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Do antioxidants and polyunsaturated fatty acids have a combined association with coronary atherosclerosis?

Frans J. Kok [1], Geert van Poppel [1], Johan Melse [2], Ellen Verheul [2], Evert G. Schouten [2], Dick H.C.M. Kruyssen [3] and Albert Hofman [4]

[1] *Epidemiology Section, TNO-CIVO Toxicology and Nutrition Institute, Zeist,* [2] *Department of Environmental and Tropical Health, Agricultural University, Wageningen,* [3] *Department of Cardiology, Zuiderziekenhuis Hospital, Rotterdam and* [4] *Department of Epidemiology, Erasmus University Medical School, Rotterdam (The Netherlands)*

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Summary

To evaluate the antioxidant hypothesis with regard to atherosclerosis, we compared plasma selenium, serum α-tocopherol, serum polyunsaturated fatty acids (PUFA), and the ratios of selenium and α-tocopherol to PUFAs in subjects with varying degrees of coronary atherosclerosis. Cases had more than 85% stenosis in at least one coronary vessel and controls had less than 50% stenosis in all three vessels. Plasma selenium was significantly lower in cases than controls (95.1 ± 21.0 μg/l and 108.8 ± 29.3 μg/l, respectively). Though α-tocopherol and PUFA levels were similar in both groups, the ratios Se/linoleic acid, Se/total PUFA and Se/total $n-6$ acids were significantly lower in cases. In particular, these differences were observed in subjects with low serum α-tocopherol level (below the median; 1452 μg/dl). Moreover, in this subgroup the ratio Se/PUFA was significantly lower in cases than in controls for all PUFAs except eicosapentaenoic and docosahexaenoic acid. Though definitive conclusions cannot be drawn from our data, it is hypothesized that high PUFA levels, when insufficiently protected by antioxidants against peroxidation, may indicate a higher risk of atherosclerosis.

Key words: α-Tocopherol; Selenium; Polyunsaturated fatty acid; Coronary atherosclerosis; Angiography; Lipid peroxidation; Antioxidant

Introduction

Lipid peroxidation reactions may have an important role in the pathogenesis of atherosclerosis [1–3]. Increased lipid peroxidation products have been observed in plasma of patients with occlusive arterial disease [4] and other vascular complications [5,6]. Oxidative injury of endothelial cells membranes may be an important mechanism [1,2]. In addition, recent investigations have strongly implicated lipid peroxidation of low-density lipoprotein in atherogenesis. Oxidized LDL is taken up rapidly by macrophages by means of the

Correspondence to: Dr. Frans J. Kok, Epidemiology Section, TNO-CIVO Toxicology and Nutrition Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

scavenger receptor; macrophages are thus converted to foam cells of the fatty streaks. Moreover, oxidized LDL is cytotoxic and may thus cause denudation of the endothelium [3].

Polyunsaturated fatty acids (PUFA) in membranes [2] or lipoproteins [3] are the main substrates for oxidation reactions. Antioxidants, e.g. α-tocopherol and the selenium-dependent enzyme glutathione peroxidase, inhibit oxidative reactions by scavenging oxygen-derived free radicals and/or by interfering with the chain reaction of peroxidation [7]. The antioxidant hypothesis with regard to atherosclerosis, therefore, imply an adequate antioxidant status in order to protect polyunsaturated fatty acids from peroxidation. To evaluate this hypothesis, we compared plasma selenium, serum α-tocopherol and PUFA levels in patients with varying degrees of coronary atherosclerosis.

Subjects and methods

Study population

The study was conducted in 1986–1987 in the Zuiderziekenhuis hospital, Rotterdam, The Netherlands. Of all 562 patients who had coronary angiography in the study period because of possible ischaemic heart disease, 47 patients refused participation, while 266 were not eligible for the following reasons: age over 75 years ($n = 7$), not of Dutch nationality ($n = 24$), under medical treatment for longer than 2 years ($n = 111$) and history of myocardial infarction longer than 3 months before angiography ($n = 124$). The latter two criteria were chosen because of possible drug and dietary influences. From the remaining patients ($n = 249$), cases were selected on the basis of having more than 85% stenosis in at least one coronary vessel, and controls on having less than 50% stenosis in all three vessels, leaving 163 subjects for data analysis; 91 cases and 72 controls. Prior to the angiography results, information on medical history, use of medication, smoking habits and alcohol use was collected. In addition, a non-fasted venous blood sample and anthropometric and blood pressure measurements were taken.

Laboratory analysis

Selenium concentration was measured by neutron activation analysis after freeze-drying of aliquots of 0.75 ml of plasma as previously described [8]. α-Tocopherol was determined by high-pressure liquid chromatography [9]. The instrumentation (Merck-Hitachi 655A-11,22; E. Merck, Amsterdam, The Netherlands) was equipped with reversed phase and guard columns of 30 × 4 mm (Merck-Hibar LiChro CART RP 18, E. Merck, Amsterdam, The Netherlands). Total serum lipids were determined according to the method of Folch et al. [10]. Serum fatty acids were determined as their methyl esters with gas-liquid

TABLE 1

CARDIOVASCULAR RISK FACTORS AND MEDICAL HISTORY OF PATIENTS WITH DIFFERENT DEGREES OF CORONARY ATHEROSCLEROSIS

Mean ± SD.

	Cases ($n = 91$) [a]	Controls ($n = 72$) [a]
Age (years)	54.8 ± 8.8	53.3 ± 9.2
Serum total cholesterol (mmol/l)	7.4 ± 1.5	7.0 ± 1.3 [b]
Serum LDL-cholesterol (mmol/l)	5.6 ± 1.5	5.1 ± 1.4 [b]
Serum HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.2 ± 0.3 [b]
Serum total triglycerides (mmol/l)	2.2 ± 1.0	2.1 ± 1.2
Systolic blood pressure (mmHg)	130 ± 22	136 ± 22
Diastolic blood pressure (mmHg)	80 ± 13	86 ± 13 [b]
Body mass index (kg/m2)	25.8 ± 2.4	26.0 ± 2.8
Proportion (%) of		
Males	70	60
Cigarette smokers	28	28
Past smokers	62	44 [b]
Alcohol drinkers	62	67
Use of antihypertensive medication	36	35
History of hypertension	48	42
History of myocardial infarction	35	15 [b]
Family history of myocardial infarction	34	31
Family history of bypass surgery	14	7

[a] Cases: > 85% stenosis in at least one coronary vessel; controls: < 50% stenosis in all 3 vessels. Some of the individual parameters have missing data.

[b] Student's *t*-test for unpaired samples ($P < 0.05$).

chromatography. The chromatograph (Carlo Erba, 4160 series) was equipped with a 50-m capillary column and a CPSIL 88 stationary phase (Crompack, The Netherlands) and a temperature programming from 70 to 200 °C.

Data analysis

First, baseline characteristics and levels of selenium, α-tocopherol and PUFAs were compared. Second, to study relationships between PUFA and antioxidant status, the ratio of selenium and α-tocopherol to PUFA levels was calculated and compared between both groups. Finally, synergism between selenium and α-tocopherol with respect to protection of PUFAs was evaluated by comparing the ratio Se/PUFA between cases and controls in subgroups with either low or high α-tocopherol levels (division at the median). The Student *t*-test for unpaired samples was used for statistical testing.

TABLE 3

RATIOS OF PLASMA α-TOCOPHEROL (μg/dl) TO PLASMA FATTY ACIDS (μmol/l) IN PATIENTS WITH DIFFERENT DEGREES OF CORONARY ATHEROSCLEROSIS

Mean ± SD.

	Cases ($n = 91$) [a]	Controls ($n = 72$) [a]
α-Tocopherol/linoleic	0.396	0.396
α-Tocopherol/arachidonic	2.321	2.144
α-Tocopherol/eicosapentaenoic	39.417	30.902
α-Tocopherol/docosahexaenoic	8.551	8.517
α-Tocopherol/total PUFA	0.277	0.292
α-Tocopherol/total $n-3$	4.601	4.187
α-Tocopherol/total $n-6$	0.300	0.313

[a] Cases: > 85% stenosis in at least one coronary vessel; controls: < 50% stenosis in all 3 vessels: Some of the individual parameters have missing data.

Results

Cardiovascular risk factors and medical history of cases and controls are presented in Table 1. Cases, compared to controls, had significantly ($P < 0.05$) higher levels of total cholesterol and LDL cholesterol and lower levels of diastolic blood pressure and HDL cholesterol. Among cases there were significantly more past smokers. On average, past smokers stopped smoking 3.7 ± 1.8 years ago. Table 2 shows that plasma selenium levels were significantly lower in cases than in controls. No important differences in serum α-tocopherol (with

TABLE 2

PLASMA SELENIUM, SERUM α-TOCOPHEROL AND SERUM FATTY ACID LEVELS IN PATIENTS WITH DIFFERENT DEGREES OF CORONARY ATHEROSCLEROSIS

Means ± SD.

	Cases ($n = 91$) [a]	Controls ($n = 72$) [a]
Selenium (μg/l)	95.1 ± 21.0	108.8 ± 29.3 [b]
α-Tocopherol (μg/dl)	1526.8 ± 395.4	1513.3 ± 470.2
α-Tocopherol/cholesterol (μg/mmol)	2080.3 ± 540.2	2206.7 ± 779.7
Linoleic acid (μmol/l)	4099.5 ± 1050.5	3965.8 ± 1091.8
Arachidonic acid (μmol/l)	725.6 ± 208.7	736.8 ± 183.3
Eicosapentaenoic acid (μmol/l)	60.7 ± 40.7	63.4 ± 31.5
Docosahexaenoic acid (μmol/l)	218.1 ± 97.4	222.3 ± 90.4
Total polyunsaturated fatty acids (μmol/l)	5513.8 ± 1143.0	5260.9 ± 1218.1
Total $n-3$ fatty acids (μmol/l)	379.0 ± 138.6	379.7 ± 118.5
Total $n-6$ fatty acids (μmol/l)	5175.5 ± 1135.2	4954.7 ± 1242.1
P/S ratio	1.28 ± 0.25	1.25 ± 0.22

[a] Cases: > 85% stenosis in at least one coronary vessel; controls: < 50% stenosis in all 3 vessels. Some of the individual parameters have missing data.

[b] Student's *t*-test for unpaired samples ($P < 0.01$).

TABLE 4

RATIOS OF PLASMA SELENIUM (μg/l) TO PLASMA FATTY ACIDS (μmol/l) IN PATIENTS WITH DIFFERENT DEGREES OF CORONARY ATHEROSCLEROSIS

Mean ± SD.

	Cases ($n = 91$) [a]	Controls ($n = 72$) [a]
Se/linoleic	0.025	0.029 [b]
Se/arachidonic	0.145	0.156
Se/eicosapentaenoic	2.451	2.200
Se/docosahexaenoic	0.528	0.660
Se/total PUFA	0.018	0.021 [c]
Se/total $n-3$	0.286	0.307
Se/total $n-6$	0.019	0.023 [c]

[a] Cases: > 85% stenosis in at least one coronary vessel; controls: < 50% stenosis in all 3 vessels. Some of the individual parameters have missing data.
Student's t-test for unpaired samples: [b] $P < 0.05$ ([c] $P < 0.01$).

and without adjustment for cholesterol) or fatty acid levels were observed.

Ratios of plasma α-tocopherol to plasma fatty acids, shown in Table 3, did not differ substantially between cases and controls. The ratios Se/linoleic acid, Se/total PUFA and Se/total $n-6$ were lower in cases than in controls (Table 4). Tables 5 and 6 show that these differences occur only in the subgroup with low α-tocopherol levels (under the median; 1452 μg/dl). Moreover, in the subgroup with low α-tocopherol levels, the ratio Se/PUFA was significantly lower in cases than in controls for all PUFAs except eicosapentaenoic acid ($P = 0.66$) and docosahexaenoic acid ($P = 0.18$).

TABLE 5

RATIO'S OF PLASMA SELENIUM (μg/l) TO PLASMA FATTY ACIDS (μmol/l) IN PATIENTS WITH DIFFERENT DEGREES OF CORONARY ATHEROSCLEROSIS AND LOW α-TOCOPHEROL STATUS (PLASMA α-TOCOPHEROL < 1521 μg/dl)

Mean ± SD.

	Cases ($n = 48$) [a]	Controls ($n = 34$) [a]
Se/linoleic	0.026	0.032 [c]
Se/arachidonic	0.143	0.171 [b]
Se/eicosapentaenoic	2.256	2.408
Se/docosahexaenoic	0.502	0.848
Se/total PUFA	0.019	0.024 [c]
Se/total $n-3$	0.280	0.347 [b]
Se/total $n-6$	0.021	0.026 [c]

[a] Cases: > 85% stenosis in at least one coronary vessel; controls: < 50% stenosis in all 3 vessels. Some of the individual parameters have missing data.
Students's t-test for unpaired samples: [b] $P < 0.05$ ([c] $P < 0.01$).

TABLE 6

RATIOS OF PLASMA SELENIUM (μg/l) TO PLASMA FATTY ACIDS (μmol/l) IN PATIENTS WITH DIFFERENT DEGREES OF CORONARY ATHEROSCLEROSIS AND HIGH α-TOCOPHEROL STATUS (PLASMA α-TOCOPHEROL > 1521 μg/dl)

Mean ± SD.

	Cases ($n = 43$)	Controls ($n = 38$) [a]
Se/linoleic	0.024	0.026
Se/arachidonic	0.146	0.144
Se/eicosapentaenoic	2.671	2.004
Se/docosahexaenoic	0.559	0.487
Se/total PUFA	0.017	0.019
Se/total $n-3$	0.294	0.271
Se/total $n-6$	0.018	0.021

[a] Cases: > 85% stenosis in at least one coronary vessel; controls: < 50% stenosis in all 3 vessels. Some of the individual parameters have missing data.

Discussion

We observed markedly lower selenium levels in patients with severe atherosclerosis compared with controls, whereas PUFA levels and α-tocopherol levels were similar. In contrast to the relatively large number of epidemiological studies on the association between selenium and acute myocardial infarction or death from cardiovascular disease [8,11–15], its relationship with coronary atherosclerosis has hardly been investigated. Moore et al. [16] observed an inverse association between plasma selenium levels and the degree of atherosclerosis in patients who underwent coronary angiography. This relationship, however, was not found in a similar Finnish study [17]. None of these earlier investigations considered the interrelationship between PUFA and antioxidant level.

Though we did not observe differences between cases and controls in PUFA levels or α-tocopherol levels, the ratio of selenium to PUFA levels was negatively correlated with coronary atherosclerosis. Moreover, this association was observed only in the subgroup having low α-tocopherol levels. According to the antioxidant hypothesis, protection against oxidative damage is important because damage of the endothelial cell membrane [2] and/or peroxidation of low-density lipoprotein [3] may lead to coronary atherosclerosis. Apart from their beneficial role with regard to athero- and thrombogenesis, PUFAs are the major substrate for free-radical lipid peroxidation [2]. This oxidation may be prevented by the selenium-dependent enzyme glutathione peroxidase and by α-tocopherol, two important enzymes in the body's multiple defence against free radical damage [2,7,18–21]. Evidence from experiments both in vitro and in vivo show a certain mutual dependence of α-tocopherol and glutathione peroxidase [22–26]. Furthermore, human and animal experiments indicate that both antioxidants are required to prevent PUFA oxidation and loss of membrane integrity. In accordance, our data suggest a combined association of PUFA, α-tocopherol and selenium with coronary atherosclerosis. This association is in accordance with the antioxidant hypothesis, i.e. that polyunsaturated fatty acids will only be able to exert a beneficial effect with regard to atherosclerosis if protection against oxidation is sufficient.

From our data, it could be hypothesized that high PUFA levels, when insufficiently protected against oxidation, indicate a higher risk of atherosclerosis. Several precautions, however, have to be taken when interpreting our data. The different pattern for eicosapentaenoic acid and, to a minor extent, docosahexaenoic acid as compared with the other PUFAs is surprising. As an explanation, a different sensitivity to oxidation seems improbable, given the chemical similarities to other polyunsaturated fatty acids.

Dietary differences arising from case and control selection may have biased our results. However, both groups had coronary angiography recommended by the cardiologist for reasons of possible ischaemic heart disease, which implies equal motivation for dietary changes in both groups. Moreover, we did not observe differences in fatty acid levels between subjects with or without previous myocardial infarction (data not shown), which does not indicate dietary advice specifically to MI patients. In addition, our data show comparable results when the analysis is restricted to subjects without previous MI (data not shown).

In this study, we used blood levels of selenium, α-tocopherol and PUFAs. Clearly, measurements of these parameters in target tissues would have provided a better measure of antioxidant protection and fatty acids. With regard to the postulated LDL oxidation pathway [3], specifically plasma levels of antioxidants and PUFAs may be relevant to determine the resistance of LDL against oxidative modification. However, with regard to the pathway of oxidative injury to endothelial cell membranes, blood levels of antioxidants and PUFAs may be less suitable.

A final consideration in interpreting our data is that there are no direct ways to determine oxidant stress. As an approximation, we determined PUFAs, the substrate for peroxidation, and the antioxidant compounds selenium and α-tocopherol. Other factors will, however, influence the balance between antioxidant protection and oxidant stress. Lipid-soluble antioxidants which we did not measure (e.g. carotenoids) may be important. Also, ascorbic acid, which can regenerate α-tocopherol [26], may contribute to the antioxidant defence. Furthermore, smoking is known to induce oxidant stress [27]. Though the proportion of smokers was equal in cases and controls, a larger proportion of cases were ex-smokers who, on average, stopped smoking more recently. All ex-smokers, however, had stopped smoking at least one year before, so we expect that smoking induced oxidant stress will not have biased our results.

In conclusion, our data suggest a combined association of selenium, α-tocopherol and polyunsaturated fatty acids with coronary atherosclerosis. From this association it can be hypothesized that high polyunsaturated fatty acid levels, when insufficiently protected against oxidation, may indicate a higher risk of atherosclerosis. However, further research, giving attention to total antioxidant potential and total oxidant stress in target

tissues is required to be conclusive about this association.

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