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# Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease

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Free radicals may be involved in the aetiology of cancer and cardiovascular diseases. In epidemiological studies poor plasma levels of all essential antioxidants are associated with increased relative risks; in particular, low levels of carotene and vitamin E with the risk of cancer and ischemic heart disease, respectively. The studies suggest that for optimal synergistic protection the plasma antioxidant levels should simultaneously exceed the threshold values of 28–30 μmol/l lipid-standardized vitamin E, 40–50 μmol/l vitamin C, 0.4–0.5 μmol/l carotene and 2.2–2.8 μmol/l lipid-standardized vitamin A. However the preventive efficacy of an optional antioxidant status is still to be proven in randomized intervention trials. Although these antioxidant micronutrients may be the primary protective components of vegetable-rich 'preventive' diets, the potentials of other plant components await exploration, eg carotenoids other than β-carotene, bioflavonoids and oxygen-sensitive B-vitamins.

Aggressive oxygen species (superoxide anion radical, hydroxyl radical, hydrogen peroxide and singlet oxygen, carbon-centered and thiyl radicals can damage all biochemical components, ie DNA/RNA, proteins, carbohydrates, unsaturated lipids and micronutrients such as carotenoids, vitamins A, $B_6$, $B_{12}$ and folate. Lipid peroxidation can proceed as a chain reaction producing hydroperoxides and aldehydes which can damage at distance.[1–3] Free radicals have been implicated in the aetiology of many diseases.[1–3] Unfortunately the extremely short

half-life of most aggressive radical species prevents direct investigation. But indirect information is available from measuring antioxidants which are crucial in the body's multilevel defence against radicals. Lines of defence include enzymes (eg superoxide dismutase, catalase, glutathione peroxidase), non-essential endogenous antioxidants (glutathione, proteins, uric acid, ubiquinol-10 etc) and essential radical scavengers, ie vitamins C (ascorbic acid) and E (α-tocopherol), carotenoids (eg β-carotene, a potential vitamin A precursor with singlet oxygen-quenching effects, and non-vitamin A precursors, e.g. lycopene) and finally vitamin A (retinol, potentially a thiyl radical scavenger).[1–3] The first line of essential antioxidants is formed by vitamin C, the principal radical scavenger of the aqueous phase,[4–5] which is also primarily exhausted by coronary bypass surgery (a special reperfusion injury of heart/lung).[6] Vitamin E represents the principal chain-breaking antioxidant in the liposoluble phase.[3] This review summarizes inverse associations between essential antioxidants in plasma and cancer (International Classification of Diseases: ICD 140–239), cardiovascular diseases (CVD) such as ischemic heart disease (IHD; ICD 410–414)[7–12] and stroke (cerebrovascular disease; ICD 430–438)[12] which suggest a pathogenetic involvement of free radicals. If free radicals are aetiologically involved in the major causes of death, optimization of antioxidant status should offer preventive prospects for 'optimum health' as defined by WHO. The status of vitamin antioxidants (in contrast to that of other antioxidative defence lines) can easily, and without risking side effects, be modified by dietary measures.

## VEGETABLE–RICH DIETS

Many nutritional surveys demonstrate a higher life expectancy and lower risk of cancer and IHD in subjects preferring special diets such as a vegetable-rich (not necessarily truly vegetarian) type and the Mediterranean diet. The latest dietary guidelines recommend a lowering of total dietary fat (primarily the mammalian fat, but with a relative increase of monoene-rich vegetable oils) together with an increased consumption of green-yellow vegetables and fruits. An optimized diet might theoretically reduce the incidence of cancer by 30–60%.[13] Vegetable/fruit-enriched diets may increase plasma levels of vitamin C and carotene, and vegetarians have also a higher ratio of vitamin E/polyunsaturated fatty acids (PUFAs, the major substrate of lipid peroxidation).[14, 15]

Although the principal antioxidant vitamins, vitamins C and E, together with β-carotene and vitamin A may be primarily related to the reduction of radical-related pathogenicity, supplementary contributions could come from further plant components, eg carotenoids other than

β-carotene such as lycopene, bulky strong antioxidants such as common phenols and mostly poorly absorbable bioflavonoids (potentially protecting principal antioxidants such as vitamin C), oxygen radical-sensitive B-vitamins such as folate and vitamin $B_6$ (probably requiring protection by a fair vitamin C status in vivo).[16, 17]

## CALCULATED DIETARY INTAKE OF ESSENTIAL ANTIOXIDANTS

Dietary surveys based on Food Composition Tables consistently reveal inverse correlations between essential nutrient antioxidants and the risk of cancer[16–20] and IHD.[7–10] Preliminary data of the US Nurses Study[21] suggest a reduced risk of IHD at the highest intake of either vitamin E or A or carotene. The First National Health and Nutrition Examination Survey (NHANES I) reveals that an increased vitamin C intake gives substantial risk reductions for all causes of death, all cancers and all CVD.[22] According to a preliminary subset analysis of the US Physicians Study supplementation of β-carotene (alternately with aspirin) can also reduce the IHD risk.[23]

## OBSERVATIONAL DATA ON PLASMA LEVELS OF ANTIOXIDANT NUTRIENTS

Several complementary types of epidemiological studies in westernized countries have revealed that increased risks for cancer, IHD and stroke are associated with a poor plasma status of essential antioxidants such as vitamins C and E as well as carotene and vitamin A. Clearly plasma levels are more conclusive indicators of the antioxidant status than the calculated dietary intake since the actual status in vivo is the outcome of the bioavailability of antioxidants (depending on dietary supply, intestinal absorption, hepatic secretion and metabolic regulations) and of the individual requirement which can be increased by peroxidation-prone nutrients such as PUFAs, by 'oxidative stress', by the environment (including smoking) or by life-style factors, eg exhaustive exercise and possibly catecholamine-related stress.

## PREDICTION OF CANCER BY LOW PLASMA ANTIOXIDANT NUTRIENTS

### Case–control studies

Several studies found lower plasma carotene and vitamin E in patients with existing cancers, eg of the lung,[24–26] presumably at least in part because of a lower dietary intake.[24, 26] But retrospective case-control studies of malignant tumors are of limited value since they cannot

distinguish between cause and result of the disease which is mostly diagnosed only decades after its initiation.

**Prospective studies**

The longitudinal follow-up of initially apparently healthy subjects up to 12 years in about 10 study populations revealed that the subsequent cancer mortality was, when compared to surviving controls, inversely related to initial levels of β-carotene (or α -plus β-carotene), and in part also to levels of vitamins E, C and A (Table 1).[27–50] Whereas prospective cohort studies have mostly a 'blood-bank' design (with a great loss of carotenoids and a marked vitamin E decay in frozen serum stored for years) this handicap was avoided by the Basle Prospective Study, and the latter was also unique in the simultaneous assay of plasma vitamin C. Sequential evaluations of the Basle Study[27, 30–32, 35, 37] consistently revealed a very strong predictive power of a poor carotene status for most cancers (Figs. 1 & 2) thus confirming other studies with the storage handicap (Table 1) and further favouring the idea that β-carotene is a substantial component of preventive vegetable-rich diets.[13] In addition, the Basle Study established a significantly increased relative risk of gastrointestinal cancers at low plasma concentrations of vitamin C[30–32, 37] and at low levels of lipid-standardized vitamin A[37] (Figs. 1 & 2). This substantiates nutritional data on inverse correlations between dietary vitamin A[18] or vitamin C[22, 51] and risk of subsequent cancers. It is unknown why the cancer risk is clearly predicted by lipid-standardized plasma vitamin A but rather not by absolute plasma vitamin A which revealed no consistent correlations or, at best, trends.[16, 18, 28, 33, 36, 43, 47] Finally, the Basle Study may indirectly support results from other study cohorts with a presumably poor vitamin E status which revealed an increased cancer risk at the lowest percentiles of vitamin E (Table 1). Thus, the unusually and uniformly high vitamin E levels in Basle lacked (at most follow-ups) a significant correlation to subsequent cancer (Figs. 1 & 2), most probably since all vitamin E values of the Basle population were above the risk threshold[35, 37] similar to other populations with high vitamin E status, e.g. in Hawaii.[28] Clearly, an increased risk of a poor level of any antioxidant can only be revealed if the latter occurs in a statistically sufficient percentage of study subjects. This happened in the Basle Study for the antioxidants mentioned above but not for vitamin E.[35] Since the Basle population (men of 30–65 years of age) did not show any age-dependent variation of all the plasma vitamins measured (Gey KF, Stähelin HB, Lüdin E, unpublished data, 1990), the differences of the antioxidant status between deceased cases and surviving controls may have been rather permanent ones during all stages of carcinogenesis.

**Table 1** Prospective studies on subsequent cancer mortality: a poor plasma antioxidant status at baseline is associated with a significantly increased relative risk

| Antioxidant | Cancer (in 5–12 years) | Reference |
|---|---|---|
| Carotene | Lung | 27–39 |
| | Gastrointestinal | 27, 28, 37 |
| | Skin melanoma | 39 |
| | All (in males) | 30–37 |
| Vitamin A | Lung | 30–32, 37, 40 |
| | Stomach | 30–32, 37, 40, 41 |
| | Prostate | 42 |
| | All (in males) | 37, 39 |
| Vitamin C | Gastrointestinal | 30–32, 37 |
| | All (in males) | 30–32, 37 |
| Vitamin E | Lung | 29–32, 38, 39, 43 |
| | Gastrointestinal | 39, 40, 44–46 |
| | Colorectal | 46 |
| | Pancreas | 40 |
| | Skin melanoma | 39 |
| | Urinary organs | 44 |
| | All (in males) | 39, 43–45, 47 |
| | Breast | 48, 49 |
| | Female reproductive | 49 |
| | All (in females) | 49 |

Taking together all prospective studies on subsequent cancers the correlation is strongest for carotene but there are marked differences between cancer sites.[37, 50] For the relative cancer risk of the Basle population (Fig. 2) the rank order of correlations was:

- bronchus cancer: carotene (β-, possibly also α-carotene) >lipid-standardized vitamin A (and vitamin E in areas with poor vitamin E status)
- gastrointestinal cancer: vitamin C = carotene >vitamin A (= vitamin E in areas with poor vitamin E status)
- all cancers: carotene = vitamin A >vitamin C.

Furthermore, the combination of low levels of several antioxidants, eg of both carotene and vitamin A, increased the risk additively.[32, 37] In conclusion, preventive attempts should simultaneously optimize the status of all essential antioxidants.

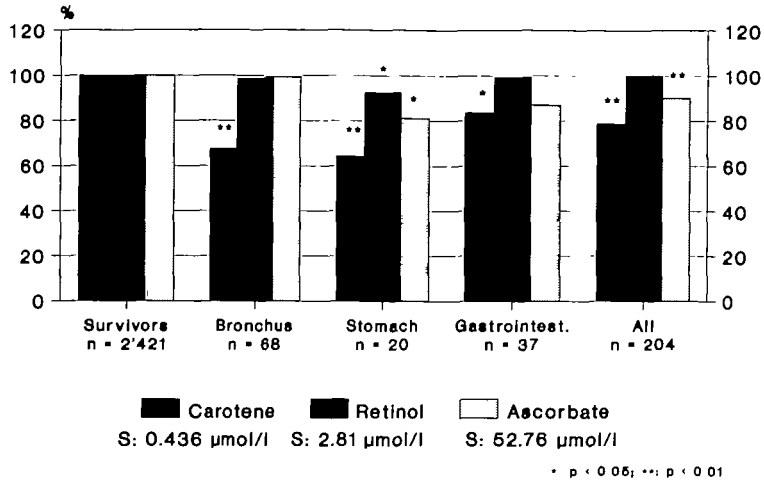


**Fig. 1** Mean base-line values of plasma carotene, vitamin A (retinol; lipid-standardized) and vitamin C in cases of subsequent cancer in % of means of survivors: 12-year follow-up of the Prospective Basel Study.[37] The absolute plasma values of survivors (S) are indicated at the bottom. The mean values for lipid-standardized vitamin E were in all subsequent cancer cases almost identical to that of survivors, ie unusually high (mean of survivors 34.6 µM) and thus presumably far above any critical threshold.[37]

## PREDICTION OF CARDIOVASCULAR DISEASE BY LOW PLASMA ANTIOXIDANT NUTRIENTS

Consistent evidence for associations of a poor plasma status of essential antioxidants and an increased risk of IHD has emerged from 3 complementary types of studies:

### Cross-cultural epidemiology

This can reveal differences in risk factors that may be important for early pathogenic stages, ie initiation and/or promotion of atherogenesis (provided that younger age strata are included). Data of several countries suggest that in given study populations the antioxidant status is fairly constant in men aged 20–65 years.[10] The Vitamin Substudy of the WHO/MONICA Project (a standardized and by far the largest trial for Monitoring trends and determinants of Cardiovascular disease) compared in randomly-selected representatives of 16 European study populations the plasma antioxidant status with the concurrent age-specific IHD mortality. The latter varied up to 6-fold (as in the whole world) and antioxidant levels up to about 2-fold. In the majority (ie in 12 study populations) the classical risk factors of plasma cholesterol, blood

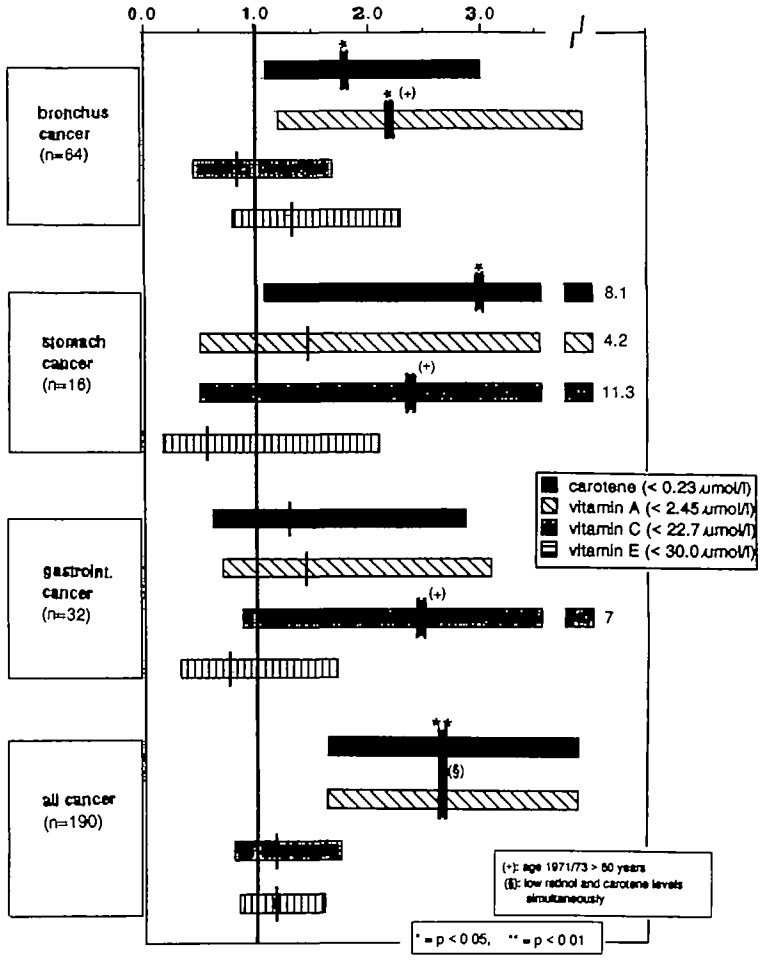


**Fig. 2** Relative risk of low plasma levels of essential antioxidants (at base-line) for subsequent cancer death (first 2 years excluded) after adjustment for age, smoking and lipids, with 95%-confidence intervals: 12-year follow-up of the Prospective Basel Study.[37] Horizontal scale and numbers at the right end of the bars of 95%-confidence: Relative risk of quartile 1 (threshold levels in the larger box at the right edge) in comparison to quantiles 2 to 5 (with higher antioxidant levels) in the Cox proportional hazard regression model.

pressure and smoking did not differ significantly and could thus not sufficiently explain the up to 6-fold differences in IHD mortality.[7, 8, 11] The differences could, however, be predicted by a hitherto unique de-

gree when the plasma status of essential antioxidants was simultaneously considered. Most impressive and consistent in all sequential evaluations[7–11, 31, 52–54] was the strong inverse correlation of vitamin E ($r^2$>0.6; $P$<0.0003) which occurred for both absolute vitamin E in populations with comparable plasma lipoproteins and lipid-standardized vitamin E in all study populations (Fig. 3). In univariate analysis vitamin E was clearly a stronger predictor of IHD mortality than the classical risk factors total plasma cholesterol ($r^2$=0.29; $P$=0.03) and diastolic blood pressure ($r^2$=0.25; 0.25; $P$=0.05), even after their combination in multiple regression analysis ($r^2$=0.44; $P$=0.02).[54] There was practically no overlap of the distribution pattern of vitamin E when study populations with high and low risk of IHD were compared (Fig. 4). 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 evaluations according to the accidental composition of the available study populations.[7–11, 31, 53–54] When a special, presumably in part genetic, 'Finland factor' is tentatively considered,[11] vitamin C comes close to vitamin E (Fig. 3). The stepwise regression analysis of the latest report,[11] further complemented by data from additional study populations (KF Gey et al., unpublished evaluations) suggests the following tentative rank order for IHD: vitamin E >>cholesterol and/or a 'genetic Finland factor' >vitamin C >carotene = diastolic blood pressure >vitamin A. 'Finland factor' is suggested by the fact that Finnish study populations behave as outlyers in almost all variables and are significantly different from total cholesterol.[11] This rank order of antioxidants, is, however, limited by their known interdependence: although plasma antioxidants can vary independently from each other in healthy individuals their medians in populations tend to correlate ($rP$= 0.4–0.5), and this happens presumably also in IHD. Nevertheless, the combination of the above mentioned variables (emerged from stepwise regression analysis but without vitamin A) can predict the existing IHD mortality to 90% (Fig. 5). Thus this combination of risk factors describes cross-cultural IHD in Europe to a degree that has never been achieved before.

### Case-control study in early undiagnosed angina pectoris

Common case-control studies on patients with long-standing coronary obstruction or even acute infarction (with hormonal and metabolic consequences) cannot differentiate between cause and result of the disease (and/or previous therapy possibly affecting the antioxidant status.)[10] Such weaknesses are avoidable by studying subjects whose symptoms had previously been disregarded and whose moderate IHD had not been diagnosed and thus not yet resulted in life style changes and/or treatment. In the Edinburgh Case-Control Study on Previously Undiagnosed

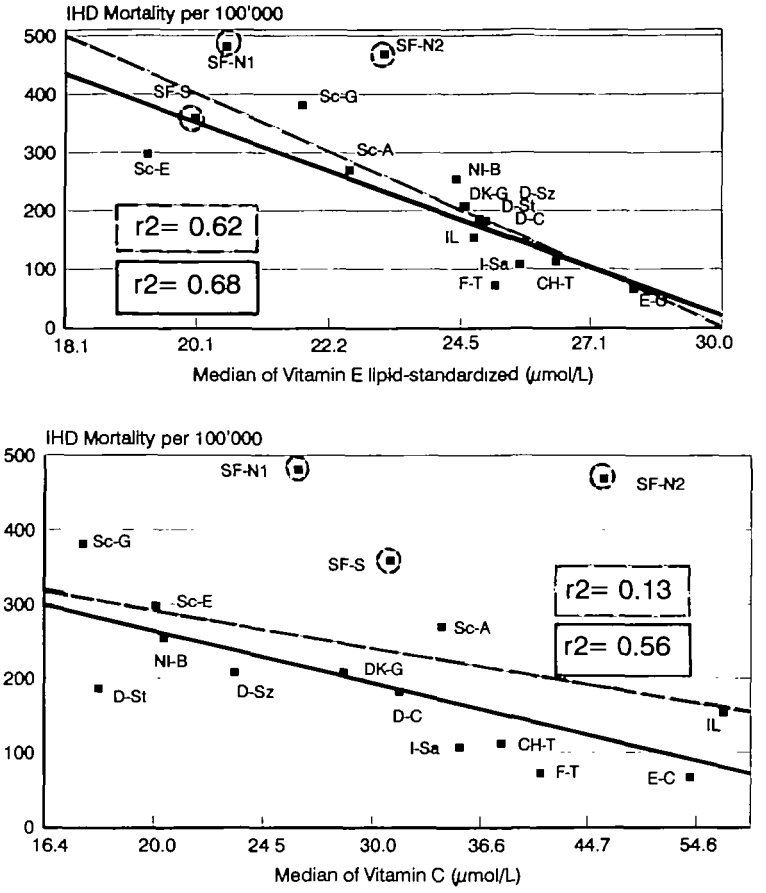


**Fig. 3** Inverse correlation between age (40–59 years)-specific IHD mortality and the medians of (logarithms of lipid-standardized) plasma vitamin E (above) and plasma vitamin C (below): cross-cultural comparisons of male European study populations in the Vitamin Substudy of the WHO/MONICA Project.[9, 11]
Solid regression lines: all study populations (n=16). Dotted regression lines: correlations of non-Finnish populations (n-=13), ie after tentative exclusion of the Finnish study sites (dotted circles) which might have another special risk factor of IHD, the 'Finland factor'.[11]

Angina Pectoris[55] middle-aged men from a population with plasma antioxidant levels between sufficient/fair to poor (due to rare consumption of fresh fruits and vegetables[56]) and a very high IHD morbidity were screened for cases with previously undiagnosed angina pectoris and compared 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 IHD

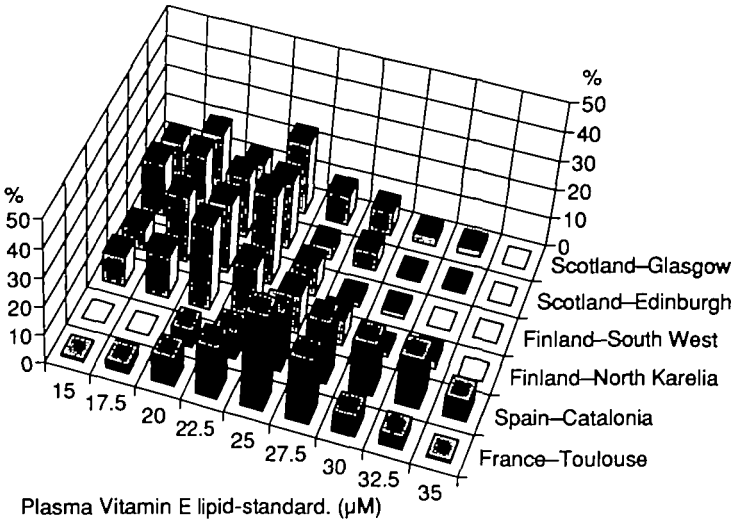


**Fig. 4** Different distribution of lipid-standardized plasma vitamin E values in populations with high IHD risk (Scotland – Glasgow, Edinburgh; Finland – South West and North Karelia) and low IHD risk (Spain – Catalonia, France – Toulouse): Cross-cultural comparisons of the Vitamin Substudy MONICA Project. The altitude of the columns represents the % of subjects within any study population (Ordinate) with different levels of plasma levels of lipid-standardized vitamin E (Abscissa).

as compared to high antioxidant levels (Fig. 6). Whereas the increased risk of low vitamin C and carotene were confounded by (and most likely mainly due to) cigarette smoking, the statistically-significant linear risk attributable to low vitamin E was independent of classical risk factors.[55] If in these Scotsmen the IHD risk of smoking is mainly mediated by poor levels of vitamin C and carotene[56] the rank order of essential antioxidants should be similar to that in the cross-cultural MONICA Vitamin Substudy, ie vitamin E >vitamin C = carotene >vitamin A.

## Prospective data

In the 12-year follow-up of initially healthy men in the above mentioned Basle Prospective Study the lowest quartile of lipid-standardized plasma carotene or of vitamin C showed (after adjustment for age, cholesterol and smoking) a statistically-significant association with an increased IHD mortality (relative risk of low carotene 1.53, of low vitamin C 1.25, and the combination of both 1.96; $P$=0.02).[12] Interestingly, death by stroke behaved correspondingly (relative risk of low carotene 2.07, low vitamin C 1.28, of low levels of both 4.17; $P$<0.01 for overmultiplica-

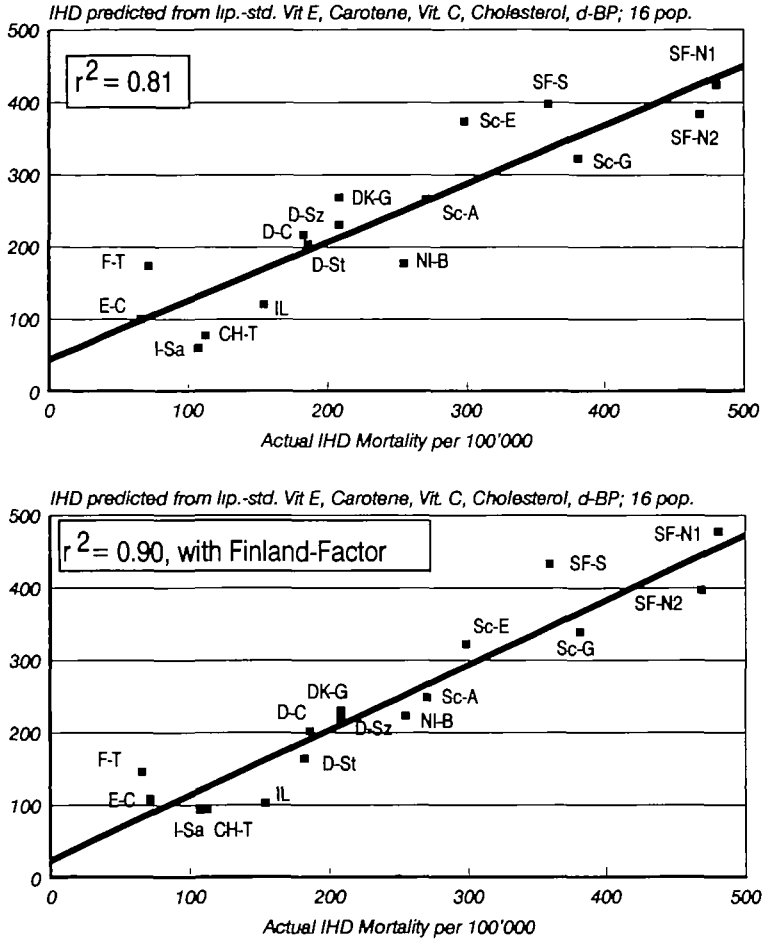


**Fig. 5** Correlation between actual age-specific IHD mortality in middle-aged males of 16 European study populations of the Vitamin Substudy of the MONICA/WHO Project (Abscissa) and the IHD mortality predicted by the combination of lipid-standardized vitamins E, total cholesterol, carotene, vitamin C and diastolic blood pressure in multiple regression analysis (Ordinate). Recalculation of basic data from a previous publication.[11] Upper graph: the combination of the variables mentioned above predicts 81% of the actual IHD mortality. Lower graph: additional inclusion of 'Finland factor' into the model of multiple regression analysis increases the predictive power of the model ($P$=0.057) to 90%.

tive interaction).[12] 'Optimal' vitamin A levels may reduce mortality and improve neurological recovery after stroke.[57] At vitamin E levels above 30 μmol/l no correlation has been found to death from CVD in the Basle Prospective Study,[11, 12] from IHD in a prospective Dutch study[58] and for survival after stroke[57] as may be expected from (as discussed above)

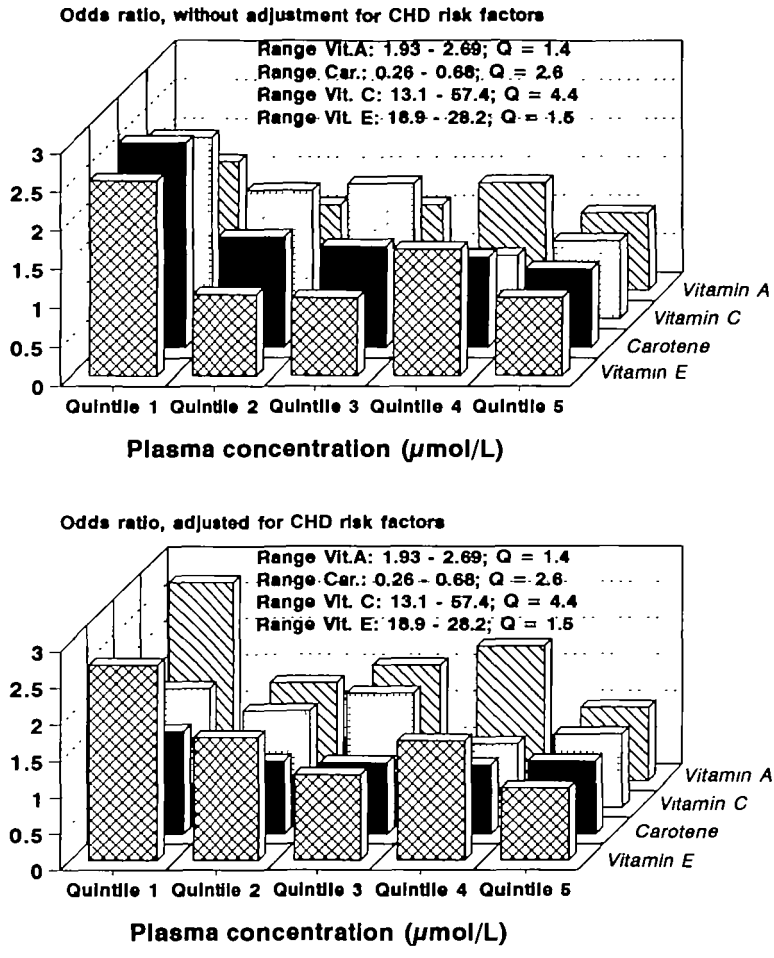


**Fig. 6** Increased risk of previously undiagnosed angina pectoris in Edinburgh, Scotland, at suboptimal plasma levels of essential antioxidants levels. The odds ratio (ordinate) was calculated in relation to the vitamin levels in the healthy control population. Upper graph: no adjustment for concurrent risk factors of IHD. Lower graph: adjustment by logistic regression for age, blood pressure, total plasma cholesterol and HDL cholesterol, triglycerides, body weight, season and smoking (the latter as major confounder being primarily responsible for the drop of carotene- and vitamin C-related risk by adjustment). Obviously a clear linear trend existed without adjustment for vitamins E, C and carotene but after the adjustment the linear trend remained statistically significant ($P$=0.02) only for vitamin E. 'Range' indicates plasma levels below the first and above the fifth quintile and their quotient Q.

uniformly high vitamin E levels in both study populations (mean 35 μmol/l being markedly above any critical threshold). In a prospective

Finnish study[59] the individual risk of IHD was not correlated to plasma vitamin E either but presumably the inter-individual variation of the notoriously poor vitamin E status in Finland[8–11, 14] was too small or within a low range of similarly increased IHD risk.

Taking the available (still incomplete) prospective findings together, they may concur with the working hypothesis of CVD-protective potentials of a high status of antioxidant vitamins. Broader prospective data may be expected from the Prime Study which currently compares middle-aged men in Northern Ireland (with poor antioxidant status but high IHD risk) and France (with high antioxidant status but low IHD risk).

## INTERVENTION TRIALS

Epidemiological observations, even if complementary and consistent, cannot reveal any causal relationship. The latter remains to be proven by properly designed population-based intervention trials which encounter, however, many problems, eg apparently healthy subjects at high risk (in primary prevention studies) may poorly comply in contrast to either health-oriented subjects at low risk or elderly patients (secondary prevention) whose disease might be too advanced and/or too heavily superimposed by age-related processes to be modified.

At present at least 20, mostly primary intervention trials in randomized subjects with high cancer risk (eg in smokers), are sponsored only by the National Cancer Institute, NIH, Bethesda, Maryland, testing cancer-preventive potentials of β-carotene and/or of vitamins A, C and E.[60] Only one trial, testing secondary prevention of non-melanoma skin cancer by β-carotene supplements (after surgical removal of the primary tumour) has been terminated: doubling of the initially rather high (presumably 'safe') β-carotene status for 6 years lacked any beneficial effect.[61] This could be related to various design problems:

- *no protective benefit when the plasma concentration already initially exceeds the risk threshold level?* Likely, by definition of threshold (meaning minimal risk),
- *testing a single component instead of an improved overall antioxidant status?*

Single components deserve great scientific interest but the chance of prevention may be greater for the combination of several synergistic antioxidants.[10, 17, 18, 62–67] The first step towards IHD prevention may be the combination of vitamins E, C and β-carotene[68]—logically aiming at multirisk factor intervention in the multifactorial multi-stage IHD.

- *supplementation period (of a few years) too short or (for secondary prevention) too late at fully developed disease (as established cancer)?*

This crucial question could be answered by successful primary prevention trials. Ideally any preventive measure should begin before initiation and promotion of cancerogenesis and of arteriosclerotic plaques, respectively, ie in childhood to early adulthood but this is unrealistic. Primary intervention trials are feasible only for about 6–8 years in middle-aged subjects shortly before the number of diagnosable cases will substantially increase, ie when 'sleeping' cancers start to progress (by infiltration or metastases) or/and when previously uncomplicated, asymptomatic arterial plaques are aggravated by age-related unspecific arterial thickening and sclerosis and thus progress faster into complicated 'abscess'-like lesions prone to fissures or eruption (causing coronary thrombosis and/or extreme vasospasms and ventricular fibrillation).

Results from several other cancer prevention trials can be expected within a few years. A fairly big cancer intervention study testing β-carotene and/or vitamin E in Finnish smokers may be evaluated about 1994.

Large-scale IHD-related interventions are also certainly warranted.[68] For primary prevention most conclusive results may be expected from an ascertained rise of plasma antioxidants in randomized subjects at high IHD risk at comparable age but poor antioxidant status. Secondary prevention in IHD patients may meet many more problems, eg risk of being too late (no experimental evidence has suggested regression of advanced arterial lesions by antioxidant vitamins), great inhomogeneity of complicated arterial lesions and their clinical symptoms, difficulties to interpret antioxidative interactions of concurrent drug therapy.[10] The high cost of intervention trials makes money-saving compromises and/or combinations tempting but after a badly-designed study with negative outcome there is little chance for another trial even if the latter is perfectly designed.

## THRESHOLD LEVELS OF PLASMA ANTIOXIDANT VITAMINS AND PRUDENT DIET

The IHD-related cross-cultural findings in the Vitamin Substudy of the WHO/MONICA Project[11, 54] and the individual data from the Edinburgh Case-Control Study on Previously Undiagnosed Angina Pectoris[65] suggest consistent thresholds of 'effective', ie presumably 'optimum' plasma levels which may be in the order of >28–30 μmol/l lipid-standardized vitamin E, >40–50 μmol/l vitamin C, >0.4–0.5

μmol/l (α- plus β-) carotene, >2.2–2.8 μmol/l lipid-standardized vitamin A. These levels agree also with individual data regarding both cancers[37] and CVD[12] in the Basle Prospective Study.

Although preventive potentials of an optimized antioxidant status remain still conclusively to be proven, the presently still incomplete observational data appear consistent enough for amendments of previous dietary guidelines. The latter have given general recommendations to lower dietary fat (particularly of mammalian origin) with partial replacement by monoene-rich vegetable oils and to increase the percentage of green-yellow vegetables/fruits. An updated prudent diet should more specifically aim at a daily intake of antioxidant micronutrients which can achieve the above mentioned 'safe' plasma levels: about 1 mg vitamin A (1 RDA), 60–250 mg vitamin C (1–4 times the present RDA), at least 60–100 IU vitamin E (4–6 times the present RDA) and about 6–15 mg β-carotene.[10, 31, 69] These suggestions may, of course, require adjustments for actual individual requirements, eg the desirable plasma status of vitamin E should be defined not regarding concurrent plasma lipids (as in common standardizations) but more specifically by the vitamin E/PUFA ratio,[14] similarly, the 'efficacy' of plasma vitamin C or β-carotene should also consider any extra source of exogenous radicals, eg dietary oxidized lipids, smoking-derived and other environmental radicals. Future studies will be needed on whether the WHO goal of 'optimum human health' requires also 'effective' plasma levels of oxygen-sensitive (presumably vitamin C-protected) micronutrients such as folic acid or vitamin $B_6$[17] as well as a minimal intake of possibly supportive plant antioxidants such as phenols/bioflavonoids[64] or carotenoids other than β-carotene.[3, 20, 50]

A different rank order of essential antioxidants regarding CVD and cancer (and further variation by cancer site) and the likelihood of synergistic interactions between all antioxidants suggests that public health measures should not improve the status of any single antioxidant but rather aim at a general optimization of the overall antioxidant potential.[7] The latter may be possible by common diets with suitable amounts and variations of common fruit and vegetable items but in individuals or populations at risk special recommendations may be needed, based on the assay of the actual antioxidant status.

## CONCEIVABLE MECHANISMS OF ACTION OF DIETARY ANTIOXIDANTS IN DISEASE PREVENTION

### Cancer

The in vivo mechanisms by which principal essential antioxidants and singlet oxygen-quenching carotenoids could counteract mutagenesis and cancerogenesis are poorly understood. Radicals can certainly

damage DNA[70] but may independently act as tumour promoters.[71] Deficiency of vitamin C causes oxidative DNA damage (eg 8-hydroxydeoxyguanosine[5]) but impairs also immunoresponses.[5, 16, 17] Improvement of the latter and possibly of overall tumour surveillance has actually been reported for all essential antioxidants.[16, 18] Experimental vitamin A deficiency is well known to result in metaplasia and facilitation of experimental tumours and, in the human, vitamin A and/or β-carotene reverse the precancerous leukoplakia and reduce the occurrence of pathological micronuclei even on continuous exposure to mutagens from betel nuts and/or tobacco chewing.[72] The conceivable anti-cancer properties of β-carotene are presumably independent from its potential function as provitamin A.[17] The protective effects of vitamin A (as related retinoids) and in part also of β-carotene may be due to direct modulation of the expression of proto- and antioncogenes although the intriguing underlying mechanisms are unclear.[73] All essential antioxidants might also, at least in part, modulate secondary messengers, eg by inhibition of adenylate cyclase.[74] In some organs special mechanisms may be of additional importance, eg protection of the gastrointestinal tract against mutagens by vitamins C and E through reduction of nitrosamine formation[16, 75] or diminution of endogenous faecal mutagens.[16, 17] In conclusion, the previously–studied principal antioxidants may reduce tumour initiation and/or promotion by several mechanisms. Cancer-preventive potentials remain further to be explored for a series of hitherto widely neglected compounds from plants, eg carotenoids other than β-carotene such as lycopene[2, 3, 20, 50] as well as the large fraction of mostly poorly absorbed but potent antioxidants and metal chelators, such as common phenols and bioflavonoids.[64] Special attention should be devoted to some oxygen radical-sensitive B-vitamins such as folate and vitamin $B_6$ which might require protection by a fair vitamin C status[5, 17, 67] and which are required to prevent chromosomal damage and metaplasia.[17]

**Cardiovascular disease**

Although arteriosclerosis-like lesions due to chronic marginal deficiency of vitamins C and E have been described in several animal species[10] the mechanisms of action have not been clarified. Free radical-induced oxidation of low density lipoproteins (LDL) can clearly provoke unregulated lipid accumulation in monocyte-derived macrophages and thus presumably initiate atherogenesis by the formation of foam cells and fatty streaks.[76, 77] The quantitative importance of oxidative LDL modifications in vivo, however, is still uncertain. Thus the (copper-induced) oxidation rate of isolated LDL correlates only weakly ($r^2$=0.2) with coronary atherosclerosis in young survivors of myocar-

dial infarction,[78] and the considerable inter-individual variation of the oxidative susceptibility of isolated LDL from healthy volunteers is normally not determined by its vitamin E content, although experimental doubling of vitamin E in LDL can markedly improve the oxidation resistance of isolated LDL, half of which is then explained by vitamin E.[79] Since the first antioxidative defence line of plasma lipoproteins consists actually of vitamin C[4, 12, 79] it remains to be elucidated whether the oxidation resistance of LDL in plasma depends in vivo on vitamin C rather than on vitamin E but this may also be true for ubiquinol-10,[81] an endogenous antioxidant that can be increased by supplementation. Further exploration is merited on the vitamin C-protected B-vitamins such as folate, which normalizes moderate homocysteinemia[82] and thus another strong risk factor of CVD which is independent from classical risk factors. Although protection of LDL has recently gained wide interest as new potential antiatherosclerotic mechanism, essential antioxidants have been reported to have many other beneficial effects that could also counteract numerous crucial steps in atherogenesis[10]:

- in endothelium, eg inhibition of lipoxygenase(s) and thus of oxidative LDL modification,[76, 77] prevention of cellular transitions, reduction of monocyte adhesion
- in macrophages, eg modulation of their migration into the subintimal space, reduction of the LDL-modifying 'respiratory burst' of radicals; improvement of the catabolism of modified LDL by resident macrophages resulting in diminished foam cell formation, reduced production of crucial cytokines such as interleukin-1 and thus regulation of cell-cell interactions
- in smooth muscle cells, eg inhibition of proliferation and of the signal transducing protein kinase
- in blood platelets, eg inhibition of adhesion
- improvements of immunoresponses.

## CONCLUSIONS

The observational data reviewed above may corroborate the working hypothesis that in the human a suboptimal status of essential antioxidants (although with different rank orders for both CVD and cancer as well as cancer sites) is involved in both cancerogenesis and atherogenesis. Thus preventive potentials of essential antioxidants may be expected which are, however, hardly restricted to single specific compounds but rather due to various synergistic interactions of all antioxidant micronutrients. Correspondingly, a variety of sequential and interdependent mechanisms of action is conceivable for all essential

antioxidants in both diseases and may even be needed since both cancerogenesis and atherogenesis have been accepted to be multifactorial multi-stage processes. In consequence a steady optimization of the overall antioxidant potential (possibly still to be supplemented by other plant components) rather than optimization of any single component seems at present to offer the best preventive prospects. The above-described observational data justify current and forthcoming intervention trials which, if properly designed, may be hoped to verify the actual preventive potentials of innocuous antioxidant micronutrients. All prophylactic measures deserve high priority because of the great limitations in the therapy of both major causes of death.

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