA of 60 mg. But the vitamin C intake was particularly low in large subgroups of the population, i.e., <42 mg of vitamin C (<70% of RDA) were consumed by 31% of nonsmokers and by 41% of smokers.[154]

Smoking increases the vitamin C requirement substantially, i.e., in fact an additional 40 to 68 mg is needed to achieve serum vitamin C levels comparable to nonsmokers.[101,108,111,112,156] For the Public Health it is undesirable that 16% of nonsupplementing male smokers in NHANES II had a plasma vitamin C <14 μmol/L, i.e., levels of marginal to overt vitamin C deficiency,[108–110] and this was also true in 24% of subjects with very low income.[111,154] When the plasma/intake response to vitamin C in NHANES II[111] is applied to the 46% nonsupplementing participants in NHANES I who consumed <50 mg/day,[146] their actual mean vitamin C intake of 22 mg daily should yield a plasma concentration of approximately 45 μmol/L in non-smokers, i.e., a level slightly below the desirable range of ≥50 μmol/L; but an intake of 22 mg daily in smokers will result in only 28 μmol/L, i.e., a plasma level associated with about a 2 fold greater CHD mortality in observational studies *(Table 1)*.

In a random sample of Scotsmen,[84] an intake of 22 mg (the mean of NHANES I subjects with an intake <50 mg) corresponded in smokers to only <10 μmol/L of plasma vitamin C, i.e., values typical of vitamin C deficiency,[108–110,155] but even in nonsmokers to only 18 μmol/L,[84] i.e., still a level of marginal vitamin C deficiency with increased CHD risk.

This holds equally true for 40- to 49-year-old Germans in the VERA Study (National Consumption Study combined with Risk Factor Analysis; plasma measurements in a subsample of n = 2,000 out of the total dietary survey of n = 24,632 subjects from 10,000 households in 16 centers all over former West Germany, with a 70% response rate).[157] In these Germans who were mostly well provided with all

the principal antioxidant micronutrients, a daily intake of 22 mg of vitamin C corresponded to <15 μmol/L in smokers and nonsmokers, which is similar to the response in Scotsmen. An intake in the order of the present US RDA of 60 mg daily in the above-mentioned study cohorts would result in plasma levels below or near to the desirable 50 μmol/L of vitamin C in nonsmokers but markedly lower values in smokers. First, the US RDA of 60 mg would yield in the nonsmoking Americans of NHANES II a plasma vitamin C of 50 μmol/L, but in smokers only around 35 μmol/L,[111] i.e., levels that may have some CVD risk *(Tables 1, 2, and 4)*. Second, in nonsmoking Scotsmen (also suboptimal for vitamin E and carotene)[84] *(Table 3)* the present US RDA corresponded to 37 to 45 μmol/L, but in current smokers to only 18 to 27 μmol/L, i.e., levels associated with at least a doubled CVD risk *(Tables 1, 2, and 4)*. Third, in Germans (mostly with a fair overall status of antioxidants),[157] the US RDA only resulted in plasma levels of about 40 μmol/L, regardless of smoking status, i.e., slightly below the desirable safety threshold.

In contrast, the CVD preventive intake of vitamin C of >130 mg resulted in all study populations in the optimum plasma levels of >50 μmol/L, even in smokers. First, ~130 mg of vitamin C achieved in nonsmoking Americans of NHANES II[111] plasma vitamin C concentrations near the renal threshold of approximately >55 to 70 μmol/L,[101] and with that intake even in smoking Americans still stayed in the CHD-preventive range of 50 (−60) μmol/L. Second, almost the same plasma vitamin C response was seen with a daily intake of 130 mg of vitamin C in smoking and nonsmoking Scotsmen.[84] Third, in Germans in the VERA Study,[157] an intake of ~130 mg of vitamin C gave plasma values of >80 μmol/L in all subjects, i.e., levels of tissue saturation. The reason German smokers uniquely did not require extra vitamin C remains to be clarified. Racial influences on vitamin C may concern tissue concentration rather than the response of plasma level to a given intake.[101] Consequently, nutritional differences may primarily be responsible for the different response of plasma vitamin C in German and Anglo-American smokers. It may be important that in all Germans the mean values of both plasma vitamins C and E were in the optimum range, whereas this applies for neither Scotsmen *(Table 3)* nor Americans of NHANES I and NHANES II. Consequently, in German smokers vitamins C and E could have mutually spared each other. In contrast, this is hardly true for β-carotene in German smokers (below), being too low to exert any substantial sparing of vitamin C. Comparing in the three cohort studies the vitamin C intake required to achieve an optimal plasma level of ≥50 μmol/L, similar amounts are found for nonsmokers, i.e., approximately 60 mg in NHANES II,[111] 79 mg in Germans (with mostly fair antioxidant status),[157] and 85 mg in Scotsmen.[84] This indicates that in nonsmokers the vitamin C requirement for desirable plasma levels depends primarily neither on concurrent levels of other antioxidants nor on conceivable genetic differences. In contrast, the vitamin C requirement of smokers clearly depends on levels of other antioxidants. Hence, attaining the desirable plasma vitamin C seems to require approximately >110 mg in smokers of NHANES II[111] and >120 mg in smoking Scotsmen,[84] i.e., in populations being notably low in both vitamin E and β-carotene. The latter may be excluded since German smokers, despite being lower in β-carotene, require no extra vitamin C.[157] As a result, a sparing of vitamin C by vitamin E remains as the most likely reason for an unimpaired plasma/intake response in German smokers, and this might also be true for the reduction of CVD mortality in NHANES I by vitamin C supplements,[146] presumably in combination with substantial amounts of vitamin E, as discussed above. Sparing of vitamin C by vitamin E is conceivable for an excess of vitamin E at which vitamin C is no longer needed for recycling of vitamin E. Nevertheless, such sparing interactions between vitamins C and E may be limited or conditioned. Hence, in the prospective Basel Study, the abundance of vitamin E could not prevent an increased CHD risk at low plasma levels of vitamin C and both vitamin C and carotene.[51,125] In any case, regarding the present US RDA of 60 mg, the available data suggest that the latter is almost scarce for nonsmokers, but obviously insufficient for smokers in the USA as in Scotland. The latter seem to require a daily intake of >125 to 140 mg of vitamin C in order to approach the optimum plasma range of >50 (−60) μmol/L,[84,111] but this might also be true for Australia.[108] Whereas in 95% of healthy middle-aged men, the body's vitamin C pool size is saturated by 100 mg daily,[112] and an intake of ≥125 mg is required to assure adequate body reserves of vitamin C in healthy Americans aged 60 years and older,[158] i.e., at an age when morbidity and mortality start to increase exponentially. Since these consumption rates correspond to the CVD-preventive intake in NHANES I, a ROI of this order seems to be justified, at least for smokers and/or the elderly. Since the above comparisons are based on regression means, any final recommendation levels will require some upgrading if they are to be applied to everyone. For instance, 50% of German male nonsmokers require a median intake of 79 mg daily to achieve an optimum plasma vitamin C of >50 μmol/L, but 95% of Germans would require 88 mg.[157]

Multivitamin self-supplementation in American study cohorts

Harvard Study of Health Professionals. The Harvard Study of Health Professionals[159] recorded coronary events (infarcts, cardiac deaths) over 4 years in initially healthy males aged 40 to 75 years (n = 39,910) some of whom took multivitamin supplements, as part of a health-oriented lifestyle that included avoiding smoking (only 9% smoked), taking physical exercise, aspirin consumption, and prudent low-fat diets. The generally healthier risk profile of supplementing men[159] included, for example, also higher consumption of dietary fibers and plant-derived magnesium which were inversely correlated to blood pressure.[160]

Vitamin C in the Health Professionals Study. The intake of vitamin C in the health-oriented study subjects lacked any significant correlation to coronary events in multivariate analysis, as could only be expected from a mean vitamin C intake of >92 mg daily, even in nonsupplementing health professionals who were mostly nonsmokers. Consequently, the majority of study subjects (in contrast to the majority of supplement nonusers of the US[153]) most

probably had an optimum plasma level of >50 μmol/L of vitamin C. In conclusion, in this study population with its fair vitamin C supply, the relative risk of a poor vitamin C status could not be tested,[159] which indirectly supports the vitamin C-related conclusions of *NHANES I* and those of the European observational studies reviewed earlier.

Vitamin E in the Health Professionals Study. Among all vitamins evaluated by univariate analysis for all Health Professionals, only vitamin E showed statistically significant inverse correlations. This strongly confirms and extends the observational data reviewed above which indicate that an optimal vitamin E status is a prerequisite for cardiovascular health (as far as micronutrients are concerned). Hence, a daily consumption of >60 IU of vitamin E (>40 mg of RRR-α-tocopherol equivalents, equal to >44 mg of RRR-α-tocopheryl acetate, or to >60 mg dl-α-tocopheryl acetate) reduced the multivariate relative risk of all coronary events to 0.64 (95% CI 0.49 to 0.83) in comparison with subjects consuming a median of 6.4 IU (range 1.6 to 6.9 IU).[159] Daily supplements of >100 IU of vitamin E (>67 mg of RRR-α-tocopherol equivalents, equal to >74 mg of RRR-α-tocopheryl acetate, or to >100 mg of dl-α-tocopheryl acetate) reduced the multivariate relative risk of all coronary events to 0.54 (CI 0.33 to 0.88) as compared with nonsupplementing men.[159] The meager vitamin E intake from diet of nonsupplementing Health Professionals reflects fully the low category of vitamin E supply in the USA which had in NHANES II in nonsupplementing males a median of 11 IU, and after inclusion of fortified foods 12 IU.[149,163,161] This order compares unfavorably with male adults in Western Germany (18 IU)[157] and even with the United Kingdom (median 14 IU).[113] If a conservative order of ≥0.4 mg of vitamin E/g of PUFA is considered desirable, 23% of men of NHANES II had diets with low ratios.

All these data arouse the suspicion about whether the particularly low dietary vitamin E in Americans, if corresponding to suboptimal plasma levels, might be a quantitatively important CHD risk factor in the USA, according to the Antioxidant Hypothesis.[1] The vitamin E supply of nonsupplementing Health Professionals was insufficient even with respect to both the present and previous RDA. Hence, the nonsupplementors consumed only roughly a third of the present US RDA (1989), which recommends 15 IU of vitamin E daily, and only a fifth of the scientifically based[162] recommendation of 30 IU in the US RDA (1968).[148] The critically low vitamin E intake of the US Health Professionals may involve several factors.

(i) Restriction of calories from fat, which is sensibly recommended.[5] The actual 35% of total calories derived from fat[124] in Health Professionals should scarcely limit the intestinal absorption of vitamin E.[163] In any case, the dietary vitamin E of Health Professionals clearly did not depend on the total fat consumption.[159] In NHANES II dietary vitamin E was consumed in common fats and oils (20% of α-tocopherol intake), vegetables (another 15%), and meat, poultry and fish (additional 13%).[161] In conclusion, a low intake of dietary fat is unlikely to be a major cause of the low vitamin E intake in the USA.

(ii) Dietary fats being poor in both absolute and net α-tocopherol. The latter was defined by *Bässler*[164] as extra α-tocopherol above the requirement for concurrent PUFAs (essential polyunsaturated fatty acids). Net vitamin E seems preferable to the α-tocopherol/PUFA ratio since it precisely indicates absolute amounts of α-tocopherol available for the body's α-tocopherol balance taking PUFA consumption into account. Contrary to a common belief,[5] the increased consumption of vegetable PUFAs does not necessarily improve vitamin E intake,[165] because many plants protect their PUFAs primarily by γ-tocopherol,[166] which lacks vitamin E activity in mammals.[155] For instance, if US Health Professionals with low dietary vitamin E consumed a polyunsaturated dietary fat with a negative net α-tocopherol value, such as soy bean oil (or products based on it such as margarine, mayonnaise, and French dressing), their vitamin E balance will become negative, too. Since the Health Professionals' actual fat intake was 13.6 g (6% of a total of 1,967 calories daily)[124] the balance for soy bean oil is: 13.6 g contain about 2 IU of α-tocopherol (aside from abundant γ-tocopherol), but the high PUFA content of soy bean oil (54% linoleic, 7% α-linolenic acid) requires approximately 8 IU of α-tocopherol to prevent is peroxidation in the body (according to the presently best estimate that 1 g of linoleic acid requires 0.6 mg = 0.9 IU of α-tocopherol, and 1 g of α-linolenic acid 0.9 mg = 1.34 IU[167]; 2 − 8 IU = −6 IU net α-tocopherol daily).

Other oils with a negative net vitamin E are corn, rapeseed oil, and peanut oil, whereas a net vitamin E around zero is provided by safflower, grapeseed, and cottonseed oil.[164,166] In contrast, the daily intake of 13.6 g of oils with clearly positive net vitamin E could supply the small amounts of vitamin E consumed by the Health Professionals. For instance, 13.6 g of extra virgin olive oil contain about 3 IU of vitamin E, but its minimal PUFA content requires only 1 IU; the balance is 2 IU net α-tocopherol. The same amount of sunflower oil contains 11 IU, requires 7 IU for PUFA protection: balance net α-tocopherol 4 IU.[164] If a more conservative, although widely accepted, requirement of 0.4 mg of α-tocopherol/g of PUFA were still considered sufficient, the differences in oils regarding net vitamin E would, in principal, remain the same: an additional demand of 3 IU of α-tocopherol by 13.6 g of soy bean oil, in contrast to an actual supply of 2 IU net α-tocopherol by olive oil, and 6 IU by sunflower oil respectively. But, in fact, 0.4 mg of vitamin E/g of PUFA may scarcely provide a CHD-protective plasma level of α-tocopherol, at least not in Americans. Hence, based on the worldwide and current US recommendation that PUFAs should account for ≤10% of total calories, Health Professional had a PUFA allowance of ≤197 calories or 21.9 g daily,[124] and with the conservative ratio of 0.4 mg of vitamin E/g of PUFA their theoretical vitamin E requirement should be 8 IU. This was the vitamin E intake in nonsupplementing Health Professionals whose relative CHD risk was roughly doubled as compared with users of supplements of >100 IU. Even the more generous guideline of 0.6 mg of vitamin E/g of PUFA[164,167] is debatable since the latter would demand 13 IU of vitamin E for Health Professionals. This was actually the British median,[113] which is, however, still associated with at least 3 fold CHD risk in comparison with Southern Europe. If an ROI of 36 IU of vitamin E, as considered desirable for Germans[157] (see below), was sufficient for US Health Professionals they should consume 1.6 IU of vitamin E/g of

PUFA. These simple calculations show clearly that previous recommendations of either 0.4 or 0.6 mg of vitamin E/g of PUFA may require review if they are not replaced by the more easily understood absolute amounts of net vitamin E. Future recommendations may have to take into account that not only newly consumed PUFAs require adequate protection by vitamin E, but there is an additional demand for replacing the continuous vitamin E losses which may be unavoidable during the steady-state vitamin E recycling while protecting PUFAs within the body.

Previously recommendations such as "five serving of fruits and vegetables daily"[5] unfortunately underestimated the problem of dietary vitamin E. If this should be made good, forthcoming health-promoting recommendations will at least have to specify the types of preferable dietary fats, i.e., those with high net vitamin E content. For the consumer, food labels could become a great practical help if they indicated (not only the cholesterol content but also) the minimum net vitamin E content in every commercial oil and prefabricated lipid-containing food item, respectively. Unfortunately, the present system of food tables may be completely overcharged by a demand of net vitamin E after subtraction of the very variable losses by processing and storage, with the present speed of developments by food industry.[166] In any case, >36 IU vitamin E daily is a minimum for any recommendation focusing on health maintenance.

(iii) Losses of vitamin E during preparation, preservation, and storage of foods. Once the chain reaction of lipid peroxidation is initiated (e.g., by γ-irradiation or by any common food processing and cooking) it can proceed even in the frozen state and exponentially produce ROS. The latter will react with all available antioxidants, e.g., by formation of α-tocopherol free radical as a first step, followed by irreversible oxidization of the latter to quinone derivatives as a second step. Since at present approximately 95% of food in the USA (atmost 70% in Europe) is processed or industrially modified, and as preservation by γ-irradiation becomes frequent, the risk of chain reactions of lipid peroxidation seems appreciable. It is increased by growing shelf lives of processed food, ranging presently from months through years. It may be interesting to look into the concentrations of α-tocopherol and lipid peroxides, respectively, in popular prefabricated fast food products that are kept frozen and finally warmed up, such as hamburgers and pizza. If regulatory bodies wanted to implement an adequate dietary vitamin E supply they might oblige the food industry by replacing any α-tocopherol lost through technical procedures or longer storage.

(iv) Increased requirement of vitamin E caused by other dietary constituents, i.e., mainly the above-mentioned lipid peroxides formed during storage and/or reheating, but in addition plant preservatives, biocides, and drugs, many of which can yield peroxides in the human liver, as well as by excessive alcohol consumption.

Dietary vitamin E only moderately predicts ($r_P \geq 0.5$) the lipid-standardized plasma level of α-tocopherol,[136,137,139–141,144,145,168] which depends, of course, also on homeostatic and hormonal factors, age, etc.[56,155] In case of vitamin E supplements, however, the intake becomes a major predictor of the plasma vitamin E level.[144,163,168,180] Therefore, the pertinent question of this review is about plasma levels of vitamin E in nonsupplementing versus supplementing persons. According to European data and several American publications, a daily intake of vitamin E as low as 1.6 to 6.9 IU by US Health Professionals very probably resulted in critically low plasma values, i.e., <20 to 25 μmol/L being associated with a several-fold higher risk of CHD *(Tables 1, 2, and 4)*. Hence, in randomized middle-aged male Germans in the VERA Study, 8 to 11 IU of dietary vitamin E achieved plasma levels of about 10 μmol/L.[157] Furthermore, in Scottish surveys a dietary vitamin E intake of 6.0 to 7.6 IU yielded 12 to 26 μmol/L in plasma and an equally poor α-tocopherol/cholesterol ratio *(Table 3)*. Similarly, in the Dietary and Nutritional Survey of British Adults[113] studying a random sample of men 16 to 64 years of age from all over Britain ($n = 946$; consuming as much fat, i.e., 38% fat calories including 14.4 g of PUFAs, as US Health Professionals) the median intake (±SE of mean) of 14 (±0.4) IU of vitamin E corresponded to a plasma median of 25.5 (±0.3) μmol/L and an α-tocopherol/cholesterol ratio of 4.5 (±0.04).[113] These European data match those from several American studies. First, the Nutritional Status Survey in the Greater Boston, MA Area[169] observed in nonsupplementing younger and older males, medians of plasma α-tocopherol (±SE of mean) of 22 (±1) and 24 (±1) μmol/L, respectively, with a corresponding α-tocopherol/cholesterol ratio as poor as 4.4 (±0.2) and 4.5 (±0.1), respectively, and similarly at baseline of a supplementation study in Washington, DC,[170] a plasma level of 24 μmol/L with an α-tocopherol/cholesterol ratio of about 4.6 ± 0.2 was found. In the NCI-USDA Study, subjects without any supplement had a plasma vitamin E of 22.5 μmol/L (CI 15 to 37 μmol/L), subjects with common multivitamin supplements including 10 to 60 IU of vitamin E with 23.0 μmol/L (CI 18 to 49 μmol/L) revealed no increase, and subjects on special vitamin E supplements of 100 to 230 IU had plasma levels of only 27.0 μmol/L (CI 20 to 42 μmol/L),[153,172] i.e., still levels below the threshold of CHD risk. Similarly, in a subsample of Health Professionals consuming a mean ± SE of 70 ± 17 IU of vitamin E daily, only a plasma level of 27 ± 1 μmol/L was detected, corresponding to an α-tocopherol/cholesterol ratio of 5.1 ± 0.1,[145] i.e., to values at the upper end of the suboptimal vitamin E range. The poor plasma/supplement response of subjects in the NCI-USDA Study and in the Health Professionals Study corresponds largely to Bostonians whose plasma vitamin E was only 26 ± 1 μmol/L (similar to the Americans quoted above) at a dietary vitamin E of 24 ± 6 IU, and who required supplementation with 107 ± 22 IU of vitamin E in order to achieve the desirable plasma level of 30 ± 2 μmol/L.[144]

Such amazingly poor plasma responses differ considerably from that in a supplement study in Washington, DC, and even more from the plasma response to dietary vitamin E in Germans of the VERA Study.[157] Hence, in Washingtonians given daily supplements of only 30 IU of vitamin E (in addition to presumably ≤11 IU from diet), the plasma vitamin E rose from 24 ± 1 μmol/L, i.e., the common range of nonsupplementing Americans, to the threshold band of CHD risk, i.e., 29 ± 1 μmol/L.[170] When the same study subjects in Washington, DC, received subsequently

daily supplements of 100 IU (i.e., the dosage of CHD-protective supplements of Health Professionals) their plasma vitamin E increased to values substantially above the threshold of CHD risk, i.e., 36 ± 2 μmol/L.[170] In the VERA Study (where fat intake was 38% of total calories from fat; mean PUFAs 13 g, P/S ratio 0.3,[157] i.e., everything similar to that of Health Professionals) a daily intake of 70 IU of vitamin E resulted in a "saturation" level at about 60 μmol/L of cholesterol-standardized plasma α-tocopherol, i.e., an approximately 2 fold higher plasma vitamin E than in supplementing Health Professionals. In these representative adult Germans[157] the desirable optimum plasma status of ≥30 μmol/L of cholesterol-standardized vitamin E could be achieved by a median dietary vitamin E intake of only 23 IU, which is equivalent to 1.53 times the present US RDA/RDI of 15 IU. The same plasma status resulted in nearly all Germans (85%) who had a daily intake of 36 IU.[157] This order is only slightly higher than the US RDA (1968) of 30 IU which was based on balance studies.[162]

In summary, the consistent data in Americans and Europeans indicate that the meager vitamin E provision of either nonsupplementing Health Professionals (<10 IU daily) or of Americans in NHANES II (11 to 12 IU, recalculated by including fortified foods[153]) can only result in plasma vitamin E levels as low as approximately 23 to 26 μmol/L, which is associated with a several-fold higher CHD risk *(Tables 1–3)*. In Americans, even 30 IU of the higher RDA (1968)[148] do not bring about any substantial increase of plasma vitamin E, in contrast to Germans. In most Americans, including the subsample of Health Professionals, the threshold of desirable plasma levels of 29 to 30 μmol/L of plasma vitamin E can only be approached by supplements in the order of (60-) 100 IU,[145] which prevented also the relative CHD risk of nonsupplementing Health Professionals. With such a poor plasma/supplement response in the Health Professionals Study,[159] corresponding findings in the NCI-USDA Study[153] and in supplementing Bostonians,[144] it seems premature to assume a general "associated between a high intake of vitamin E and lower risk of CHD in men."[159] In fact, the available plasma data rather suggest that the correction of a previously suboptimal vitamin E status, as one prevalent CHD risk factor in Health Professionals, helped to reduce the relative CHD risk, in line with the Antioxidant Hypothesis.[1] The apparently weak plasma/intake response of vitamin E in Health Professionals[145] and comparable American surveys[144,153] suggests awareness of crucial nutritional problems, at least in some Americans. Several factors should be considered:

(i) Differences of analytical methodology. They could hardly explain the striking discrepancy.
(ii) Inadequacy of present food tables for assessing net α-tocopherol after cooking, processing, storage, and reheating.[166] Although this handicap may have been very relevant in the calculation of small amounts of dietary vitamin E intake, it does not exist for the estimate of appreciable quantities of stable supplements in capsules.
(iii) The amount and type of dietary fat[165] and its net vitamin E.[164] This factor may be of crucial importance for the majority of Americans who do not consume vitamin supplements or fortified foods and who exclusively consume fats low in vitamin E (e.g., from animals, salad dressing, mayonnaise, and margarine)[153] or even vegetable fat with a negative net vitamin E (such as soy bean oil).
(iv) Increased vitamin E requirement of Health Professionals due to larger amounts of dietary lipid peroxides. Dietary lipid peroxides, hydroperoxides, and/or reaction products have organ toxicity. They will not only increase the requirement of vitamin E, but also have been thought to increase the risk of CHD, e.g., by alteration of vascular eicosanoids.[1,44–48] Differences in dietary lipid peroxidation products are possible between the USA and European countries as the amount of oxidation products is only legally restricted in European communities. An increased vitamin E requirement in Americans has to be considered as a major causal factor in the poor plasma/supplement response in Health Professionals, Bostonians, etc.
(v) Unidentified dietary life style factors increasing the vitamin E requirement.
(vi) Genetic factors affecting bioavailability of vitamin E. If they exist, they could be derived from Britain where smokers' handling of vitamin C (above) and of β-carotene (below) indicates extra requirements similarly as in US smokers.

Vitamin E in smoking Health Professionals. The reduction of CHD risk by vitamin E supplements >100 IU was less and without statistical significance (relative risk 0.67, 95% CI 0.34 to 1.31) in comparison with nonsmokers (relative risk 0.52, CI 0.34 to 0.78).[159] This might be related to the fact that smoking can somehow deplete vitamin E despite the fact that plasma vitamin E of a smoker is occasionally,[141] but not consistently, decreased[84,98,100,107,168,173–175] (as typical for vitamin C and carotene). When vitamin E is reduced in LDL the latter is more easily oxidizable and leads to foam cell-like cholesterylester accumulation in macrophages,[176] but when smokers have normal vitamin E levels in LDL, its copper-induced oxidation is normal, too.[175] Smokers reveal an increased plasma concentration of conjugated dienes which are reduced by substantial vitamin E supplements.[173] Smoking also acutely increases TBARs in plasma and LDL, and in turn LDL is liable to cell-induced LDL oxidation and increased metabolism by macrophages; again these abnormalities can be prevented by massive supplements of vitamin C or E.[177] The lung lavage fluid from smokers is poorer in vitamin E,[178] suggesting lower antioxidant protection of the smoker. Erythrocytes of smokers have a higher sensitivity to peroxidation which is normalized by huge vitamin E supplements.[179] Accordingly, it is conceivable that smokers have an increased vitamin E requirement, e.g., on the cellular site, which is not present at the plasma level.

β-Carotene in the Health Professionals Study. The dietary intake of β-carotene of the total study population of the *Health Professionals Study*[159] was fair, i.e., >2.4 mg from the diet, increasing by selfsupplementation to 8.6 mg. This difference did not affect CHD events in nonsmokers as

could be expected from the measurement of plasma levels. Hence, a subsample of the Health Professionals (including 9% current smokers only) had a mean consumption ± SE of 6.1 ± 0.7 mg of β-carotene with plasma of 0.46 ± 0.03 μmol/L,[145] i.e., a value in the presumed optimum plasma range >0.40 μmol/L of β-carotene. Similarly, in a nonsmoking random sample of Scotsmen,[84] the dietary β-carotene intake of about 2.5 mg provided the threshold plasma levels of 0.40 μmol/L, and in 50% of a sample of Germans in the VERA Study this level was achieved by orders of 1 to 2 mg of β-carotene daily, and in 90% of this German population by 3.0 mg of dietary β-carotene.[157] A subsample of smoking Health Professionals revealed, however, a plasma β-carotene of only 0.30 μmol/L, i.e., 37% less their nonsmoking colleagues[168] which may be associated with an increased CHD risk. Current smokers in the Health Professionals Study[159] required an increased β-carotene intake, i.e., of 4.2 to 5.8 mg in order to have the multivariate relative CHD risk reduced to 0.46 (95% CI 0.23 to 0.91) and >8.6 mg to lower the risk to 0.30 (0.11 to 0.82). This finding concurs with the well-established fact that current smokers among Americans,[107,138,180] Scotsmen,[84] and some Finns[127] typically respond by a given dietary intake of β-carotene with a smaller increase of plasma levels than nonsmokers. Aggressive radicals from tobacco smoke[102–105] may be causally involved in the increased requirement of β-carotene in smokers, as has generally been accepted for vitamin C. A poorer plasma/intake response in smokers may be partially responsible for the fact that plasma levels of nonsupplementing smokers are typically low in most westernized peoples.[84,107,127,138,141,168,174,181–187]

This fact, which is usually associated with a higher CHD risk, was extended to the adipose tissue level in the EURAMIC Study.[118] As already mentioned, liposoluble antioxidants were measured in adipose tissue of coronary infarct patients in various European centers shortly after the clinical event, in comparison with controls. The relative CHD risk of smokers was, regardless of the geographical region, inversely related to the sequestration of β-carotene in the adipocytes which could be an integral marker of life-long β-carotene intake.[119] Low plasma β-carotene levels can even further deteriorate with a reduced intake that occurs frequently,[107,127,181,188] but not always[100,173,181] in smokers. A reduced β-carotene intake results in lower plasma β-carotene even when the smokers' plasma/intake response does not differ from that of nonsmokers. Hence, in male Germans of the VERA Study the plasma/intake response for β-carotene (as well as vitamins C and E) lacked any statistically significant difference between nonsmokers and smokers,[157] since the optimum plasma level of ≥0.4 μmol/L of β-carotene in smokers (>10 cigarettes daily) required a median consumption of 1.2 mg (95% CI 1.0 to 1.6), and in nonsmokers 1.7 mg (CI 1.2 to 1.4). But nevertheless, the actual median of plasma β-carotene in all German smokers was substantially (28%) lower than in nonsmokers, and the smokers' absolute plasma value of 0.36 μmol/L of β-carotene[157] was as undesirably low as that of smoking Scotsmen with increased CHD risk *(Table 1)*. This plasma median of German smokers was strongly related to a lower β-carotene intake, being 40% lower in heavy smokers (>30 cigarettes), since the latter consumed daily approximately 0.7 mg as compared with 1.3 mg in nonsmokers), whereas the intake in the remaining smokers was 21% less (actually 1.0 ± 0.02 mg) than in nonsmokers. But it must be noted that even in nonsmokers the β-carotene intake in multivariate analysis predicts at most only 10 to 25% of the variation in plasma β-carotene and much less in smokers.[107,145,181]

Individual factors are strongly involved in the degree of the plasma response to dietary and even supplemented β-carotene,[107,137–139,188,190,191] and homeostatic factors maintain interindividual differences of plasma β-carotene despite similar intakes.[180,192,193] With excessive β-carotene supplementation, the relatively great interindividual variation can disappear, e.g., in Finns supplementing with 20 mg daily,[181] but Americans have mostly maintained individual response rates, even at doses as pharmacological as 50 or 180 mg.[180,188,189] After adjustment for all known factors determining plasma β-carotene (such as dietary intake, serum cholesterol, body mass index, smoking, or alcohol consumption), up to 25 to 50% of the variation of the plasma response to β-carotene supplements was predictable by the β-carotene level category before supplementation.[185,187,191] It has been thought that the biological mechanism behind the individual response pattern to dietary β-carotene partly reflects the degree of absorption, hepatic secretion into, and clearance from, plasma by extrahepatic tissues and particularly by adipocytes, and that this individual pattern is also preserved after supplementation.[181] If this were true, it could partly explain the fact that former smokers among Health Professionals needed almost as much extra β-carotene as smokers in order to reduce the relative CHD risk to 0.60 (CI 0.38 to 0.94),[159] although ex-smokers by definition no longer actively inhale the radicals of tobacco smoke. But this fact could also be related to the ex-smokers' tendency further to adhere[194] to the smokers' life-style. The latter is characterized on one hand by the low consumption of β-carotene as well as of all the other constituents of fruits/vegetables/"brown bread" (vitamins A, C, and E, folate and other B vitamins, fibers, bioflavonoids, etc.), and on the other hand by a higher intake of alcohol, fried foods, and rather irregular life style, as seen in subjects of NHANES II and British study cohorts.[173,194–196] In consequence, other dietary constituents, e.g., synergistic antioxidants, possibly play an important role in the homeostatic modulation of plasma β-carotene.

This may also be inferred from the German smokers in the VERA Study whose plasma/intake response to dietary β-carotene was identical with that of nonsmokers. They were otherwise characterized by optimum plasma medians of vitamin C (63 μmol/L in all smokers, versus 71 μmol/L in nonsmokers) and vitamin E (cholesterol-standardized α-tocopherol 31 μmol/L in smokers versus 30 μmol/L in nonsmokers). They also consumed only about 10% less riboflavin, pyridoxin, and folic acid than nonsmokers, i.e., practically the same amounts of B vitamins as nonsmokers.[157] In consequence, the undiminished response of plasma β-carotene in German smokers to a given intake of β-carotene could be related to their concurrent optimum plasma status of principal antioxidants other than β-carotene. This possibility concurs with the fact that any deteri-

oration in the plasma response to β-carotene in smokers in other studies has regularly been associated with a poor status of vitamin E, but only in part with suboptimal vitamin C. Hence, smoking Scotsmen (with plasma vitamin E as critical as 23 ± 1 μmol/L)[84] and smokers in Massachusetts (plasma vitamin E as poor as 22 ± 8 μmol/L)[107] required at least six times more β-carotene than nonsmokers to achieve the desirable plasma β-carotene level of >0.4 μmol/L, i.e., ≥12 mg as compared with approximately 1 to 3 mg in matched nonsmokers.

In Finland, another country with high CHD risk, a smoker's lack of response might be inversely related to vitamin E plasma levels.[127] In contrast, the undiminished response of German smokers occurred at optimum plasma vitamin E (median 30 μmol/L).[157] Synergistic interactions between β-carotene and vitamin E have already been suggested by experiments in vitro and ex vivo.[72–74] Accordingly, in hypercholesterolemic patients massive supplements of vitamin E were observed to triple serum β-carotene within 3 months,[75] which could suggest β-carotene sparing by vitamin E. Anyway, the principal geographic differences mentioned above suggest that the requirement of extra β-carotene by current cigarette smokers can vary considerably, i.e., with the rank order Americans ≥ Scotsmen ≥ Finns ≥ Germans. Furthermore, the large interindividual differences in the plasma/intake response to β-carotene also point to the relative importance of homeostasis. Consequently, recommendations for an optimum β-carotene intake can hardly be generalized, and should take into account the concurrent status of other antioxidant micronutrients in any particular region. Logically individual recommendations will have to rely on the individual plasma/intake response as well. All β-carotene data from studies on self-supplementation, if taken together with plasma/intake responses, seem further to support the hypothesis that correcting suboptimal plasma β-carotene levels to >0.40 μmol/L reduces the risk of CHD.[51,98]

Vitamins other than the principal antioxidants in the Health Professionals Study. Antioxidant supplements in the US Health Professionals Study[159] were not consumed singly but as part of multivitamin supplements. The preparations especially improved the intake of vitamin E (about 8 fold) but also that of other vitamins, i.e., vitamin A (almost twice and thus approaching the RDA), vitamins B_1 (tripling the RDA), B_2 and B_{12} (doubling the RDA), B_6 (four times the RDA), and folate (1.35 fold increase slightly exceeding the RDA of 400 mg).[124] Although the increased intake of these vitamins in multivariate regression analysis lacked any association with the CHD risk,[159] their synergistic interaction with vitamin E and/or β-carotene still remains to be elucidated. If optimum conditions of all essential micronutrients were required for normal cell function (e.g.,of endothelial or mononuclear blood cells similar as in carcinogenesis or prevention of malformations, or for aging-related arterial phenomena, e.g., of proteoglycans/elastin/collagen corresponding to cataract formation), vitamin A and/or B vitamins would require optimization along with the principal antioxidant micronutrients. Other epidemiological data also encourage the exploration of synergistic interactions. Hence, the generally poor nutritional habits of British and American smokers may also affect these "orphan" vitamins.[113,194,195]

US Nurses' Health Study.[197] This survey dealt with US female nurses (n = 87,245) in parallel with the US Health Professionals Study,[159] with results for vitamin E that were similar.

US Physicians Study.[198] An abstract of a preliminary subset analysis of this randomized intervention trial (which has revealed CHD-protective properties of aspirin), reports a significant reduction of CHD risk by supplementation with β-carotene (50 mg every second day alternately with aspirin), but the data set is still rather limited.

An intriguing question is whether the age-standardized CHD morbidity/mortality of supplementing US Health Professionals (or of other Americans with optimized antioxidant status) is similar to that of the general population in central and Southern Europe where nonsmoking nonsupplementing people mostly have optimum plasma antioxidant levels: Mediterranean areas and Switzerland have ≤110 CHD deaths/100,000 inhabitants, Germans approximately 150 to 200/100,000 inhabitants yearly, i.e., still roughly ≥100/100,000 less than the general US population.

In conclusion, the US supplementation findings strengthen the European observational data which indicate that a suboptimal status of antioxidant micronutrients can frequently occur in populations liable to CVD. In fact, in the USA, as a country with a medium-high prevalence of CHD, a vitamin C deficit seems to be common in substantial fractions of the nonsupplementing general population, and a suboptimal vitamin E status may be almost regular in supplement nonusers. The fact that even nonsupplementing health professionals had a critically low vitamin E status reveals a remarkable gap of professional information on nutrition and health. American smokers may require markedly more β-carotene than nonsmokers as has been shown for vitamin C. Since vitamin C supplements have only demonstrable preventive properties in conjunction with other constituents of multivitamin preparations the latter may have beneficial potentials per se or by interaction. Whether this is also true for vitamin E in multicomponent supplements remains to be elucidated. Anyway, the US data taken together support again the view that CVD prevention requires the concurrent optimization of all antioxidant vitamins, at least.[1] The presently available selfsupplementation data confirm for the USA that antioxidant optimization may be a crucial complement to the prevention of classical risk factors for CVD.

Multicomponent intervention trials in Linxian, North-Central China

Nutrition Intervention Trial in the General Population. In this experiment eight groups of randomized apparently healthy subjects 40 to 69 years of age (n = 29,584) over 6 years received four randomly allocated combinations of various micronutrients whose status has been poor in this rural area in Henan Province.[199] Only groups receiving the com-

bination of vitamin E (30 mg daily) + β-carotene (15 mg) + selenium (50 mg) revealed a significant reduction in mortality, i.e., that of total mortality to 0.91 (95% CI 0.84 to 0.99), and of mortality from stomach cancers to 0.79 (CI 0.64 to 0.99), whereas the decrease in mortality from stroke (the second-most important cause of death, CHD being of marginal importance) to 0.90 (CI 0.76 to 1.07) lacked statistical significance. According to the published raw data, however, the stroke mortality tended to be lower in most intervention groups in comparison with placebos, but not only in the groups given vitamin E + β-carotene + selenium (−19%), but also in those receiving other combinations, i.e., of vitamin A + zinc (−15%), vitamin B_2 + niacin (−18%), vitamin C + molybdenum (−13%). The largest reductions were seen for vitamin E + β-carotene + selenium combined with either vitamin A + zinc (−29%) or with vitamin B_2 + niacin (−24%), and the latter combined with vitamin C + molybdenum (−22%). Forthcoming follow-ups of this study may inform on the importance of interactions. Plasma antioxidants were raised from critically low to optimum levels, e.g., vitamin C from 8.5 to 14 μmol/L at base-line (marginal vitamin C deficiency) to 31 μmol/L by combinations lacking vitamin C, and to 47 μmol/L ($P = 0.0001$; close to optimum) by vitamin C-containing preparations. Correspondingly, β-carotene was increased from about 0.1 μmol/L at base-line (i.e., a very poor status in high risk range) to 0.2 μmol/L (still very low) by supplements other than β-carotene, and to 1.5 μmol/L ($P = 0.0001$; very high) by β-carotene–containing preparations. Since the unspecific, although statistically insignificant rise in plasma levels of vitamin C and β-carotene by other micronutrients suggests mutual sparing effects of the various micronutrients, the interpretation of the mortality data is difficult. The plasma vitamin E status was unfortunately not measured; but if it were as good as in other parts of China (α-tocopherol/cholesterol ratio = 5.35 ± 0.07)[58,200] the trend for stroke reduction by the combination of vitamin E, β-carotene, and selenium should primarily be due to β-carotene and/or selenium. If this holds true, the result would concur with the above-mentioned stroke data on β-carotene from the Basel Prospective Study.

Multiple Vitamin/Mineral Supplementation Trial in Adults with Esophageal Dysplasia.[201] A mixture of all vitamins and minerals was given for 6 years in doses typically 2 to 3 times the US RDA (e.g., 10,000 IU vitamin A, 180 mg vitamin C, 60 IU vitamin E, 15 mg β-carotene daily, 50 mg selenium, 40 mg vitamin B_2, 6 mg B_6, 18 mg B_{12} 800 mg folic acid, etc.) which increased plasma levels as expected from poor levels to the optimum range, e.g., vitamin C from 35 μmol/L (upper end of risk range) to 52 μmol/L ($P = 0.0001$; optimum range), and β-carotene from 0.2 μmol/L (very poor level in high risk range) to 2.2 μmol/L ($P = 0.0001$; very high in the optimum range). Multivitamin/mineral supplementation in a previously poorly nourished population may be particularly suitable to test overall potentials including synergistic interactions of all essential micronutrients, in comparison with special fruit, and vegetable-enriched diets containing additional components such as bioflavonoids, fibers, etc. In the Linxian subjects with esophageal dysplasia, the multicomponent supplements reduced the relative risk of cerebrovascular deaths to 0.62 (CI 0.37 to 1.06) in comparison with placebos, i.e., apparently stronger than in the general population although again without statistical significance.[201] The relatively stronger trend of the multivitamin/mineral preparation toward reduced stroke mortality might suggest that the moderate stroke-protective potential of the combination of vitamin E, β-carotene, and selenium were particularly improved by synergism with vitamins A, B_2, and C which had already revealed some benefit in the general population trial (above). But other agonists in the multipreparation, which have not been tested in the general population, also remain to be considered, e.g. folate, vitamin B_6, and B_{12}. Thus, the latter may reduce plasma homocystein,[202] i.e., a strong independent risk factor for all CVD.[82,203] The parallel reduction of stroke mortality in both Linxian trials suggests that this result, in spite of lacking statistical significance, may not be a chance finding. Taken together the stroke data from the Linxian trials favor the hypothesis that the correction of a suboptimal antioxidant status (conceivably in synergistic interaction with other essential micronutrients) decreases the risk of CVD.

Interestingly, vitamin supplements in Linxian reduced the mortality from esophageal/gastric cancer (the major local cause of premature death) in the general population study,[199] but not in subjects with established dysplasia, i.e., a fairly advanced irreversible precancerous stage.[201] Since in vitro antioxidants can stabilize chromosomes against ROS and inhibit cell proliferation,[10,204] and since in humans β-carotene clearly counteracts reversible genotoxic effects such as initial abnormalities such as DNA strand breaks, micronuclei, sister chromatin exchange, and metaplasia,[204–206] it may be assumed that antioxidants can only modulate the earliest cellular events such as initiation and early promotion of carcinogenesis. If any extrapolation to atherogenesis were permitted it could be speculated whether the initial stages of atherogenesis, such as endothelial dysfunction, the differentiation of monocytes to macrophages, as well as the transformation of normal contracting smooth muscle cells into the arteriosclerotic type, had the best chance of prevention by antioxidant micronutrients.

Summing up all supplementation data, they further support the earlier evidence that the correction of suboptimal antioxidant plasma levels eliminates the risk of CVD. The supplementation data clearly suggest that the minimization of the CVD risk requires the simultaneous optimization of all antioxidant micronutrients and possibly of other synergistic nutrients as well.

General discussion and synopsis

Observational and other evidence for optimum plasma levels of antioxidant micronutrients

Considering the approximate order of standard deviation and biological variation, the available observational data permit the tentative recommendation for optimum plasma levels (thresholds to no or minimal relative risk).

Vitamin C >50 to 60 μmol/L (>0.86 to 1.00 mg/dL). This level varies in the lower half of the range of tissue saturation.[101,108,111,112] It is practically identical with that

of minimal cancer risk[4] and that needed for optimum health in other respects, e.g., for the normalization of the hydroxyproline and proline status in reducing the relative risk of periodontal disease.[207,208] The steady rise of plasma vitamin C from 31 ± 5 μmol/L to >54 μmol/L by vitamin C supplements prevents the acute rise of TBARs in plasma and LDL, respectively, due to smoking six cigarettes in 90 min, and simultaneously LDL ex vivo from vitamin C supplementors is resistant to the in vitro modification by smooth muscle cells which results in foam cell formation.[177] Tissue saturation may also be required for optimal immune functions or cataract prevention.[208] Prevention of copper-induced LDL oxidation in vitro becomes significant at a minimum of 40 μmol/L and complete at 60 μmol/L.[209] Correspondingly, 50 μmol/L of vitamin C prevent the damage to proteins by ROS from a superoxide generating system in vitro.[210]

Vitamin E >30 μmol/L (>12.9 mg/L) at common plasma lipids (220 mg/dL of cholesterol and 110 mg of triglycerides/dl) and an α-tocopherol/cholesterol ratio >5.2 (4.8 to 5.6) μmol/mmol, respectively. This plasma status has also been associated with minimal cancer risk[4] and "normal" breath pentane, i.e., of an in vivo indicator of lipid peroxidation in man.[211] The rise of plasma vitamin E from 14 ± 2 μmol/L (with an α-tocopherol/cholesterol ratio of 3.2 ± 0.5) to >35 μmol/L (α-tocopherol/cholesterol ratio >7.8) by vitamin E supplements prevents the acute rise due to acute heavy smoking of TBARs in plasma and LDL. Moreover, LDL ex vivo, from subjects supplemented with vitamin C or E, is resistant to smooth muscle cell–induced modification in vitro which results in foam cell formation.[177]

β-carotene >0.40 μmol/L (>0.22 mg/L) or α- and β-carotene >0.50 μmol/L (>0.27 mg/L). This risk threshold is practically identical with that of minimal cancer risk.[4] β-carotene status with plasma levels of >0.8 μmol/L was needed to minimize x-ray induction of micronuclei in lymphocytes ex vivo.[212]

Health benefits of vegetable-oriented diets mediated by essential antioxidants

The above reviewed observational data, if taken together with supplementation data, provide strong evidence that even within a health-oriented life style a suboptimal status of the principal essential antioxidants, either singly or in combination, can be an important, but correctable risk factor of CVD.

The principal antioxidant micronutrients form, of course, only a small portion of health-protective special diets. Plants contain many more antioxidants that deserve to be explored in more detail, i.e., on one hand carotenoids other than β-carotene, e.g., lycopene, lutein, β-cryptoxanthin, and on the other hand a series of hydroquinone derivatives, e.g., ubiqinol-10, and the bulk of hundreds of common phenols, bioflavonoids, and anthocyanins (approximately at least 1 g daily) which are not only strong antioxidants but also potent metal chelators.[213] Crucial health-protective potentials are also conceivable for some radical-sensitive B vitamins, e.g., folate, pyridoxine, and riboflavin as required for one carbon transfer reactions. Low plasma levels of folate, pyridoxine, and cobalamine are inversely correlated with the plasma status of homocysteine, which is associated with a strongly increased risk factor of CVD, and supplements optimize plasma homocysteine.[82,202,203] Homocysteine can modify lipoproteins, endothelial and other cells,[202,214] but is counteracted by reaction with nitrogen oxide–related endothelium-derived relaxing factor,[215,216] the production of which is known to be reduced in experimental atheromatosis and by oxidatively modified LDL. Cigarette smoking tends to lower plasma folate and particularly cellular folate.[217] Folate is known to require antioxidant protection[101] and can be spared in humans by vitamin C.[65,76] Riboflavin is involved in the permanent glutathione regeneration by glutathione reductase and might have been underestimated in its reactivity toward peroxy radicals.[218] Riboflavin seems to be of great importance in the prevention of nuclear cataracts in the elderly,[199,201,219] as a marker of the aging-related and UV-induced photo-oxidation processes involving ROS. Important constituents other than antioxidants in plants are, of course, fibers, such as from whole grain and pectins, minerals, such as potassium, magnesium, and selenium, the monoenic oleic acid and PUFAs, particularly of the n-3 type, special leguminous proteins, unrefined carbohydrates, special sulfur-compounds in broccoli, cabbage, garlic, etc. As for an optimally composed "functional" diet, all potentially health-protective plant constituents will have to be explored further not only in univariate analysis but by also seeking synergistic interactions.

Possible CVD-protective mechanisms of vitamins C, E and carotene

Cells strive to maintain a reducing environment, keeping glutathione, vitamins C and E, pyridine nucleotides, etc. largely in the reduced state. In blood vessels prone to CVD, however, a pro-oxidative state may predominate, at least at certain stages of atherogenesis and/or compartments (as repeatedly reviewed[1,3,19,23,27,29,220,221]) since

(i) oxidatively modified LDL occurs in arteriosclerotic arteries of animals and men,
(ii) oxidized lipids (epoxides and peroxides of cholesterol and PUFAs) accumulate in arteriosclerotic plaque.
(iii) highly oxidized lipoproteins ("ceroid") form insoluble complexes within foam cells where they may become a catabolic problem leading to plaques, and
(iv) increased antibody titers have been described in the plasma of patients with periaortitis and may also occur in advanced peripheral arteriosclerosis.

The inverse associations between clinical CVD and the intake and plasma levels of antioxidant micronutrients provide additional indirect evidence that an imbalance in pro-oxidants/antioxidants ("oxidative stress") is mechanistically involved in arteriosclerosis. The previously reviewed data[2,3] suggest that antioxidants could counteract some risk factors for CVD, as well as almost all crucial stages of atherogenesis.

I. Plasma parameters related to CVD
   A. "Electronegative" LDL (as found in CVD patients) is attenuated by vitamin E in vivo
   B. TBARs (a marker of plasma oxidizability), being increased in smokers, diabetics, hyperlipemia, and CVD patients) are decreased by vitamins C and/or E in vivo
   C. Lp(a) (an independent strong risk factor for CHD) can be diminished in vivo by vitamin E and vitamin C
   D. Glycated proteins (an LDL modification that can occur in diabetics and induces foam cell formation without oxidative LDL modification) is attenuated by vitamin E in vivo
   E. HDL_3 (associated with lower CHD risk) can be increased by vitamin E in vivo although not in all subjects
   F. Transition metals (copper and iron catalyze the formation of the most aggressive hydroxyl radicals in the metal-driven Fenton reaction, or decompose inert hydroperoxy compounds to peroxy and alkoxy radicals) are kept in a reduced state and chelated by optimal levels of vitamin C in vitro, and iron- and copper-induced toxicity is counteracted in vivo by vitamins E and C. The activity of the copper-carrier ceruloplasmin is diminished by vitamin C
   G. Oxidation resistance of plasma to all kinds of ROS depends primarily on vitamin C which thus forms the first line of antioxidative defense
   H. Resistance of isolated LDL to (copper-induced) oxidation, i.e., the crucial action demanded by Steinberg's LDL Oxidation Hypothesis[19,20] (correlating with arteriographically quantified CHD to only 20%[97]) is not always predictable by the α-tocopherol concentration in LDL but can in vivo be improved markedly by supplements of vitamin E,[27] probucol, or ubiqinol-10 but not by β-carotene[175] (although the latter is inversely correlated to CVD)

II. Blood pressure
   A. Vitamin C may have modest hypotensive properties[110,222]

III. Fatty streaks (the first although still partially reversible plaque stage)
   A. Endothelial modulation/dysfunction (the initial cellular event in atherogenesis)
      - Uptake of oxidatively modified LDL is reduced by vitamin E in vitro and in vivo
      - Injury due to oxygen radicals, oxidatively modified LDL, or monocytes/macrophages is diminished by vitamin E in culture and in vivo, and by vitamins C and E, respectively, in vivo
      - Endothelial barrier function is preserved by vitamin E in vitro, i.e., the pathological increase in lipoprotein influx is prevented
      - Calcium release from storage sites (a signal transducing mechanisms) is attenuated by vitamin E in vitro
      - Phospholipase A_2 (the hydrolytic prerequisite of eicosanoid formation) is inhibited by vitamin E in vitro and in vivo
      - Prostacyclin (the crucial vasodilatory and antithrombotic eicosanoid) is increased by vitamin E in normal endothelium in vivo, and the reduced prostacyclin production in atherosclerotic endothelium is restored by vitamin E whereas thromboxane (the antagonist of prostacyclin) is unaltered or depressed. Vitamin C acts in the same way in vitro and vivo
      - Lipoxygenases (forming "inflammatory" leukotrienes) are inhibited by vitamin E in vitro and in vivo, by vitamin C directly and/or indirectly, and possibly by β-carotene/vitamin A in vitro
      - Endothelium-induced LDL modification is reduced by vitamin E in vitro
      - Monocyte adhesion to cultured endothelial cells is decreased by vitamin E
   B. Proteoglycan structure (coresponsible for arterial filtration rate, subendothelial electronegative charges, adhesion phenomena): control of biosynthesis in vivo by vitamins A, C, and E
   C. Immunological/inflammatory responses
      - Improvements, e.g., of mitogenic response of T-cells ex vivo by vitamins A, C, E, and β-carotene
      - Prostaglandin production and lipoxygenase is inhibited in immune cells by vitamin E ex vivo, and by vitamin C
      - IL-2 production by mononuclear cells is increased by vitamin E ex vivo or inversely related to low vitamin E intake, depending on challenging mitogen
   D. Monocytes/macrophage
      - ROS-induced damage is reduced by vitamin E in vitro
      - Chemotactic response and migration is improved in vitro by vitamins A, C, and E
      - Phagocytic functions are improved by vitamin E ex vivo
      - Respiratory burst is reduced by vitamin E ex vivo
      - Monocyte/macrophage-challenged LDL oxidation is reduced in vitro by vitamin E
      - Secretion of proteolytic enzymes is reduced by vitamin E
      - Intracellular accumulation of cholesteryl esters, ceroid, and chemoluminescent lipid peroxides is counteracted by vitamin E in vitro and in vivo. Thereby vitamin E can activate cholesteryl ester hydrolysis, inhibit ACAT ex vivo, particularly attenuate the intracellular lipid peroxidation in vitro with corresponding deactivation of lysosomal enzymes ex vivo. In consequence, vitamin E reduces the secreted catabolites of oxidatively modified ApoB
      - Lipoxygenase can be inhibited by vitamin E
      - IL-1 expression is prevented by vitamin E in vitro and ex vivo
      - Protein kinase C activation can be prevented by vitamin E ex vivo

IV. Fibrous plaques/smooth muscle cell proliferation
   A. Smooth muscle cells
      - Proliferation is inhibited by vitamin E in vitro parallel to reduced activation of protein kinase C

- Calcium efflux is attenuated by vitamin E in vitro
- Lipoxygenase is inhibited by vitamin E in vitro
- Cholesteryl ester accumulation can be counteracted by vitamin E in vitro

B. Fibroblasts: prostaglandin synthesis is stimulated by vitamin E in vitro

V. Myocardial infarction

A. Arrythmia/myopathy
- Catecholamine-provoked effects are attenuated by vitamin E in vivo
- Occlusion-induced fibrillations can be decreased by vitamin E in vivo
- Left ventricular function abnormalities are improved by vitamin E in vivo

B. Platelets
- Adhesion onto collagen, fibronectin, fibrinogen is prevented by vitamin E ex vivo
- Aggregation will be inhibited by vitamin E, but probably only when the vitamin E status is poor or in contraceptive-using women
- Prostacyclin production is increased by vitamin E in vivo
- Thromboxane formation is inhibited when vitamin E status is poor
- Lipoxygenase is inhibited by vitamin C in vitro and in vivo
- PAF production in polymorphonuclear leucocytes is reduced by vitamin E in vivo

C. Coagulation factors
- Fibrinogen level can be decreased by vitamin E in vivo, fibrinolytic activity increased by vitamin C
- Antithrombotic proteoglycans are maintained by vitamin E in vivo
- Aggregatory factor synthesis is inhibited by vitamin E in vitro

D. "Reperfusion injury": infarct area, tissue damage, and peroxide level are decreased by vitamins E and/or C in vitro and in vivo

In conclusion, at present the preventive potentials of antioxidant micronutrients cannot be fully ascribed to any particular mechanism. Many experimental data would suggest that antioxidant micronutrients can prevent the initiation and/or delay the promotion of CVD. This view may be supported by the fact that on the one hand CVD remains the major cause of death in all westernized communities, but that on the other hand premature CVD (recorded as age-standardized morbidity and mortality in subjects 35 to 64 years of age) reveals up to 6 fold differences, at least in CHD, which are inversely related to essential antioxidants.

Steinberg's Hypothesis of Oxidative LDL Modification[19,20] inspired many important studies on initial antioxidant actions which may be related to the initiation of foam cell formation. However, it should be remembered that any primary role of the oxidative LDL modification involving scavenger receptors of monocytes/macrophages remains unproven.[19,20,22,23,29] The inhibition of atherosclerosis in the hyperlipemic WHHL rabbit by the antioxidant drug probucol is not reflected by an arterial decrease of oxidatively modified LDL.[39] Any antiatherosclerotic effects of β-carotene cannot be explained by the inhibition of LDL oxidation[175] but rather may be related to the known effects of β-carotene on cell differentiation and/or cell–cell communication.[204] Foam cells can also result from unregulated uptake of several other nonoxidative LDL modifications, e.g., by aggregation, "precipitation" by acidic arterial mucopolysaccharides (e.g., chondroitin, dermatan, and heparan sulfate), glycation, desialation, antibody interaction, and digestion and/or aggregation with phospholipase C.[19] The earliest subendothelial event seems to be the extracellular accumulation of aggregated, reassembled, but presumably oxidation-prone LDL.[223–226] The focal sequestration of LDL in arterial lesion may be due to oxidation-independent changes of charge and polarity of LDL,[227] possibly as a consequence by a prolonged intra-arterial transit and/or local shear and tear forces.

In animal experiments, vitamin E counteracts pathobiochemical steps such as monocyte adhesion to the endothelium and monocyte transmigration induced by LDL,[228] as well as the LDL accumulation in plaques and foam cells.[229] Presumably hand in hand, either vitamin E or β-carotene preserve also the endothelium-dependent vasodilatation in hyperlipemia,[230] i.e., another hallmark of arteriosclerotic arteries. Cellular benefits from antioxidants, e.g., on endothelial physiology[231] or on the intracellular catabolism of LDL modifications by macrophages,[232,233] could be at least as important as the prevention of LDL oxidation. It should be noted that physiological amounts of ROS can trigger transcription factors of gene expression, whereas antioxidants can counteract such ROS-modulated gene expressions.[2,6,10,16–18,234–237] The antioxidant prevention of very subtle oxidized, "minimally modified" LDL which does not provoke scavenging by macrophages (but might be TBAR related) could nevertheless abolish a variety of atherogenic cell responses. Thus, the endothelium responds even to very minimal modifications in LDL with regard to monocyte adhesion (mediated by expression of special adhesion molecules), production of monocyte-colony stimulating factor, loss of vasodilatation, etc.[238,239] The crucially important vascular transcellular signaling[240–243] is modulated by antioxidants on the level of eicosanoids,[3,244–246] of cytokines[246] and gap junctional communication.[247] The fact that antioxidants can modify gene expression of a cytokine as important as interleukin-1 in cell culture,[30] and of interleukin-2 in vivo[246] has been highlighted by Jackson's Cytokine Hypothesis.[30] All this might well incorporate antioxidants into Ross' updated version of Virchow's Inflammatory Response-to-Injury Hypothesis,[26] particularly when it is appreciated that antioxidants can improve several immunoresponses.[248–250] Antioxidants might thus in part even fit another main theory, i.e., Benditt's Monoclonal Hypothesis. But since no specific theory on initial or promotional steps in atherogenesis has been proven or discarded, it may be premature to link individual antioxidant actions with possible benefits on the chronic CVD. In this 10-year retrospective it still seems reasonable to adhere to the "Universal" Antioxidant Hypothesis of Arteriosclerosis,[1–3] which focuses primarily on pre- or subclinical protective potentials of antioxidants rather than on their specific protective mechanisms.

Geographical differences

The presently available data indicate gross differences as follows:

- Linxian/Northern China: Poor status of all essential micronutrients, some nearby deficiency, which after correction by multivitamin/mineral supplements for longer follow-up periods is likely to reveal statistically significant reduction of stroke mortality.[199,201] The effect of relatively small supplements on plasma levels is striking.
- USA: Large segments of the population have a poor vitamin C status,[111,146,148–155,158,251,252] the general population is critically low in vitamin E,[107,144,145,149,151–153,159,161,169,172,197,251,252] and smokers are particularly inadequate regarding β-carotene.[107,145,159] Suboptimal plasma levels, at least in part resulting from a frequently poor intake, are only correctable by relatively large supplements of vitamins C and E as well as β-carotene within multivitamin preparations.[145,146,159] Smokers may have an increased requirement for all principal antioxidants,[159] but possibly other micronutrients also becoming suboptimal due to the smokers' life style.[195] The amazingly weak plasma response to supplements of all principal antioxidants awaits elucidation.
- Scotland/England: Critically poor mean status in the general population, clearly exaggerated by smoking.[51,84,98,100,113,179] Plasma/intake response for β-carotene and vitamin C in smokers is as weak as in the USA, but that of vitamin E comparable to other European populations.
- Finland: Traditionally low in vitamin E, previously also poor in β-carotene and vitamin C, which have partially improved in the last years;[51,127,133,181] reports on preventive potentials of supplementation with vitamin E and/or β-carotene in smokers regarding cancer are available,[253] those on CVD are under scrutiny.
- Germany/Switzerland: Fair to optimal status of all antioxidants in non-smokers,[14,51,52,121,129,157] but at least approximately one out of four smokers has suboptimal levels of vitamin C and β-carotene, both at or below the borderline of CVD protection.[51,125] Low levels seem, however, to respond to an adequately adjusted intake both in smokers and nonsmokers.[157]
- France, Spain, Italy, probably most communities of Southern Europe, and "olive latitudes" consuming the Mediterranean diet: as far as measured,[51] mostly fair status of all principal antioxidants in nonsmokers, and smokers possibly being relatively better off than in Germany/Switzerland. Unraveling the mysteries of the Mediterranean diet, and contrasting them to the diets of Northern Britain, New England, and other parts of the US, is a worthy goal for preventive nutrition.

Brillat-Savarin postulated (Physiologie du goût, 1825): "La destinée des nations dépend de la manière dont elles se nourissent," i.e. "We are what we eat"/"Der Mensch ist, was er isst." Because gene expression is modulated by ROS, but can be balanced by antioxidants,[2,6–10,16–18,234–237] the realization of genetic potentials may indeed, at least in part, depend on food preference. For people who do not have five servings of fruit/vegetables and adequate amount of vitamin E carriers, there is a great opportunity to improve health by adequate consumption.

Regular dietary supply of antioxidants, food fortification, or vitamin supplementation

Vitamins C and β-carotene are available from fruits and/or vegetables, and vitamin E from vegetable oils with a high net vitamin E (or more simply a high α-tocopherol/PUFA ratio). Southern European communities exemplify the fact that optimal plasma levels of these antioxidants can easily be achieved by regular local diets characterized by a great variety of items, preference for fresh products, frequent consumption of fruit/vegetables/legumes and oils with their fair net vitamin E content. In contrast, major parts of populations in the USA or in the Northern parts of Europe obviously do not consume antioxidant nutrients in protective amounts. The availability of very convenient, lower-priced, storable, prefabricated, or industrially modified foods in the US seems clearly to militate against the consumption of freshly prepared native food of all kinds (still the choice of the French/Italian cuisine). Thus, only 10% of Americans follow the advice of five servings of fruit/vegetables daily,[251] only a quarter has fruits or vegetables rich in vitamin C or carotenoids, and 41% had no fruits or vegetables on the survey day.[252] A recent reanalysis (including fortified foods) of NHANES II data showed that vitamin supplements are the major contributors of the principal antioxidant micronutrients in the US diet (28% of vitamin C and 46% of vitamin E).[153] This fact hardly meets with the basic concept of previous dietary recommendations.[5] Even more importantly, only special vitamin supplements were the effective means of exceeding an intake of 100 mg of vitamin C daily (as desirable according to the mortality follow-up of NHANES I) and to achieve at least 33 IU of vitamin E (a third of the selfsupplemented 100 IU[153] which reduced the CHD risk of Health Professionals).[159]

The promise of both selfsupplementation studies in the US is that the antioxidant-related CVD risk can in principle be prevented, e.g., by supplements as part of health-oriented life style. Antioxidant supplements could, of course, be promoted as a preventive measure (although the doses of antioxidants in multivitamin preparations may require upgrading to that of special vitamin supplements).[153] Nutritionist and spokesmen of Public Health medicine are, however, rather hesitant to recommend supplements to the general population. But if nutritional education continues to fail in reaching nearly everyone, food fortification could become the best alternative, legislation permitting. Since food fortification has been employed to prevent overt clinical deficiency syndromes, e.g., of vitamins A, B_1, and D, an extension toward Optimum Health (WHO) seems logical. A minimum goal may be the elimination of most striking nutritional imbalances by making good the crucial antioxidant losses in processing, preservation procedures, and/or during the growing shelf-life of food products. No technical problems should hinder antioxidant supply to achieve the desirable plasma levels. Fortified food has already made minor although variable contributions to the

problem intake of antioxidant in the US, i.e., 10% of vitamin C (mainly from fruit drinks) in white people, and 26% in black people, 10% of vitamin E (from instant breakfast bars) in whites alone, but only <1% of β-carotene thus far.[153]

If CVD prevention should begin early, as the consensus demands, food fortification may be particularly suited to improve the antioxidant status in young adults who hardly care for nutritional quality or supplements.

Need for defining a recommended optimum intake and of food labeling

To date recommendations on micronutrient intake (RDA, RDI, Reference Intake, etc.) have primarily intended to avoid clinically overt deficiencies, e.g., scurvy, or malformations such as neural tube defects. The above-reviewed observational and supplementation data provide, however, strong and consistent evidence that the prevention of slow multistaged processes such as CVD and cancer might require a higher intake of some essential antioxidants. If any recommended intake should really refer to ". . . amounts considered sufficient for the maintenance of health for nearly all people" (FAO/WHO) the present recommendations will require either upgrading or an additional term, e.g., a recommended optimum intake (ROI) which varies, of course, with gender, age, with special requirements for smokers, "nonresponders," pregnancy, and possibly the elderly. The ROI could be defined as sufficient (culture- and/or region-specific) intake to achieve plasma levels associated with the observed minimum relative risk of disease. A ROI would simply quantify specific dietary constituents of conceivably crucial importance within the still desirable "five servings of fruit and vegetables daily."[5] As far as regional data on the effect of voluntary supplements on hard clinical end point are available, e.g., the mortality follow-up of NHANES I[146] or CHD morbidity in the Health Professionals Study,[159] they may form the most reliable justification for the ROI. Otherwise ROIs could be relatively easily established by means of the plasma antioxidant/intake response for regions with a relatively homogeneous life style, as recently done for Germany.[157] By the plasma antioxidant/intake response ROIs can also be specified for population groups and individuals. Hence, plasma values indicate the actual balance between the antioxidant intake and the requirement that can substantially vary, e.g., with factors such as life style including diet, smoking, exhaustive exercise, homeostasis, possibly genetic components, and environmental factors, e.g., ozone. With the inclusion of the antioxidant assay in plasma risk factor screening (in addition to lipoproteins, blood pressure), nutritional prevention would gain the standard of clinical medicine that presently bases diagnosis and treatment widely on plasma levels such as glucose, lipoproteins, enzymes, antibodies, etc. ROIs would have to be oriented to the most unfavorable conditions within the given community. For instance, US ROIs would have to refer to male smoking Americans in the selfsupplementation studies: >130 mg of vitamin C,[146] >100 IU of vitamin E,[159] and >9 mg of β-carotene[159] daily. At the other extreme, 85% of Germans may be equally well provided by optimum plasma levels due to ROIs of >132 mg of vitamin C, >36 IU of vitamin E, and >3 mg of β-carotene daily.[157] Differences as impressive as for vitamin E and β-carotene seem to exclude world-wide ROIs and those for continents, at least for Europe.

Interim guidelines on optimum antioxidant plasma levels will await either reconfirmation or revision, e.g., by future conclusive data on moderate supplements. Unfortunately ROIs can hardly be deduced from all ongoing intervention trials, since the latter are often designed for yes-or-no answers to abundant doses of one single "magic bullet" micronutrient, or at best of a few combined. Negative outcomes of intervention trials could also be inconclusive if benefits of test compounds depend strongly on synergistic interactions with other micronutrients or if the "too late" problem was not adequately considered in the design.

Any updated recommendations on intake can, of course, only become effective if combined with public education and regulatory support. Food labeling (as for cholesterol, for the constituents of breakfast cereals, or of fortified milk) may also help. The label should indicate the minimal content of principal antioxidant micronutrients and concurrent vitamins and minerals, and for vitamin E particularly the net amount as well as the absolute amount of PUFAs (rather than the α-tocopherol/PUFA ratio). Scientifically based regulation on food constituents may also help to minimize compounds that increase the antioxidant requirement (such as lipid peroxides using up vitamin E), and to reconstitute antioxidant vitamins and other micronutrients as far as lost by processing and storage.

Outlook toward antioxidative intervention trials: Need of early prevention

Health-maintaining properties of antioxidant micronutrients can only be proven by randomized and rigidly controlled intervention trials in adequate study populations of high risk but initially poor antioxidant status. Several studies on CHD prevention are in preparation since an NHLBI Consensus Conference recommended testing a combination of vitamins C and E with β-carotene.[135] However, the hitherto known projects have not perceived all problems of the study population's adequacy. Conclusiveness was apparently sacrificed for cost sharing. A NCI/NHLBI-supported design for primary prevention of mamma carcinoma (although valuable in itself) with menopausal, frequently estrogen-substituted women is a less-than-ideal study population to scrutinize the antioxidant benefits on CVD. When testing antioxidants for the first time in males, any concurrent drug testing seems premature because of antioxidant properties of all cardiaca.[86–96] Biostatistical models can hardly unravel multiple, biologically complex interactions in multifactorial study designs, particularly when unforeseen adverse effects appear.[253] Amazingly, no clear-cut specific project on primary prevention in younger/middle-aged men (ideally with fortified food items) has found support. But an Oxonian project is going to recrute the "general population" all over Britain (despite regional variation of the antioxidant status[113]), with admission of age categories 40–79 years, and even of overt CHD. In such a heterogeneous study population, hard clinical end points will almost exclusively be due

to most advanced and complicated CHD. There has been general concern that beginning CVD prevention in middle age is "too little too late."[254,255] More specifically, conclusive experimental evidence is missing that antioxidants can reverse progressed arteriosclerotic lesions, as reported for cholesterol lowering. In this situation it may be important to learn from disappointing results on β-carotene in primary and secondary cancer prevention. First, in Americans with prevalent nonmelanoma skin carcinoma the occurrence of secondary cancers was not prevented by excess supplements with β-carotene.[256] Second, in Chinese with esophageal dysplasia, its progression was not inhibited by optimized antioxidant plasma levels (by multivitamin/mineral supplementation),[201] whereas in the general population equivalent supplements (particularly of the combination β-carotene/vitamin E/selenium) clearly counteracted stages earlier than dysplasia, i.e., initiation and promotion of carcinogenesis.[199] This finding agrees with reports that precancerous lesions can be controlled by β-carotene under experimental conditions and in study populations of different races.[10,204–206] Third, in middle-aged Finnish smokers the clinical appearance of primary carcinoma was not prevented by β-carotene and/or vitamin E, and some prevalent, but at baseline undiagnosed, small cancer cell clones possibly progressed even faster under the antioxidant supply.[253] Assuming that an optimized antioxidant status mainly counteracts initial and reversible cell abnormalities (as presumable for cancer at least), no material benefit from antioxidants can be expected in late, complicated arterial disease or for secondary CVD prevention. It would be a tragedy if any negative results (to be expected from inadequately conceived secondary prevention-type trials in advanced CHD) were to be used as a justification for not mounting adequate primary prevention trials of antioxidants. The present state-of-the-art only recommends early prevention.

Acknowledgments

The observational data in this paper have only been obtained thanks to the collaboration with many experts and reliable principal investigators, i.e., in the cross-cultural Vitamin Substudy of the WHO/MONICA Project (initiated as the International Collaborative Study on the Fatty Acid-Antioxidant Hypothesis of Arteriosclerosis) with G. Alfthan, I. Balaguer-Vintro, F. Bernasconi, C. Bolton-Smith, D. Brunner, E. Bühler, J.-P. Cambou, C. Casey, I.M. Eckstein, A. Evans, F. Fidanza, P. Fuchs, H. Georgi, H. Heine, L. Heinemann, P. Jordan, K. Klarlund, M. Mancini, U. Moser, C. Morrison, R. Muggli, J. Müller, T. Nikkari, M.F. Oliver, P. Puska, R.A. Riemersma, G. Ritzel, P. Rubba, J.-B. Ruidavets, M. Salo, S. Sans, M. Schroll, W.C.S. Smith, H. Tunstall-Pedoe, E. Vartiainen, C. Wagenknecht, and D.A. Wood; in the Edinburgh Case-Control Study on Previously Undiagnosed Angina Pectoris with R.A. Elton, M.F. Oliver, R.A. Riemersma, and D.A. Wood; in the Basel Prospective Study with F. Bernasconi, G.B. Brubacher, M. Eichholzer, E. Lüdin, and H.B. Stähelin; in all studies the antioxidant assays provided by F. Hoffman-La Roche, Ltd., Basel, Switzerland, as generous technical support, and carried out with great expertise initially by J.-P. Vuilleumier, and more recently by W. Schüep. Discussion with I. Gyarfas, WHO, Geneva, Switzerland, as well as with G.B. Brubacher, C.E. Reilly, H.B. Stähelin, Basel, Switzerland, and particularly with A. Evans, Belfast, Northern Ireland, have also proved most helpful.

Note added in proof: In discussing the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study[253] (quoted above as "too late" for primary prevention) the authors emphasized that poor plasma antioxidants at base line predicted in the placebo group the subsequent cancer risk (as in previous prospective studies), whereas the relatively short-termed rectification of only two antioxidants did not attenuate the presumably fairly advanced lung cancer and CVD in chronic heavy smokers. Thus, the "lifelong" antioxidant supply through the common but complex, balanced diet was clearly superior to supplementing two antioxidants shortly before death.[257,258] A recent re-evaluation of stroke mortality in the Linxian Trials[259] revealed a reduction of the relative risk to 0.45 ($P < 0.05$) by multivitamin/mineral supplementation, i.e., the latter had a significantly stronger preventive effect than combinations of a few components. These studies strongly support the point of this review that full benefits of adequacy of all antioxidants (synergistically integrated in the overall antioxidant defense potential[1]) may require the concurrent adequacy of other essential micronutrients.

References

1 Gey, K.F. (1986). On the antioxidant hypothesis with regard to arteriosclerosis. *Bibl. Nutr. Diet.* **37**, 53–91

2 Gey, K.F. (1990). The antioxidant hypothesis of cardiovascular disease: Epidemiology and mechanisms. *Biochem. Soc. Trans.* **18**, 1041–1045

3 Gey, K.F. (1993). Vitamin E and other essential antioxidants regarding coronary heart disease: risk assessment studies. Epidemiological basis of the antioxidant hypothesis of cardiovascular disease. In *Vitamin E in Health and Disease* (L. Packer, and J. Fuchs, eds.), p. 589–633, Dekker, New York, NY USA

4 Gey, K.F. (1993). Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. In *Free Radicals in Medicine* (K.H. Cheeseman, and T.F. Slater, eds.) Brit. Med. Bull. **49**(3), p. 679–699, Churchill Livingstone, Edinburgh, Scotland, for Brit. Med. Council

5 National Research Council, U.S.A., Committee on Diet and Health (1989). *Diet and Health: Implications for Reducing Chronic Disease*. National Academy of Sciences, Washington, DC

6 Cheeseman, K.H., and Slater, T.F., eds. (1993). *Free Radicals in Medicine*. Brit. Med. Bull. **49**(3), p. 479–724, Churchill Livingstone, Edinburgh, for British Medical Council

7 Sies, H., ed. (1985, 1991). *Oxidative Stress*, 1st ed. *Oxidative Stress: Oxidants and Antioxidants*, 2nd ed., Academic Press, London, UK

8 Sies, H. (1993). Strategies of antioxidative defense. *Eur. J. Biochem.* **215**, 213–219

9 Halliwell, B., and Gutteridge, J.M.C. (1989). *Free Radicals in Biology and Medicine*, 2nd ed., Clarendon Press, Oxford, UK

10 Ames, B.N., Shigenaga, M.K., and Hagen, T.M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* **90**, 7915–7922

11 Arntzenius, A.C., Kromhout, D., Barth, J.D., et al. (1985). Diet, lipoproteins and the progression of coronary atherosclerosis: The Leyden Intervention Trial. *N. Engl. J. Med.* **312**, 805–811

12 Ornish, D., Brown, S.E., Scherwitz, L.W., Billings, J.H., Armstrong, W.Z., Ports, T.A., McLanahan, S.M., Kirkeeide, R.L., Brand, R., and Gould, K.L. (1990). Can life-style changes reverse

coronary heart disease? The Lifestyle Heart Trial. *Lancet* **336,** 129–133
13 Watts, G.F., Lewis, B., Brunt, J.N.H., Lewis, E.S., Coltart, D.J., Smith, L.D.R., Mann, J.I., and Swan, A.V. (1992). Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St. Thomas' Atherosclerosis Regression Study (STARS). *Lancet* **339,** 563–569
14 Ballmer, P.E., Reinhart, W.H., Jordan, P., Bühler, E., Moser, U.K., and Gey, K.F. (1994). Depletion of plasma vitamin C but not of vitamin E in response to open heart surgery. *J. Thorac. Cardiovasc. Surg.* **108,** 311–320
15 Clemens, M.R., Ladner, C., Ehninger, G., Einsele, H., Renn, W., Bühler, E., Waller, H.D., and Gey, K.F. (1990). Plasma vitamin E and β-carotene concentrations during radiochemotherapy preceding bone marrow transplantation. *Am. J. Clin. Nutr.* **51,** 216–219
16 Allen, R.G. (1991). Oxygen-reactive species and antioxidant response during development: The metabolic paradox of cellular differentiation. *Proc. Exp. Soc. Biol. Med.* **196,** 117–129
17 Janssen, Y.M.W., Houten, B. van, Borm, P.J.A., and Mossman, B.T. (1993). Biology of disease. Cell and tissue responses to oxidative damage. *Lab. Invest.* **69,** 261–274
18 Collins, T. (1993). Biology of disease. Endothelial nuclear factor-κB and the initiation of the atherosclerotic lesion. *Lab. Invest.* **68,** 499–508
19 Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989). Beyond cholesterol: Modifications of low-density lipoprotein that increases its atherogenicity. *New Engl. J. Med.* **320,** 915–924
20 Witztum, J.L., and Steinberg, D. (1991). Role of oxidized low density lipoproteins in atherogenesis. *J. Clin. Invest.* **88,** 1785–1792
21 Steinbrecher, U.P., Parthasarathy, S., Leake, D.S., Witztum, J.L., and Steinberg, D. (1984). Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc. Natl. Acad. Sci. USA* **81,** 3883–3887
22 Steinbrecher, U.P. Zhang, H., and Lougheed, M. (1990). Role of oxidatively modified LDL in atherosclerosis. *Free Radical Biol. Med.* **9,** 155–168
23 Ylä-Herttuala, S. (1991). Macrophages and oxidized low density lipoproteins in the pathogenesis of atherosclerosis. *Ann. Med.* **23,** 561–567
24 Yagi, K., ed. (1982). *Lipid peroxides in biology and medicine.* Academic Press, New York
25 Yagi, K. (1987). Lipid peroxides and human diseases. *Chem. Phys. Lipids* **45,** 337–351
26 Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* **362,** 801–809
27 Esterbauer, H., Gebicki, J., Puhl, H., and Jürgens. G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biol. Med.* **13,** 341–390
28 Kuzuya, M., and Kuzuya, F. (1993). Probucol as antioxidant and antiatherogenic drug. *Free Radical Biol. Med.* **14,** 67–77
29 Janero, D.R. (1991). Therapeutic potential of vitamin E in the pathogenesis of spontaneous atherosclerosis. *Free Radical Biol. Med.* **11,** 129–144
30 Jackson, R.L., Ku, G., and Thomas, C.E. (1993). Antioxidants: a biological defense mechanisms for the presention of atherosclerosis. *Med. Res. Rev.* **13,** 161–183
31 Rath, M., and Pauling, L. (1990). Immunological evidence for the accumulation of lipoprotein(a) in the atherosclerotic lesion of the hypoascorbic guinea pig. *Proc. Natl. Acad. Sci. USA* **87,** 9388–9390
32 Wojcicki, J., Rozewicka, B., Barcew-Wiszniewska, L., Samochowiec, S., Kadlubowska, S., Tustanowski, S., and Juzyszyn, Z. (1991). Effect of selenium and vitamin E on the development of experimental atherosclerosis in rabbits. *Atherosclerosis* **87,** 9–16
33 Williams, R.J., Motteram, J.M., Sharp, C.H., and Gallagher, P.J. (1992). Dietary vitamin E and the attenuation of early lesion development in modified Watanabe rabbits. *Atherosclerosis* **94,** 153–159
34 Verlangieri, A.J., and Bush, M.J. (1992). Effects of d-alpha-tocopherol supplementation on experimentally induced primate atherosclerosis. *J. Am. Coll. Nutr.* **11,** 130–137
35 Smith, T.L., and Kummerow, F.A. (1989). Effect of dietary vitamin E on plasma lipids and atherogenesis in restricted ovulator chicken. *Atherosclerosis* **75,** 105–109
36 Mao, J.T., Yates, M.T., Parker, R.A., Chi, E.M., and Jackson, R.L. (1991). Attenuation of atherosclerosis in an modified strain of hypercholesterolemic Watanabe rabbits with use of a probucol analogue (MDL 29,311) that does not lower serum cholesterol. *Arteriosclerosis* **11,** 1266–1275
37 Hodis, H.N., Chauhan, A., Hashimoto, S., Crawford, D.W., and Sevanian, A. (1992). Probucol reduces plasma and aortic wall oxysterol levels independently of its plasma cholesterol lowering effect. *Atherosclerosis* **96,** 125–132
38 Nagano, Y., Kume, N., Otani, K., et al. (1990). Probucol does not act on lipoprotein metabolism of WHHL rabbit macrophages. *Ann. NY Acad. Sci.* **598,** 503–531
39 O'Brien, K., Nagano, Y., Gown, A., Kita, T., and Chait, A. (1991). Probucol treatment affects the cellular composition but not anti-oxidized low density lipoprotein immunoreactivity of plaques from Watanabe heriditable hyperlipidemic rabbits. *Arteriosclerosis* **11,** 751–759
40 Parthasarathy, S. (1993) Evidence for an additional intracellular site of action of probucol in the prevention of oxidative modification of low density lipoprotein. Use of a new water-soluble derivative. (1992). *J. Clin. Invest.* **89,** 1618–1621
41 Wiklund, O., Mattsson, L., Björnheden, T., Camejo, G., and Bondjers, G. (1991). Uptake and degradation of low density lipoproteins in atherosclerotic rabbit aorta: Role of local LDL modification. *J. Lipid Res.* **32,** 55–62
42 Stein, Y., Stein, O., Delplanque, B., Fesmire, J.D., Lee, D.M., and Alaupovic, P. (1989). Lack of effect of probucol on atheroma formation in cholesterol-fed rabbits kept at a comparable plasma cholesterol levels. *Atherosclerosis* **75,** 145–166
43 Björkhem, I., Henriksson-Freyschuss, A., Breuer, O., Diczfalusy, U., Berglund, L., and Henriksson, P. (1991), The antioxidant Butylated Hydroxytoluene protects against atherosclerosis. *Arteriosclerosis* **11,** 15–22
44 Addis, P.B., and Warner, G.J. (1990). The potential health aspects of lipid oxidation products in food. In *Free Radicals and Food Additives,* (O.I. Auroma, and B. Halliwell, eds.), p. 77–119, Taylor & Francis, Ltd., London, UK
45 Kubow, S. (1992) Routes of formation and toxic consequencies of lipid oxidation products in foods. *Free Radical Biol. Med.* **12,** 63–81
46 Yoshida, H., and Kajimoto, G. (1989). Effect of dietary vitamin E on the toxicity of autoxidized oil to rats. *Ann. Nutr. Metab.* **33,** 153–162
47 Sevanian, A. (1993). Chemistry of fatty acids and cholesterol oxidation: toxicological implications. In *Dietary Lipids, Antioxidants, and the Prevention of Atherosclerosis* (F. Ursini, and E. Cadenas, eds.), p. 135–142, CLEUP, University Publisher, Padova, Italy
48 Giani, E., Masi, I., and Galli, C. (1985). Heated fat, vitamin E and vascular eicosanoids. *Lipids* **20,** 439–448
49 Gey, K.F., Stähelin, H.B., Puska, P., and Evans, A. (1987). Relationship of plasma vitamin C to mortality from ischemic heart disease. *Ann. NY Acad. Sci.* **498,** 110–123
50 Gey, K.F., Puska, P., Jordan, P., and Moser, U.K. (1991). Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.* **53** (suppl), 326S–334S
51 Gey, K.F., Moser, U.K., Jordan, P., Stähelin, H.B., Eichholzer, M., and Lüdin, E. (1993). Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: an epidemiological update with special attention to carotene and vitamin C. *Am. J. Clin. Nutr.* **57** (Suppl), 787S–797S
52 Gey, K.F., Brubacher, G.B., and Stähelin, H.B. (1987). Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. *Am. J. Clin. Nutr.* **45,** 1368–1377
53 Gey, K.F., and Puska, P. (1989). Plasma vitamins E and A inversely related to mortality from ischemic heart disease in cross-cultural epidemiology. *Ann. NY Acad. Sci.* **570,** 268–282
54 Gey, K.F. (1989). Inverse correlation of vitamin E and ischemic heart disease. *Int. J. Vitamin Nutr. Res.* **30** (Suppl.), 224–231
55 Riemersma, R.A., Oliver, M., Elton, R.A., Alfthan, G., Vartiainen, E., Salo, M., Rubba, P., Mancini, M., Georgi, H.,

Vuilleumier, J.P., and Gey, K.F. (1990). Plasma antioxidants and coronary heart disease: vitamins C and E, and selenium. *Eur. J. Clin. Nutr.* **44,** 144–150

56 Horwitt, M.K., Harvey, C.C., Dahm, C.H., Jr., and Searcy, M.T. (1972). Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann. NY Acad. Sci.* **203,** 223–236

57 Massey, J.B. (1984). Kinetics of transfer of α-tocopherol between model and native plasma lipoproteins. *Biochim. Biophys. Acta* **793,** 387–392

58 Thurnham, D.I. (1990). Antioxidants and prooxidants in malnourished populations. *Proc. Nutr. Soc.* **49,** 247–259

59 Sato, K., Niki, E., and Shimasaki, H. (1990). Free-radical mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and vitamin C. *Arch. Biochem. Biophys.* **279,** 402–405

60 Kagan, V.E., Serbinova, E.A., Forte, T., Scita, G., and Packer, L. (1992). Recycling of vitamin E in human low density lipoproteins. *J. Lipid Res.* **33,** 385–397

61 Buettner, G.R. (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol and ascorbate. *Arch. Biochem. Biophys.* **300,** 535–543

62 Sharma, M., and Buettner, G.R. (1993). Interaction of vitamin C and vitamin E during free radical stress in plasma. *Free Radical Biol. Med.* **14,** 649–653

63 Igarashi, O., Yonekawa, Y., and Fujiyama-Fujihara, Y. (1991). Synergistic action of vitamin E and vitamin E in vivo using a new mutant of Wistar-strain rats, ODS, unable to synthesize vitamin C. *J. Nutr. Sci. Vitamol.* **37,** 359–369

64 Vatassery, G.T., Smith, W.E., and Quach, H.T. (1989). Ascorbic acid, glutathione and synthetic antioxidants prevent the oxidation of vitamin E in platelets. *Lipids* **24,** 1043–1047

65 Jacob, R.A., Otradovec, C.I., and Russell, R.M. (1988). Vitamin C status and nutrient interactions in healthy elderly populations. *Am. J. Clin. Nutr.* **48,** 36–42

66 Kunert, K.J., and Tappel, A.L. (1983). The effect of vitamin C on in vivo lipid peroxidation in guinea pigs as measured by pentane and ethane production. *Lipids* **18,** 271–274

67 Chen, L.H. (1989). Interaction of vitamin C and ascorbic acid. *In Vivo* **3,** 199–210

68 Reed, D.J. (1993). Interaction of vitamin E, ascorbic acid, and glutathione in protection agains oxidative damage. In *Vitamin E: Biochemistry and clinical applications* (L. Packer, and J. Fuchs, eds). p. 269–281, Marcel Dekker, New York, NY USA

69 Tappel, A. (1993). Combinations of vitamin E and other antioxidant nutrients in protection of tissues. In *Vitamin E: Biochemistry and clinical applications* (L. Packer, and J. Fuchs, eds). p. 313–325, Marcel Dekker, New York, NY USA

70 Frei, B. (1991). Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. *Am. J. Clin. Nutr.* **54** (Suppl), 1113S–1118S

71 Frei, B.B., and Ames, B. (1991). Relative importance of vitamin E in antiperoxidative defenses in human blood plasma and low-density lipoprotein (LDL). In *Vitamin E: Biochemistry and clinical applications* (L. Packer, and J. Fuchs, eds). p. 131–139, Marcel Dekker, New York, NY, USA

72 Palozza, P., and Krinsky, N.I. (1992). β-Carotene and α-tocopherol are synergistic antioxidants. *Arch. Biochim. Biophys.* **297,** 184–187

73 Palozza, P., and Krinsky, N.I. (1992). Antioxidant effects of carotenoids in vitro and in vivo: an overview. *Meth. Enzymol.* **213,** 403–420

74 Chen, H., Pellett, L.J., Andersen, H.J., and Tappel, A.L. (1993). Protection by vitamin E, selenium and β-carotene against oxidative damage in rat liver slices and homogenate. *Free Radical Biol. Med.* **14,** 473–482

75 Bierenbaum, M.L., Reichstein, R.P., Bhagavan, H.N., and Watkins, T.R. (1992). Relationship between serum lipid peroxidation products in hypercholesterolemic subjects and vitamin E status. *Biochem. Int.* **28,** 57–66

76 Jacob, R.A., Kelley, D.S., Pianalto, F.S., Swendeid, M-E., Henning, S.M., Zhang, J.Z., Ames, B.N., Fraga, C.G., and Peters, J.H. (1991). Immunocompetence and oxidant defense during ascorbate depletion of healthy men. *Am. J. Clin. Nutr.* **54,** 1302S–1309S

77 Martensson, J., Han, J., Griffith, O.W., and Meister, A. (1993). Glutathion ester delays the onset of scurvy in ascorbate-deficient guinea pigs. *Proc. Natl. Acad. Sci. USA* **90,** 317–321

78 Johnston, C.S., Meyer, C.G., and Srilakshmi, J.C. (1993). Vitamin C elevates red blood cell glutathione in healthy adults. *Am. J. Clin. Nutr.* **58,** 103–105

79 Costagliola, C., and Menzione, M. (1990). Effects of vitamin E on the oxidative state of glutathione in plasma. *Clin. Physiol. Biochem.* **8,** 140–143

80 Burton, G.W., and Ingold, K.U. (1989). Vitamin E as in vitro and in vivo antioxidant. *Ann. NY Acad. Sci.* **570,** 7–22

81 Burton, G.W., and Ingold, K.U. (1993). Biokinetics of vitamin E using deuterated tocopherols. In *Vitamin E: Biochemistry and clinical applications* (L. Packer, and J. Fuchs, eds.), p. 329–344, Marcel Dekker, New York, NY, USA

82 Stampfer, M.J., Malinow, M.R., Willett, W.C., Newcomer, L.M., Upson, B., Ullmann, D., Tishler, P.V., and Henekens, C.H. (1993). A prospective study on plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *J. Am. Med. Ass.* **268,** 877–881

83 Jost, J.P., Simon, C., Nuttens, M.C., Bingham, A., Ruidavets, J.B., Cambou, J.P., Arveiler, D., Lecerf, J.M., Schlienger, J.L., and Douste-Blazy, P. (1990). Comparison of dietary patterns between populations samples in the three French MONICA nutritional surveys. *Rev. Epidém. et Santé Publique* **38,** 517–523

84 Bolton-Smith, C., Casey, C.E., Gey, K.F., Smith, W.C.S., and Tunstall-Pedoe, H. (1991). Antioxidant vitamin intakes assessed using food frequency questionnaire: correlation with biochemical status in smokers and non-smokers. *Brit. J. Nutr.* **65,** 337–346

85 McClean, R., McCrum, E., Scally, G., McMaster, D., Patterson, C., Jackson, N., and Evans, A. (1990). Dietary patterns in the Belfast MONICA Project. *Proc. Nutr. Soc.* **49,** 97–305

86 Weglicki, W.B., Mak, I.T., and Simic, M.G. (1990). Mechanisms of cardiovascular drugs as antioxidants. *J. Mol. Cell. Cardiol.* **22,** 1199–1208

87 Nègre-Salvayre, A., and Salvayre, R. (1992). Protection of Ca^{2+} channel blockers (nifedipine, diltiazem and verapamil) against the toxicity of oxidized low density lipoprotein to cultured lymphoid cells. *Brit. J. Pharmacol* **107,** 738–744

88 Mazière, C., Auclair, M., and Mazière, J.C. (1992). Lipophilic β-blockers inhibit monocyte and endothelial-cell mediated modifications of low density lipoproteins. *Biochim. Biophys. Acta* **1126,** 314–318

89 Sobotka, P.A., Brottman, M.D., Weitz, Z., Birnbaum, A.J., Skosey, J.L., and Zarling, E.J. (1993). Elevated breath pentane in heart failure reduced by free radical scavenger. *Free Radical Biol. Med.* **14,** 643–647

90 Kempen, H.J.M., Vermeer, M., DeWit, E., and Havekes, L.M. (1991). Vastatins inhibit cholesterol ester accumulation in human monocyte-derived macrophages. *Arteriosclerosis* **11,** 146–153

91 Aviram M., Dankner, G., Cogan, U., and Brook, J.G. (1992). Lovastatin inhibits low-density lipoprotein oxidation and alters its fluidity and uptake by macrophages: in vitro and in vivo studies. *Metabolism* **41,** 229–235

92 Giroux, L.M., Davignon, J., and Naruszewic, M. (1993). Simvastatin inhibits the oxidation of low-density lipoproteins by activated human monocyte-derived macrophages. *Biochim. Biophys. Acta* **1165,** 335–338

93 Naruszewicz, M., Mirkiewicz, E., and Klosiewiczf-Latoszek, L. (1989). Modification of low-density lipoproteins from hypertriglyceridemic patients by macrophages in vitro and the effect of bezafibrate treatment. *Atherosclerosis* **79,** 261–265

94 Croft, K.D., Dimmitt, S.B., Moulton, C., and Beilin, L.J. (1992). Low density lipoprotein and oxidizability in coronary disease—apparent favourable effect of beta blockers. *Atherosclerosis* **97,** 123–130

95 Halliwell, B. (1991). Drug antioxidant effects. A basis for drug selection? *Drugs* **42,** 569–605

96 Lavy, A., Brook, G.J., Dankner, G., Amotz, A.B., and Aviram, M. (1991). Enhanced in vitro oxidation of plasma lipoprotein derived from hypercholesterolemic patients. Metabolism **40,** 794–799

97 Regnström, J., Nilsson, J., Tornvall, P., Landou, C., and Ham-

sten, A. (1992). Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet* **339,** 1183–1186

98 Riemersma, R.A., Wood, D.A., Macintyre, C.C.A., Elton, R.A., Gey, K.F., and Oliver, M.F. (1991). Risk of angina pectoris and plasma concentrations of vitamins A, C and E and carotene. *Lancet* **337,** 1–5

99 Crombie, J.K., Smith, W.C.S., Tavendale, R., and Tunstall-Pedoe, H. (1990). Geographic clustering of risk factors and lifestyle for coronary heart disease in the Scottish Heart Health Study. *Brit. Heart J.* **64,** 199–203

100 Duthie, G.G., Shortt, C.T., Robertson, J.D., Walker, K.A., and Arthur, J.R. (1992). Plasma antioxidants, indices of lipid peroxidation and coronary heart disease risk factors in a Scottish population. *Nutr. Res.* **12** (suppl. 1), S61–S67

101 Basu, T.K., and Schorah, C.J. (1982). Vitamin C in Health and Disease. Croom Helm, London, and AVI Publ. Co., Westport, CT USA

102 Church, D.F., and Pryor, W.A. (1985). Free radical chemistry of tobacco smoke and its toxicological implications. *Environm. Health Perspect.* **64,** 11–126

103 Frei, B., Forte, T.M., Ames, B.N., and Cross, C.E. (1991). Gas phase oxidants of cigarette smoke induced lipid peroxidation and changes in lipoprotein properties in human blood plasma. *Biochem. J.* **277,** 133–138

104 Anderson, R., and Theron, A.J. (1990). Physiological potential of ascorbate, β-carotene and α-tocopherol individually and in combination in the prevention of tissue damage, carcinogenesis and immune dysfunction mediated by phagocyte-derived reactive oxidants. *Wld. Rev. Nutr. Diet.* **62,** 27–58

105 Kalra, J., Chadhary, A.K., and Prasad, K. (1991). Increased production of oxygen free radicals in cigarette smokers. *Int. J. Exp. Path.* **72,** 1–7

106 Tunstall-Pedoe, H., Smith, W.C.S., and Tavendale, R. (1989). How-often-that-high graphs of serum cholesterol. Findings from the Scottish Health Heart and Scottish MONICA Studies. *Lancet* **i,** 540–542

107 Stryker, W.C., Kaplan, L.A., Stein, E.A., Stampfer, M.J., Sobner, A., and Willett W.C. (1988). The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am. J. Epidemiol.* **127,** 283–296

108 Nobile, S., and Woodhill, J.M. (1981). *Vitamin C.* MTP Press, Lancaster, PA USA

109 Committee for the Revision of the Dietary Standard for Canada, Bureau of Nutritional Sciences, Food Directorate, Health Protection Branch, Department of Health and Welfare (1983). *Recommended Nutrient Intakes for Canadiens.* Minister of Supply and Services Canada, Ottawa.

110 Simon, J.A. (1992). Vitamin C and cardiovascular disease: a review. *J. Am. Coll. Nutr.* **11,** 107–125

111 Smith, J.L., and Hodges, R.E. (1987). Serum vitamin C in relation to dietary and supplemental vitamin C in smokers and nonsmokers. *Ann. NY Acad. Sci.* **498,** 144–152

112 Kallner, A.B., Hartmann, D., and Hornig, D.H. (1981). On the requirement of ascorbic acid in man: steady-state turnover and body pool in smokers. *Am. J. Clin. Nutr.* **34,** 1347–1355

113 Gregory, J., Foster, K., Tyler, H., Wiseman, M. (1990). *The Dietary and Nutritional Survey of British adults.* A survey of the dietary behaviour, nutritional status and blood pressure of adults aged 16 to 64 living on Great Britain. Commissioned jointly by the Ministry of Agriculture, Fisheries and Food, and the Department of Health. HMSO, London UK

114 Donna, P.T., Thompson, M., Fowkes, F.G.R., Prescott, R.J., and Housley, E. (1993). Diet as a risk factor for peripheral arterial disease in the general population: The Edinburgh Artery Study. *Am. J. Clin. Nutr.* **57,** 917–921

115 Smith, F.B., Lowe, G.D.O., Fowkes, F.G.R., Rumley, A., Rumley, A.G., Donnan, P.T., and Housley, E. (1993). Smoking, haemostatic factors and lipid peroxides in a population case control study of peripheral arterial disease. *Atherosclerosis* **102,** 155–162

116 Salonen, J.T., Salonen, R., Seppänen, K., Kantola, M., Parviainen, M., Alfthan, G., Mäenpää, P.H., Taskinen, E., and Rauramaa, R. (1988). Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischemic heart disease in Eastern Finnish men. *Atherosclerosis* **70,** 155–160

117 Kok, F.J., VanPoppel, G., Melse, J., Verheul, E., Schouten, E.G., Kruyssen, D.H.C.M., and Hofman, A. (1991). Do antioxidants and polyunsaturated fatty acids have a combined association with coronary atherosclerosis? *Atherosclerosis* **31,** 85–90

118 Kardinaal, A.F.M., Kok, F.J., Ringstad, J., Gomez-Aracena, J., Mazaev, V.P., Kohlmeier, L., Martin, B.C., Aro, A., Kark, J.D., Delgado-Rodriguez, M., Riemersma, R.A., Veer, P. van't, Huttunen, J.K., and Martin-Moreno, J.M. (1993). Antioxidants in adipose tissue and risk of myocardial infarction: the EURAMIC study. *Lancet* **342,** 1379–1384

119 Hume, E.M., and Krebs, H.A. (1949). Vitamin A requirements of human adults. An experimental study of vitamin A deprivation in man. *Med. Res. Counc. Spec. Rep. Ser.* **264**

120 Widmer, L.K., Stähelin, H.B., Nissen, C., and Silva, A. da, eds. (1981) *Venen-, Arterien-Krankheiten, koronare Herzkrankheit bei Berufstätigen. Prospektiv-epidemiologische Untersuchung Basler Studie I–III* (in German). Verlag Hans Huber, Bern, Germany

121 Stähelin, H.B., Gey, K.F., Eichholzer, M., Lüdin, E., Bernasconi, F., Thurneysen, J., and Brubacher, G. (1991). Plasma antioxidant vitamins and subsequent cancer mortality in the 12-year follow-up of the Prospective Basel Study. *Am. J. Epidemiol.* **133,** 766–775

122 Willett, W. (1990). *Nutritional Epidemiology.* Oxford University Press, New York, NY USA

123 Stähelin, H.B., Eichholzer, M., and Gey, K.F. (1992). Nutritional factors correlating with cardiovascular disease: results of the Basel Study. *Bibl. Nutr. Dieta.* **49,** 24–35

124 Rimm, E.B., Giovannucci, E., Stampfer, M.J., Colditz, G.A., Litin, L.B., and Willett, W.C. (1993). Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am. J. Epidemiol.* **135,** 1114–1126

125 Gey, K.F., Stähelin, H.B., and Eichholzer, M. (1993). Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Basel Prospective Study. *Clin. Invest.* **71,** 3–6

126 Gey, K.F., Stähelin, H.B., Eichholzer, M., and Lüdin, E. (1994). Prediction of increased cancer risk in humans by interacting suboptimal plasma levels of retinol and carotene. In *Retinoids: New From Basic Science to Clinical Application* (M.A. Livrea and G. Vivaldi, eds.), p. 137–163, Birkhäuser Verlag, Basel, Switzerland

127 Järvinen, R., Knekt, P., Heinonen, M., and Aaran, R.K. (1992). Dietary response of serum β-carotene and serum retinol. *Europ. J. Clin. Nutr.* **47,** 31–47

128 DeKeyser, J., DeKlippel, N., Merkx, H., Vervaeck, M., and Herroelen, L. (1992). Serum concentrations of vitamins A and E and early outcome after ischemic stroke. *Lancet* **339,** 1562–1565

129 Hense, H.W., Stender, M., Bors, W., and Keil, U. (1993). Lack of association between serum vitamin E and myocardial infarction in a population with high vitamin E levels. *Atherosclerosis* **103,** 21–28

130 Kok, F., DeBruijn, A.M., Vermeeren, R., Hofman, A., VanLaar, A., DeBruin, M., Hermus, R.J.J., and Valkenburg, H.A. (1987). Serum selenium, vitamin antioxidants and cardiovascular mortality: A 9-year follow-up study in the Netherlands. *Am. J. Clin. Nutr.* **45,** 462–468

131 Street, D.A., Comstock, G.W., Salkeld, R.M., Schüep, W., and Klag, M.A. (1991). A population-based case-control study of the association of serum antioxidants and myocardial infarction. *Am. J. Epidemiol.* **134,** 719–720

132 Salonen, J.T., Salonen, R., Penttilä, I., Herranen, J., Jauhiainen, M., Kantola, M., Lappetaläinen, R., Mäenpää, P.H., Alfthan, G., and Puska, J. (1985). Serum fatty acids, apolipoproteins, selenium and vitamin antioxidants and the risk of death from coronary artery disease. *Am. J. Cardiol.* **56,** 226–231

133 Salonen, J.T., Salonen, R., Seppänen, K., Rinta-Kiikka, S., Kuukka, M., Korpela, H., Alfthan, G., Kantola, M., and Schalch, W. (1991). Effects of antioxidant supplementation on platelet function: a randomized pair-matched, placebo-controlled double-blind trial in men with low antioxidant status. *Am. J. Clin. Nutr.* **53,** 1222–1229

134 Evans, A.E., Richard, J.L., McMaster, D., Cambien, F., McCrum, E.E., Sykes, D., Bingham, A., Bard, J.M., Kee, F., Fruchart, J.C., Arveiler, D., Shaffer, P., Amouyel, P., Luc, G., Scarabin, P.Y., Ducimetière, P., Cambou, J.P., Douste-Blazy, P., Ruidavets, J.B., Gey, K.F., Juhan, A., Perret, B., McConville, M., and Yarnell, J.W.G. (1993). The PRIME Study. *Ulster Med. J.* **63,** 206

135 Steinberg, D., and Workshop Participants (1992). Antioxidants in the prevention of human atherosclerosis. Summary of the Proceedings of a NHLBI Workshop: September 5–6, 1991, Bethesda MD. *Circulation* **85,** 2338–2344

136 Willett, W.C., Stampfer, M.J., Underwood, B.A., Speizer, F.E., Rosner, B., and Hennekens, C.H. (1983). Validation of a dietary questionnaire with plasma carotenoid and α-tocopherol levels. *Am. J. Clin. Nutr.* **38,** 631–639

137 Tangney, C.C., Shekelle, R.B., Raynor, W., Gale, M., and Betz, E.P. (1986). Intra- and interindividual variation in measurement of β-carotene, retinol, and tocopherols in diet and plasma. *Am. J. Clin. Nutr.* **45,** 764–769

138 Roidt, L., White, E., Goodman, G.A., Wahl, P.W., Omenn, G.S., Rollins, B., and Karkeck, J.M. (1988). Association of food frequency questionnaire estimates of vitamin A intake with serum vitamin A levels. *Am. J. Epidemiol.* **128,** 645–654

139 Brown, E.D., Micozzi, M.S., Craft, N.E., Bieri, J.G., Beecher, G., Edwards, B.K., Rose, A., and Smith, J.C., Jr. (1989). Plasma carotenoids in normal men after a singly ingestion of vegetables and purified β-carotene. *Am. J. Clin. Nutr.* **49,** 1258–1265

140 Romieu, I., Stampfer, M.J., Stryker, W.C., Hernandez, M., Kaplan, L., Sober, A., Rosner, B., and Willett, W.C. (1990). Food predictors of plasma beta-carotene and alpha-tocopherol;validation of a food frequency questionnaire. *Am. J. Epidemiol.* **131,** 864–876

141 Herbeth, B., Chavance, M., Musse, N., and Vernhès, G. (1990). Determinants of plasma retinol, beta-carotene, and alpha-tocopherol. *Am. J. Epidemiol.* **132,** 394–395

142 Micozzi, M.S., Brown, E.D., Edwards, B.K., Bieri, J.G., Taylor, P.R., Khachik, F., Beecher, G.R., and Smith, J.C., Jr. (1992). Plasma carotenoid response to chronic intake of selected foods and β-carotene supplements. *Am. J. Clin. Nutr.* **55,** 1120–1125

143 Forman, M.R., Lanza, E., Yong, L.C., Holden, J.M., Graubard, B.I., Beecher, G.R., Melitz, M., Brown, E.D., and Smith, J.C. (1993). The correlation between two dietary assessments of carotenoid intake and plasma carotenoid concentrations: application of a carotenoid-food composition table. *Am. J. Clin. Nutr.* **58,** 519–524

144 Jacques, P.F., Sulsky, S.I., Sadowski, J.A., Phillips, J.C.C., Rush, D., and Willett, W.C. (1993). Comparison of micronutrient intake by a dietary questionnaire and biochemical indicators of micronutrient status. *Am. J. Clin. Nutr.* **57,** 182–189

145 Ascherio, A., Stampfer, M.J., Colditz, G.A., Rimm, E.B., Litin, L., and Willett, W.C. (1992). Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J. Nutr.* **122,** 1792–1801

146 Enstrom, J.E., Kanim, L.E., and Klein, M.A. (1992). Vitamin C intake and mortality among a sample of the United States population. *Epidemiology* **3,** 194–202

147 Vanderslice, J.T., and Higgs, D.J. (1991). Vitamin C content of foods: sample variability. *Am. J. Clin. Nutr.* **54,** 1323S–1327S

148 Committee on Dietary Allowances. Food and Nutrition Board. National Research Council (1968). *Recommended dietary allowances.* 7th ed., p. 21–39, National Academy Press, Washington, DC USA

149 U.S. Department of Agriculture (1986). Continuing survey of foods by individuals. Report 86-3. Hyattsville, MD USA

150 Block, G., and Sorensen, A. (1987). Vitamin C intake and dietary sources by demographic characteristics. *Nutr. Cancer* **10,** 53–68

151 Block, G. (1992). The data support a role for antioxidants in reducing cancer risk. *Nutr. Rev.* **50,** 207–213

152 National Center for Health Statistics, Fulwood, R., Johnson, C.L., and Bryner, J.D. (1982). Hematological and nutritional biochemistry reference data for persons 6 months–74 years of age: United States, 1976–1980. Vital and Health Statistics. Series 11-No. 232. DHHS Pub. No. (PHS) 83-1682. Public Health Service, Washington DC

153 Block, G., Sinha, R., and Gridley, G. (1994). Collection of dietary-supplement data and implications for analysis. *Am. J. Clin. Nutr.* **59** (Suppl), 232S–239S

154 Woteki, C., Johnson, C., and Murphy, R. (1986). A nutritional status of the U.S. population: iron, vitamin C, and zinc. In *What Is America Eating?* (Food and Nutrition Board, Commission on Life Sciences, National Research Council, eds), p. 21–39, National Academy Press, Washington, DC USA

155 Machlin, L.J., ed. (1990). *Handbook of Vitamins,* 2nd ed., Dekker, New York, NY USA

156 Cross, C.E., and Halliwell, B. (1993). Nutrition and human disease: How much extra vitamin C might smokers need? *Lancet* **341,** 1091

157 Kübler, W. (1993). Report to National Research Council, Committee on Diet and Health, National Academy of Sciences, Washington, DC, regarding VERA Study. In *VERA Publication Series* (W. Kübler, H.J. Anders, W. Heeschen, and M. Kohlmeyer, eds.), vol III *Lebensmittel- und Nährstoffaufnahme Erwachsener in der Bundesrepublik Deutschland* (Heseker, H., Adolf, T., Eberhardt, W., Hartmann, S., Herwig, A., Kübler, W., Matiaske, B., Moch, K.J., Schneider, R., and Zipp, A., eds.), Wissenschaftlicher Fachverlag Fleck, Niederkleen, Germany

158 Garry, P.J., Vanderjagt, D.J., and Hunt, W.C. (1987). Ascorbic acid intakes and plasma levels in healthy elderly. *Ann. NY Acad. Sci.* **498,** 90–99

159 Rimm, E.B., Stampfer, M.J., Ascherio, A., Giovannucci, E., Colditz, G.A., and Willett, W.C. (1993). Vitamin E consumption and the risk of coronary heart disease in men. *New Engl. J. Med.* **328,** 1450–1456

160 Ascherio, A., Rimm, E., Giovannucci, E., Colditz, G.A., Rosner, B., Willett, W.C., Sacks, F., and Stampfer, M.J. (1992). A prospective study of nutritional factors and hypertension among US men. *Circulation Res.* **86,** 1475–1484

161 Murphy, S.P., Subar, A.F., and Block, G. (1990). Vitamin E intakes and sources in the United States. *Am. J. Clin. Nutr.* **52,** 361–367

162 Horwitt, M.K. (1988). Supplementation with vitamin E. *Am. J. Clin. Nutr.* **47,** 1088–1089

163 Lehmann, J., Marshall, M.W., Slover, H.T., and Jacono, J.M. (1977). Influence of dietary fat level and dietary tocopherols on plasma tocopherols in human subjects. *J. Nutr.* **107,** 1006–1015

164 Bässler, K.H. (1991). On the problematic nature of vitamin E requirements: net vitamin E. *Z. Ernährungswiss.* **30,** 174–180

165 Lehmann, J., Martin, H.L., Lashley, E.L., Marshall, M.W., and Judo, J.F. (1986). Vitamin E in foods from high and low linoleic acid diets. *J. Am. Diet. Assoc.* **86,** 1208–1216

166 Sheppard, A.J., Pennington, J.A.T., and Weihrauch, J.L. (1993). Analysis and distribution of vitamin E in vegetable oils and foods. In *Vitamin E: Biochemistry and Clinical Applications,* (L. Packer, and J. Fuchs, eds.), p. 9–31, Marcel Dekker, New York, NY USA

167 Muggli, R. (1989). Dietary fish oils increase the requirement for vitamin E in humans. In *Health Effects of Fish Oils* (Chandra, R.K., ed.), p. 203–210, ARTS Biomedical Publications, St. John's, Newfoundland

168 Rimm, E., and Colditz, G. (1993). Smoking, alcohol, and plasma levels of carotene and vitamin E. *Ann. NY Acad. Sci.* **686,** 323–334

169 Krasinski, S.D., Russell, R.M., Otradovec, C.L., Sadowski, J.A., Hartz, S.C., Jacob, R.A., and McGandy, R.B. (1989). Relationship of vitamin A and vitamin E intake to fasting plasma retinol, retinol-binding protein, retinyl esters, carotene, α-tocopherol, and cholesterol among elderly and young adults: increased plasma retinyl esters among vitamin A-supplement users. *Am. J. Clin. Nutr.* **49,** 112–120

170 Lehmann, J., Rao, D.D., Canary, J.J., and Judd, J.T. (1988). Vitamin E and relationship among tocopherols in human plasma, platelets, lymphocytes and red blood cells. *Am. J. Clin. Nutr.* **47,** 470–474

171 Deleted.

172 Sinha, R., Patterson, B.H., Mangels, A.R., Levander, O.A., Gibson, T., Taylor, P.R., and Block, G. (1993). Determinants of plasma vitamin E in healthy males. *Cancer Epidemiol. Biomarkers Prev.* **2,** 473–479

173 Duthie, G.G., Arthur, J.R., Beattie, J.A.G., Brown, K.M., Morrice, P.C., Robertson, J.D., Shortt, C.T., Walker, K.A., and James, W.P.T. (1993). Cigarette smoking, antioxidants, lipid per-

oxidation, and coronary heart disease. *Ann. NY Acad. Sci.* **686,** 120–129

174 Comstock, G.W., Menkes, M.S., Schober, S.E., Vuilleumier, J.P., and Helsing, K.J. (1988). Serum levels of retinol, beta-carotene, and alpha-tocopherol in older adults. *Am. J. Physiol.* **127,** 114–123

175 Princen, H.M.G., VanPoppel, G., Vogelezang, C., Buytenhek, R., and Kok, F.J. (1992). Supplementation with vitamin E but not β-carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro. Effect of cigarette smoking. *Arterioscler. Thromb.* **212,** 554–562

176 Scheffler, E., Wiest, E., Woehrle, J., Otto, J., Schulz, I., Huber, L., Ziegler, R., and Dresel, H.A. (1992). Smoking influences the athrogenic potential of low-density lipoprotein. *Clin. Invest.* **70,** 236–268

177 Harats, D., Ben-Naim, M., Dabach, Y., Hollander, G., Havivi, E., Stein, O., and Stein, Y. (1990). Effect of vitamin C and E supplementation on susceptibility of plasma lipoproteins to peroxidation induced by acute smoking. *Atherosclerosis* **85,** 47–54

178 Pacht, E.R., Kaseki, H., Mohammed, J.R., Cornwell, D.G., and Davis, W.B. (1986). Deficiency of vitamin E in the alveolar fluid of cigarette smokers. Influence on alveolar macrophage cytotoxicity. *J. Clin. Invest.* **77,** 789–796

179 Duthie, G.G., Arthur, J.R., James, W.P.T., and Vint, H.M. (1989). Antioxidant status of smokers and nonsmokers. Effects of vitamin E supplementations. *Ann. NY Acad. Sci.* **570,** 435–438

180 Willett, W.C., Stampfer, M.J., Underwood, B.A., Taylor, J.O., and Hennekens, C.H. (1983). Vitamins A, E and carotene: Effects of supplementation on their plasma levels. *Am. J. Clin. Nutr.* **38,** 559–566

181 Albanes, D., Virtamo, J., Rautalathi, M., et al. (1992). Serum beta-carotene before and after beta-carotene supplementation. *Eur. J. Clin. Nutr.* **46,** 15–24

182 Russell-Briefel, R., Bates, M.W., and Kuller, L. (1985). The relationship of plasma carotenoids to health and biochemical factors in middle-aged men. *Am. J. Epidemiol.* **122,** 741–749

183 Chow, C.K., Thacker, R.R., Changchit, C., Bridges, R.B., Rehm, S.R., Humble, J., and Turbek, J. (1986). Lower levels of vitamin C and carotenes in plasma of cigarette smokers. *J. Am. Coll. Nutr.* **5,** 305–312

184 Aoki, K., Ito, Y., Sasaki, R., Ohtani, M., Hamajima, N., and Asano, A. (1987). Smoking, alcohol drinking and serum carotenoid levels. *Japan J. Cancer Res.* **78,** 1049–1056

185 Nierenberg, D.W. (1989). Determinants of plasma levels of beta-carotene and retinol. *Am. J. Epidemiol.* **130,** 511–521

186 Shibata, A., Paganini-Hill, A., Ross, R.K., Yu, M.C., and Henderson, B.E. (1992). Dietary β-carotene, cigarette smoking, and lung cancer in men. *Cancer Causes Control.* **3,** 207–214

187 Constantino, J.P., Kuller, L.H., Begg, L., Redmond, C.K., and Bates, M. (1988). Serum level changes after administration of a pharmacological dose of β-carotene. *Am. J. Clin. Nutr.* **48,** 1277–1283

188 Dimitrov, N.V., Meyer, C., Ullrey, D.E., Chenoweth, W., Mickelakis, A., Malone, W., Boone, C., and Fink, G. (1989). Bioavailability of β-carotene in humans. *Am. J. Clin. Nutr.* **48,** 298–304

189 Mathews-Roth, M.M. (1990). Plasma concentrations of carotenoids after large doses of β-carotene. *Am. J. Clin. Nutr.* **52,** 500–559

190 Micozzi, M.S., Albanes, D., and Stevens, R.G. (1989). Relationship of body size and composition to clinical, biochemical and hematological indices in U.S. men and women. *Am. J. Clin. Nutr.* **60,** 12167–12181

191 Nierenberg, D.W., Stukel, T.A., Baron, J.A., Dain, B.J., Greenberg, E.R., and the Skin Cancer Prevention Study Group. (1991). Determinants of increase in plasma concentration of β-carotene after chronic oral supplementation. *Am. J. Clin. Nutr.* **53,** 1443–1449

192 Brown, E.D., Micozzi, M.S., Craft, N.E., Bieri, J.G., Beecher, G., Edwards, B.K., Rose, A., and Smith, J.C., Jr. (1989). Plasma carotenoids in normal men after a single ingestion of vegetables and purified β-carotene. *Am. J. Clin. Nutr.* **49,** 1258–1265

193 Mangels, A.R., Block, G., Levander, O.A., Taylor, P.R., Morris, V.C., and Patterson, B.H. (1991). Differences in plasma beta-carotene maintained despite similar dietary beta-carotene. *FASEB J.* **5,** A 1322

194 Cade, J.E., and Margetts, B.M. (1991). Relationship between diet and smoking—Is the diet of smokers different? *J. Epidemiol. Commun. Health* **45,** 270–272

195 Subar, A.F., Harlan, L.C., and Mattson, M.E. (1990). Food and nutrient intake differences between smokers and non-smokers in the US. *Am. J. Public Health* **80,** 1323–1329

196 Whichelow, M.J., Golding, J.F., and Treasure, F.P. (1988). Comparison of some dietary habits of smokers and non-smokers. *Brit. J. Addict.* **83,** 295–304

197 Stampfer, M.J., Hennekens, C.H., Manson, J.-A.E., Colditz, G.A., Rosner, B., and Willett, W.C. (1993). Vitamin E consumption and the risk of coronary heart disease in women. *New Engl. J. Med.* **328,** 1444–1449

198 Gaziano, J.M., Manson, J.E., Ridker, P.M., Buring, J.E., and Hennekens, C.H. (1990). Beta-carotene therapy for chronic stable angina. *Circulation* **82** (Suppl. III:201) Abstract 079

199 Blot, W.J., Li, J.Y., Taylor, P.R., Guo, W., Dawsey, S., Wang, G.Q., Yang, C.S., Zheng, S.F., Gail, M., Li, G.Y., Yu, Y., Liu, B.-q., Tangrea, J., Sun, Y.-h., Liu, F., Fraumeni, J.F., Jr., Zhang, Y.H., and Li, B. (1993). 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.* **85,** 1483–1492

200 Thurnham, D.I., Munoz, N., Lu, J.B., Wahrendorf, J., Zheng, S.-F., Hambridge, K.M., and Crespi, M. (1988). Nutritional and haematological status of Chinese farmers: the influence of 13.5 months treatment with riboflavin, retinol and zink. *Eur. J. Clin. Nutr.* **42,** 647–660

201 Li, J.Y., Taylor, P.R., Li, B., Dawsey, S., Wang, G.Q., Ershow, A.G., Guo, W., Liu, S.F., Yang, C.S., Shen, Q., Wang, W., Mark, S.D., Zuo, X.N., Greenwald, P., Wu, Y.P., and Blot, W.J. (1993). Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *J. Natl. Cancer Inst.* **85,** 1492–149

202 Ubbink, J.H., Vermaak, W.J.H., VanDerMeerwe, A., and Becker, P.J. (1993). Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. *Am. J. Clin. Nutr.* **57,** 47–53

203 Ueland, M., Refsum, H., Stabler, S.P., Malinow, M.R., Andersson, A., and Allen, R.H. (1993). Total homocystein in plasma or serum: methods and clinical applications. *Clin. Chem.* **39,** 1764–1779

204 Krinsky, N.I. (1993). Effects of carotenoids on cells. *Mol. Aspects Med.* **14,** 241–246

205 Stich, H., Mathew, B., Sankaranarayanan, R., Nair, K.K. (1991). Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of β-carotene and vitamin A. *Am. J. Clin. Nutr.* **53** (Suppl), 298S–304S

206 Singh, V.N., and Gaby, S.K. (1991). Premalignant lesions: role of antioxidant vitamins and β-carotene in risk reduction and prevention of malignant transformations. *Am. J. Clin. Nutr.* **53** (suppl), 386S–390S

207 Buzina, R., Aurer-Kozelj, J., Srdak-Jorgic, K., Bühler, E., and Gey, K.F. (1986). Increase of gingival hydroxyproline and proline by improvement of ascorbic acid status in man. *Int. J. Vitam. Nutr. Res.* **56,** 367–372

208 Gaby, S., Bendich, A., Singh, V.N., and Machlin, L.J. (1991). *Vitamin intake and health. A scientific review.* Dekker, New York, NY USA

209 Jialal, I., Vega, G.L., and Grundy, S.M. (1990). Physiologic levels of ascorbate inhibit the oxidative modification of low density lipoprotein. *Atherosclerosis* **82,** 185–191

210 Winter, M.L., and Liehr, J.G. (1989). Quantification of oxidative damage to protein in estrogen-treated hamster kidney. *Proc. Am. Assoc. Cancer Res.* **30,** abstr. 129

211 Lemoyne, M., Gossom, A. van, Kurian, R., Ostro, M., Axler, J., and Jeejeebhoy, K.N. (1987). Breath pentane analysis as index of lipid peroxidation: a functional test of vitamin E status. *Am. J. Clin. Nutr.* **46,** 267–272

212 Umegaki, K., Ikegami, S., Inoue, K., Ichikawa, T., Kobayashi, S., Soeno, N., and Tomabechi, K. (1994). Beta-carotene prevents

x-ray induction of micronuclei in human lymphocytes. *Am. J. Clin. Nutr.* **59,** 409–412

213 Leibovitz, B.E., and Mueller, J.A. (1993). Bioflavonoids and polyphenols: medical applications. *J. Opt. Nutr.* **2,** 17–35

214 Olszewski, A.J., and McCully, K.S. (1993). Homocystein metabolism and the oxidative modication of proteins and lipids. *Free Radical Biol. Med.* **14,** 683–693

215 Christensen, B., Refsum, H., Garras, A., and Ueland, P.M. (1992). Homocystein remethylation during nitrous oxide exposure of cells cultured in media containing various concentrations of folates. *J. Pharmacol. Exp. Ther.* **261,** 1096–1105

216 Stamler, J.S., Osborne, J.A., Jaraki, O., Rabbani, L.E., Mullins, M., Singel, D., and Loscalzo, L.E. (1993). Adverse vascular effects of homocysteine are modulated by endothelium-derived reaxing factor and related oxides by nitrogen. *J. Clin. Invest.* **91,** 308–318

217 Piyathilake, C.J., Hine, R.J., Dasanayake, A.P., Richards, E.W., Freeberg, L.E., Vaughn, W.H., and Krumdieck, C.L. (1992). Effect of smoking on folate levels in buccal mucosal cells. *Int. J. Cancer* **52,** 566–569

218 Toyosaki, T. Antioxidant effect of riboflavin in enzymic lipid peroxidation. *J. Agri. Food Chem.* **40,** 1727–1730

219 Sperduto, R.D. (1993). The Linxian cataracts studies. Two nutrition intervention trials. *Arch. Ophthal.* **111,** 1246–1253

220 Mitchinson, M.J., Ball, R.Y., Carpenter, K.H.L., and Parums, D.V. (1988). Macrophages and ceroid in atherosclerosis. In *Hyperlipidaemia and Atherosclerosis,* (K.E. Suckling, and P.H.E. Groot, eds.), p. 117–134, Academic Press, London, UK

221 Carpenter, K.H.L., Ballantine, J.A., Fussell, B., Enright, J.H., and Mitchinson, M.J. (1990). Oxidation of cholesteryl linoleate by human monocytes-macrophages in vitro. *Atherosclerosis* **83,** 217–229

222 Trout, D.L. (1992). Vitamin C and cardiovascular risk factors. *Am. J. Clin. Nutr.* **53** (Suppl), 322S–325S

223 Simionescu, M., and Simionescu, N. (1993). Proatherosclerotic events: pathobiochemical changes occurring in the arterial wall before monocyte migration. *FASEB J.* **7,** 1359–1366

224 Nievelstain, P.F.E.M., Fogelman, A.M., Mottino, G., and Frank, J.S. (1991). Lipid accumulation in rabbit aortic intima two hours after bolus infusion of low density lipoprotein. A deep-etch and immunolocalization study of ultrarapidly frozen tissue. *Arterioscler. Thromb.* **11,** 1795–1803

225 Guyton, J.R., and Klemp, K.F. (1992). Early extracellular and cellular lipid deposits in aorta of cholesterol-fed rabbits. *Am. J. Pathol.* **141,** 925–936

226 Hoff, H.F., and O'Neill, J. (1991). Lesion-derived low density lipoprotein and oxidized low density lipoprotein share a lability for aggregation, leading to enhanced macrophage degradation. *Arterioscler. Thromb.* **11,** 1209–1222

227 Chang, M.Y., Rees, A.M., and Lees, S.L. (1993). Low-density lipoprotein modification and arterial wall accumulation in a rabbit model of atherosclerosis. *Biochemistry* **32,** 8518–8524

228 Faruqui, R.M., and DiCorletto, P.E. (1993). Mechanisms of monocyte recruitment and accumulation. *Brit. Heart J.* **69** (Suppl), S19–S29

229 Wiklund, O., Mattson, L., Björnheden, T., Camejo, G., and Bondjers, G. (1991). Uptake and degradation of low density lipoproteins in atherosclerotic rabbit aorta: Role of local LDL modification. *J. Lipid Res.* **32,** 55–62

230 Keaney, J.F., Gaziano, J.M., Xu, A., Frei, B., Curran-Celentano, J., Shaery, G.T., Loscalzo, J., and Vita, J.A. (1993). Dietary antioxidants preserve endothelium-dependent vessel relaxation in cholesterol-fed rabbits. *Proc. Natl. Acad. Sci. USA* **90,** 11880–11884

231 Boissonneault, G.A., Hennig, B., Wang, Y., Wood, C.L. (1990). Aging and endothelial barrier function in culture: effects of chronic exposure to fatty acid hydroperoxides and vitamin E. *Mech. Ageing Dev.* **56,** 1–9

232 Reid, V.C., Brabbs, C.E., and Mitchinson, M.J. (1992). Cellular damage in mouse peritoneal macrophages exposed to cholesteryl linoleate. *Atherosclerosis* **92,** 251–260

233 Saito, Y., and Morisaki, N. (1989). Effect of vitamin E on the low-density lipoprotein metabolism in the arterial wall. In *CRC Handbook of Free Radicals and Antioxidants in Biomedicine,* (J. Miquel, A.T. Qintanilha, and H. Weber, eds.), Vol. II, p. 215–226, CRC Press, Inc., Boca Raton, FL USA

234 Hentze, M.W., Rouault, T.A., Harford, J.B., and Klausner, R.D. (1989). Oxidation-reduction and the molecular mechanism of a regulatory RNA-protein-interaction. *Science* **244,** 357–359

235 Storz, G., Tartaglia, L.A., and Ames, B.N. (1990). Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. *Science* **248,** 189–194

236 Schreck, R., Albermann, K., and Baeuerle, P.A. (1992). Nuclear factor kB: an oxidation-stress-responsive transcription factor of eukaryotic cells (a review). *Free Radical Res. Commun.* **17,** 221–237

237 Nagy, K., Pásti, G., Bene, L., and Nagy, I. (1993). Induction of granulocyte maturation in HL-60 human leukemia cells by free radicals: A hypothesis of cell differentiation involving hydroxyl radicals. *Free Radical Res. Commun.* **19,** 1–15

238 Rosenfeld, M. (1991). Oxidized LDL affects multiple atherogenic cellular responses. *Circulation* **83,** 2137–2140

239 Chisolm, G.M. (1993). Oxidized lipoproteins and leucocyte-endothelial interactions: growing evidence for multiple mechanisms. *Labor. Invest.* **68,** 369–371

240 Hajjar, D.P., and Pomerantz, K.B. (1992). Signal transduction in atherosclerosis: integration of cytokines and the eicosanoid network. *FASEB J.* **6,** 2933–2941

241 Marcus, A.J., and Hajjar, D.P. (1993). Vascular transclular signaling. *J. Lipid Res.* **34,** 2017–2031

242 Hansson, G.K. (1992). Role of immune responses in atherosclerosis. *Nutr. Metab. Cardiovasc. Dis.* **2,** 49–51

243 Oppenheim, J.J., and Neta, R. (1994). Pathophysiological roles of cytokines in development, immunity and inflammation. *FASEB J.* **8,** 158–162

244 Cornwell, D.G., and Panganamala, R.V. (1993). Vitamin E action in modulating the arachidonic acid cascade in *Vitamin E: Biochemistry and Clinical Applications,* (L. Packer, and J. Fuchs, eds.), p. 385–410, Marcel Dekker, New York, NY USA

245 ElAttar, T.M.A., and Lin, H.S. (1992). Effect of vitamin C and vitamin E on prostaglandin synthesis by fibroblasts and squamous cell carcinoma cells. *Prostaglandins Leukotriens Essent. Fatty Acids* **47,** 253–257

246 Meydani, S.N. (1990). Dietary modulation of cytokine production and biological function. *Nutr. Rev.* **48,** 361–339

247 Zhang, L.X., Cooney, R.V., and Bertram, J.S. (1992). Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer preventive chemopreventive action. *Carcinogenesis* **12,** 2109–2114

248 Bendich, A., Philips, M., and Tengerdy, R., eds. (1989). *Antioxidant Nutrients and Immune Functions.* Plenum Press, New York, NY USA

249 Chandra, R.K., ed. (1992). *Nutritional and Immunology.* ARTS Biomedical Publishers, St. John's, Newfoundland

250 Jacob, R.A., Kelley, D.S., Pianalto, F.S., Swendseid, M.E., Henning, S.M., Zhang, J.Z., Ames, B.N., Fraga, C.G., and Peters, J.H. (1991). Immunocompetence and antioxidant defense during ascorbate depletion of healthy men. *Am. J. Clin. Nutr.* **54,** 1302S–1309S

251 Patterson, B., Block, G., Rosenberger, R., and Pee, D. (1990). Fruit and vegetables in the American diet: data from the NHANES II Survey. *Am. J. Public Health* **80,** 1443–1449

252 Block, G. (1991). Dietary guidelines and the results of food consumption surveys. *Am. J. Clin. Nutr.* **53,** 356S–357S

253 The Alpha-Tococopherol, Beta Carotene Cancer Prevention Study Group (1994). The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N. Engl. J. Med.* **330,** 1029–1035

254 Oliver, M.F. (1992). Doubts about preventing coronary heart disease. Multiple interventions in middle aged men may do more harm than good. *Brit. Med. J.* **304,** 393–394

255 Cleland, J.G.F., and Krikler, D.M. (1993). Modification of atherosclerosis by agents that do not lower cholesterol. *Br. Heart J.* **69** (Suppl), S54–S62

256 Greenberg, E.R., Baron, J.A., Stukel, T.A., et al. (1990). A clinical trial of beta carotene to prevent basel-cell and squamous cell cancers of the skin. *N. Engl. J. Med.* **323,** 789–795

257 Kritchevsky, D. (1995). Letter to the Editor. *N. Engl. J. Med.* **331,** 611–612

258 Gey, K.F. (1995). Concurrent correction of "suboptimal" plasma antioxidant levels may, as important part of "optimal" nutrition, help to prevent early stages of cardiovascular diseases and cancer respectively. *Bibl. Dieta Nutr.*, in press

259 Blot, W.J., Li, J.Y., Taylor, P.R., Guo, W., Dawsey, S., and Li, B. (1995). The Linxian Trials: Mortality and cancer rates by multivitamin/mineral intervention group. *Am. J. Clin. Nutr.*, in press