Lipids, Lipoproteins and Antioxidants in Cardiovascular Dysfunction

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The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms

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Summary
Plasma levels of major essential antioxidants were determined in representative random samples of middle-aged men from 16 European study populations which differed up to 6-fold in age-specific mortality from ischaemic heart disease (IHD). In 12 study populations having total plasma cholesterol in the medium range (5.7–6.2 mmol/l) and usual blood pressure, both these classical risk factors lacked a significant correlation to IHD mortality, whereas the absolute level of vitamin E (α-tocopherol) showed a strong inverse correlation ($r = 0.63, P = 0.002$). On evaluation of all study populations, cholesterol and diastolic blood pressure had a moderate direct association with IHD, but their importance still remained inferior to that of vitamin E as an inversely associated, presumably protective factor. In stepwise regression and multiple regression analysis, the IHD mortality of the study populations was predictable to 62% by lipid-standardized vitamin E, to 79% by vitamin E and total cholesterol, to 83% after inclusion of lipid-standardized vitamin A (retinol) and to 87% by all the above parameters plus diastolic blood pressure. In conclusion, in the present study the plasma status of vitamin E is the most important factor to explain cross-cultural differences of IHD mortality. This finding is consistent with the hypothesis of the prevention of arteriosclerosis by antioxidant protection against oxidative lipoprotein modification, but does not exclude additional effects of antioxidant vitamins, e.g. on the cellular or immunological level.

Introduction
The risk of the majority of individuals of dying from IHD can only be ascribed to an elevated plasma level of total cholesterol, of low-density lipoprotein (LDL) cholesterol or of the LDL/HDL (high-density lipoprotein) ratio, even if combined with other classical risk factors such as hypertension, smoking, etc. This seems also to be true for the thus far greatest cross-cultural comparison of IHD mortality in the W.H.O./Monica core study (A. Stewart & K. Kuulasmaa, unpublished work). In consequence, the IDH

Abbreviations used: Apo B, LDL-apoprotein B; HDL, high-density lipoprotein; IHD, ischaemic heart disease (ICD 410–414); LDL, low-density lipoprotein; PUFAs, polyunsaturated fatty acids.

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most apolar, LDL-fraction may form some kind of ultimate barrier against the modification of the LDL (apo-B) [16–18] which may actually be caused to a great extent by hydroxyalkenals and/or malondialdehyde, i.e. by fragments of peroxidized PUFAs [16–28]. Correspondingly, LDL can be modified by incubation with cells which can produce oxygen radicals (endothelial cells, smooth muscle cells, monocytes and neutrophils) and this oxidative modification, as well as the cytotoxicity, can again be counteracted by vitamin E [28–31]. The level of malondialdehyde-containing proteins of extravascular tissues have been reported to be inversely related to the vitamin E status as well [32]. Peroxide- or peroxidized PUFAs which may actually be caused to a great extent by hydroxyaldehyde-modified LDL, in contrast to normal LDL, is taken up by monocytes/macrophages which can thereby be transformed into arteriosclerotic lipid-laden foam cells [18, 19, 21–31, 33–37]. This transformation has been assumed to be an early, if not even initial step, in the formation of the arteriosclerotic plaque [1, 13–29]. The degradation of LDL by macrophages may, at least in part, depend on the vitamin E status of LDL [36, 37]. In arteriosclerotic lesions of man and in the arteriosclerotic aorta of the Watanabe hereditary hyperlipaemic rabbit, malondialdehyde-modified and 4-hydroxynonenal-modified apo-B has been demonstrated, which is bound/degraded by macrophages [21–27]. The high immunogenicity of oxidatively modified LDL might modulate atherogenesis as well [24].

5. Probiocol, a drug with radical scavenging phenol structure, reduces (in part presumably independent of its hypcholesterolaemic potential) LDL modification and lipid storage in macrophages, as well as the progression of arteriosclerosis in the Watanabe heritable hyperlipaemic rabbit [38–41]. Thereby, probiocol may preserve plasma vitamin E (B. Finckh, M. Rath, A. Niendorf & U. Beissel, unpublished work).

6. Modified LDL was reported to occur regularly in small amounts in human plasma [43] and/or preferentially in subjects with known IHD [44]. Modified LDL can be significantly diminished by oral supplements of vitamin E [45]. Autoantibodies against modified LDL or corresponding proteins can occur regularly in human plasma [24, 25].

7. Patients with IHD have increased plasma levels of thiobarbituric acid-reactive material [1, 46], i.e. an indicator of increased susceptibility of LDL towards lipid peroxidation, which can be decreased by vitamins C and/or E [47].

8. A low plasma level of lipid-standardized vitamin E and of vitamin C is a risk factor in early angina pectoris [48], and arteriographically established IHD may be inversely related to plasma vitamin C [49].

Cross-cultural epidemiology

All the above-mentioned suggestive evidence leads to the crucial question of whether in the human a suboptimal status of antioxidant vitamins is a quantitatively important risk factor of IHD. Since the vitamin status is most properly defined by the measurement of plasma levels it is tempting to compare the well-known great demographic differences in IHD mortality with the level of antioxidant vitamins in plasma. This has actually been done in the current Collaborative Study on Antioxidant Vitamins and PUFAs of the WHO/Monica Project (initiated as the International Collaborative Study on the Fatty Acid-Antioxidant Hypothesis of Arteriosclerosis). Up to now, all essential antioxidants of plasma have been compared in 16 European populations having 6-fold differences in age-specific mortality from IHD. The interim results have shown [1, 50–54] that primarily vitamin E, is of all the essential antioxidants, the strongest inverse predictive factor of cross-cultural IHD mortality, but also that in some populations the relative risk due to a low vitamin E status may have greater quantitative importance than that of classical risk factors such as higher total cholesterol and blood pressure. The study samples consisted, in principle, of the status of the major essential antioxidants, i.e. the vitamins A, C and E, carotene, other major carotenoids, and selenium in about 100 randomized apparently healthy males, of 40–49 years of age, which represented (with a response rate > 60%) regions with different incidences of IHD mortality (mean values of at least three preceding years):

1. Highest incidence, i.e. yearly > 260 deaths from IHD (ICD 410–414) per 100 000 males, 40–59 years of age: North Karelia, Finland (481 in 1983 in rural North Karelia and 469 in 1987 in rural/hemispheric North Karelia); Graz, Austria (381); Edinburgh, Scotland (298); Aberdeen, Scotland (270).

2. Medium incidence, i.e. approx. 150–259/100 000: peripheral Belfast, Northern Ireland (254); Glostrup, Copenhagen, Denmark (208) = Schleiz. G.D.R. (208) = Gotchhus, G.D.R. (182) = Schwedt, G.D.R. (186) = Tel Aviv, Israel (154).

3. Low incidence, i.e. approx. 65–115/100 000: Thun, foothills of the Alps in Switzerland (112); Sarpi, Southern Italy (107) = Haute Garonne-Toulouse, France (72) and Catalonia, Spain (66).

More details and analytical methods for plasma vitamins A (retinol), C (ascorbic acid) and E (α-tocopherol) and the carotene fraction have been described elsewhere [1, 50–54].

In individuals (n = 1796 or 1954) biologically significant Spearman’s rank correlation coefficients (rs > 0.3) occurred among essential antioxidants between absolute plasma vitamin A (retinol) and absolute vitamin E (α-tocopherol). rS = 0.63; P = 0.0001, as well as between these lipid-soluble vitamins and cholesterol (rS = 0.24 and 0.54, respectively; both P = 0.0001), and triacylglycerols (rS = 0.37 and 0.47; P = 0.0001). Therefore, and because of biological reasons, plasma vitamin E as well as vitamin A were standardized stepwise (based on a 2-dimensional regression plane) to a plasma level of 5.7 mmol of cholesterol/l (220 mg/dl) and independently to a plasma level of 1.25 mmol of triacylglycerols/l (110 mg/dl), i.e. to the most common ‘normal’ lipid levels of European populations [54]. Thereafter the regression coefficient of cholesterol and triacylglycerols, respectively (based on 1950 plasma samples of the pooled data from all study populations) was as low as rS = 0.0075 (P = 0.74) and −0.02 (P = 0.08), respectively, for lipid-standardized α-tocopherol, and −0.021 (P = 0.40) and −0.01 (P = 0.57), respectively, for lipid-standardized retinol.

Thus, both vitamins became completely independent of cholesterol and triacylglycerols, respectively, and lipid-standardized vitamins A and E varied practically independently (rS = 0.10; P = 0.0001). This confirms that vitamin E varies independently from classical risk factors of IHD [55].

In the presently available 16 study populations two classical risk factors of IHD, i.e. total plasma cholesterol (r = 0.29; Table 1) and blood pressure [diastolic r = 0.25, systolic r = 0.19; Table 1] had a statistically significant weak direct association with IHD, whereas the habit of smoking (Table 1) lacked any significant correlation. When total cholesterol and diastolic blood pressure were combined in multivariate regression analysis, its correlation with IHD mortality remained moderate (r = 0.44; P = 0.02). The same was true for the combination of cholesterol, blood pressure and smoking (r = 0.46; P = 0.051).

Thus, in the present 16 European study populations [54] the relative importance of the above-mentioned classical risk factors was even higher than in the presently available 33 study populations of the W.H.O. Monica core study from all over the world (A. Stewart & K. Kuulasmaa, unpublished work).

In a major cluster, i.e. in 12 out of 16 study populations, the total plasma cholesterol ranged in a narrow band of usual
levels, i.e. at 5.7–6.2 mmol/l (220–240 mg/dl). In this subgroup of populations, total cholesterol lacked any statistically significant direct correlation with IHD mortality \( (r^2 = 0.04, \text{Table 1}) \) as had to be expected, but this was also true for blood pressure (diastolic \( r^2 = 0.08, \text{systolic } r^2 = 0.01; \text{Table 1} \), and for the combination in multivariate analysis of total cholesterol and diastolic blood pressure \( (r^2 = 0.10, P = 0.02) \) in this major subgroup. Since the effect of these classical risk factors is similar in this subgroup its 6-fold differences in IHD mortality must mainly be due to other factors.

In all populations \((n = 16)\), as well as in the major subgroup \((n = 12)\), most antioxidant vitamins showed in the univariate analysis an inverse correlation with IHD mortality, although with great differences. Vitamin E dominated by far, when regarding both the highly significant correlation coefficient of lipid-standardized levels \( (r^2 = 0.62) \) and 0.73 respectively; Table 1, Fig. 1), as well as of absolute levels in the population subgroup with usual cholesterol levels \( (r^2 = 0.63) \). Absolute vitamin E values may only be compared if the plasma lipids are as similar (Fig. 1) as in the subgroup \((n = 12)\). Since the medians of logarithms of vitamin E reveal practically the same inverse correlation to IHD as lipid-standardized vitamin E (Table 1, Fig. 1), or as vitamin E with previous simpler ways of lipid-standardization \([1, 50–53]\), any conceivable bias due to the lipid-standardization of vitamin E may lack any major importance. Vitamin C showed a moderately strong statistically significant inverse correlation with IHD mortality in the major subgroup of populations \( (r^2 = 0.41; \text{Table 1}) \), whereas the lipid-standardized level of vitamin A showed a weak inverse correlation only with the IHD mortality of all populations \( (r^2 = 0.24; \text{Table 1}) \). In conclusion, the interim results of the present univariate analysis reconfirm the dominant role of vitamin E, and reveal again additional weak inverse correlations of lipid-standardized vitamin A and of vitamin C \([1, 50–54]\).

Partial regression analysis (with fixation of the classical risk factors, cholesterol, diastolic blood pressure and smoking) as well as stepwise regression analysis confirmed the top rank of vitamin E as an essential antioxidant \([54]\). With the total of populations \((n = 16)\) lipid-standardized vitamin E \( (r^2 = 0.62) \) was complemented first by total cholesterol \( (r^2 = 0.79, P = 0.008 \text{ for } \text{enlarging the model}) \), then by lipid-standardized vitamin A \( (r^2 = 0.83) \) and finally by diastolic blood pressure \( (r^2 = 0.87) \). Thus, these four variables predicted the actual IHD mortality to 87\% (Fig. 2).

### Table 1. Pearson’s correlation coefficients in univariate analysis between age-specific IHD mortality of study populations and various parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Populations with usual cholesterol ((n = 12))</th>
<th>All populations ((n = 16))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r^2 )</td>
<td>( P )</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.04</td>
<td>0.53</td>
</tr>
<tr>
<td>Blood pressure, systolic</td>
<td>0.01</td>
<td>0.80</td>
</tr>
<tr>
<td>Blood pressure, diastolic</td>
<td>0.08</td>
<td>0.36</td>
</tr>
<tr>
<td>% Smokers</td>
<td>0.002</td>
<td>0.90</td>
</tr>
<tr>
<td>% Smokers (folded log)</td>
<td>0.002</td>
<td>0.89</td>
</tr>
<tr>
<td>Cigarettes/study subject</td>
<td>0.004</td>
<td>0.85</td>
</tr>
<tr>
<td>Vit. A, absolute</td>
<td>0.22</td>
<td>0.13</td>
</tr>
<tr>
<td>Vit. A, lipid-standard</td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td>Vit. E, absolute</td>
<td>0.63</td>
<td>0.002</td>
</tr>
<tr>
<td>Vit. E, lipid-standard</td>
<td>0.73</td>
<td>0.0004</td>
</tr>
<tr>
<td>Carotene, absolute</td>
<td>0.21</td>
<td>0.14</td>
</tr>
<tr>
<td>Selenium ((n = 8/12))</td>
<td>0.05</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Fig. 1. Relation between IHD mortality and α-tocopherol

Inversely correlations between age-specific IHD mortality and the medians of logarithms of (a) absolute α-tocopherol, as well as, (b) lipid-standardized α-tocopherol in 12 study samples with usual cholesterol (5.7–6.2 mmol/l = 220–240 mg/dl) and with lipid-standardized α-tocopherol respectively (round solid centre points, dotted regression lines). (b) The solid regression line represents all 16 study samples, i.e. with inclusion of hypo- and hypercholesterolaemia study samples. \( r^2 \) is a measure for the explained portion of total variance. Abbreviations: CH-T, Switzerland, Thun; DDR-C, -St and -SZ, German Democratic Republic, Cottbus, Schwedt and Schleiz; DK-G, Denmark, Glosestrup/Copenhagen; E, Spain, Catalunya; F, France, Toulouse/Haute Garonne; IL, Israel; Tel Aviv; I-Sa, Italy, Sapri; NI-B, Northern Ireland, semi-urban Belfast; SC-A, -E and -G, Scotland, Aberdeen and Edinburgh and Glasgow; SF-N1, -N2, -S, Finland rural North Karelia 1 (1983), rural/semiurban North Karelia 2 (1987) and rural Southwest Finland. From \([54]\) with permission.

**Discussion**

The present cross-cultural data of European study populations if taken in conjunction with all other data (see Introduction) become persuasive evidence that essential antioxidants, mainly vitamin E, are important, hitherto underrated factors for IHD which may substantially counteract the previously known, classical risk factors, particularly hypercholesterolaemia and hypertension. The antioxidant hypothesis of IHD (postulating antioxidant protection of LDL and possibly of other lipid compartments against peroxidative modification) does not contradict, but rather complements the classical cholesterol hypothesis. Thus the IHD risk may not only be related to the plasma level of total cholesterol and/or cholesterol- and PUFA-rich lipoproteins, but also to the relative number of protective antioxidant molecules, e.g. of α-tocopherol and carotenoids.
will, like oxidatively-modified LDL, accumulate in arterial cell types, e.g. by modulation of the eicosanoid formation by elasticity and permeability of the arterial wall but some tion, such as foam cell formation by intracellular accumula-
tion, as that of erythrocytes [56], against lipid peroxidation the susceptibility of LDL also depends considerably on the level of other lipid-soluble antioxidants, e.g. carotenoids [16-19, 57]. Therefore, the latter will have to be differentiated in more detail. Other antioxidative synergists remain to be elucidated. Vitamin C, the ‘first line of antioxidative defence’ in the water-soluble phase [15-18] presumably protects LDL by the regeneration of vitamin E at the interphase [58-59], and vitamin A is a particular thyl radical scavenger [60].

Of course, essential antioxidants may not only protect double bonds of LDL-lipids but presumably also those in cellular membrane lipid mono- and bilayers. An increased uptake of either essential antioxidant by cells may substan-
tially increase their antioxidant potential, and in consequence perhaps help to overcome critical phases of plaque formation, such as foam cell formation by intracellular accumulation of modified LDL taken up by monocytes/macrophages. Vitamin E in particular may also be able to specifically exert some well-known antiarteriosclerotic functions in various cell types, e.g. by modulation of the eicosanoid formation by inhibition of phospholipase A2 [61], increased prostacyclin formation [62-64] and/or inhibition of lipoxygenase [65], and/or by alteration of immunoresponses in general [66, 67] and perhaps also to oxidatively-modified LDL, of platelet behaviour [68], of the stabilization of membrane structures [63], of deactivation of protein kinase C [69]. Vitamin A might complement antioxidants since it is an extremely potent regulator of cell differentiation, being able to modify the expression of several proteins including arterial glyco-
proteins [70]. The latter do not only determine the visco-
elasticity and permeability of the arterial wall but some subfractions, e.g. chondroitin sulphate- and heparan sulphate proteinglycans can easily form insoluble complexes which will, like oxidatively-modified LDL, accumulate in arterial macrophages/foam cells [71]. Last, but not least, vitamin A, like so many antioxidants, may also regulate immuno-
response [70].

The available cross-cultural data clearly suggest optimal level of plasma antioxidants, particularly vitamin E, as potential beneficial factors or even preventors of IHD. Their actual IHD-preventive potentials, however, still await experi-
mental proof by population-based controlled intervention trials with specific supplements of these potential IHD preventors. Unfortunately it may be difficult to design at present. Two prospective epidemiological studies of the blood-bank type have not indicated a dependence of the very last phase of IHD on the vitamin E status [72, 73]. Although these data must be considered with great reservation because of several methodological problems [54], they could suggest that antioxidants may be most important at earlier phases of the disease, e.g. those which are still in part reversible such as fatty streaks. This would be compatible with the present state of knowledge (see Introduction) as well as with the fact that the vitamin E status in the Swiss population of the present epidemiological study does not vary significantly between early adulthood and the sixth decade of life (K. F. Gey & P. Jordan, unpublished work). Nevertheless, at least early angi a pectoris seems still to be related to low levels of plasma antioxidants [48].


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Fish oil fatty acids and cardiovascular function: epidemiology and biochemical mechanisms

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Introduction

Physiological and pathophysiological reactions like vascular resistance, thrombosis, wound healing, inflammation and allergy are modulated by oxygenated metabolites of arachidonic acid (AA; 20:4, n=6 or 20:4, ω-6) and related polyunsaturated fatty acids that are collectively termed eicosanoids. These compounds include prostaglandins (PGs), prostacyclin (PGI), thromboxane (TXA), leukotrienes and hydroxylated derivatives (epoxides) of AA. Precursors of cellular eicosanoids are essential fatty acids related polyunsaturated fatty acids that are collectively termed eicosanoids. These compounds include prostaglandins (PGs), prostacyclin (PGI), thromboxane (TXA), leukotrienes and hydroxylated derivatives (epoxides) of AA. Precursors of cellular eicosanoids are essential fatty acids composed primarily of phospholipids and cholesterol. Precursors that have important cellular signalling functions such as receptors, transporters and enzymes are embedded in the lipid bilayer. Although the following review will focus

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