Eur J Nutr 38:286–292 (1999)

ORIGINAL CONTRIBUTION

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Impact of diets with corn oil or olive/sunflower oils on DNA damage in healthy young men

Received: 4 August 1999
Accepted: 27 January 2000

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Summary *Background:* Plant fats and oils are major sources of mono- and polyunsaturated fatty acids as well as vitamin E, the major fat-soluble antioxidants in human nutrition. Dietary antioxidants are expected to reduce cancer risk by minimizing DNA damage.

Aim of the study: To compare the effects of γ-tocopherol rich corn oil and the mixture of the α-tocopherol rich olive/sunflower oil on plasma concentration of tocopherols and on the frequency of sister chromatid exchange (SCE), an indicator of DNA damage.

Methods: This study had a double-blind, cross-over design and was conducted in 20 normal healthy non-smoking males aged 19–31 years. Design included a 2-week adjustment period and two 2-week test periods in which diets containing 30% energy as fat including either 80 g of corn oil (CO) (20 mg α-tocopherol, 100 mg γ-tocopherol) or 80 g of olive/sunflower oil (OSO) (24 mg α-tocopherol, 2.4 mg γ-tocopherol), as the main fat-source, were given. Blood samples for analysis of SCE rate and content of tocopherols were collected at the beginning (T0), after adjustment (T1) and after the test period (T2) in intervals of 2 weeks.

Results: After two weeks of the corn oil diet, the plasma concentration of γ-tocopherol increased but α-tocopherol decreased significantly compared to the olive/sunflower oil diet. The concentration of α-tocopherol increased (CO: 22.99 ± 1.11 vs. OSO: 24.40 ± 1.49 µmol/l) and that of γ-tocopherol decreased (CO: 4.19 ± 0.29 vs. OSO: 2.99 ± 0.25 µmol/l) after the olive/sunflower oil diet. Intake of the corn oil diet was associated with reduced SCE rate and intensity, whereas there was no change in SCE after the olive/sunflower oil diet (CO: 7.66 ± 0.25 vs. OSO: 8.06 ± 0.47 mean SCE/cell)

Conclusions: The combination of γ-tocopherol with α-tocopherol in corn oil diet despite the lower α-tocopherol equivalents/diene acid equivalents ratio achieved better protection against DNA damage than α-tocopherol alone in the olive/sunflower oil diet.

Key words Plant oils – tocopherols – sister chromatid exchanges

Introduction

The discussion of protective effects of vitamin E in preventing chronic disease, such as cancer, heart and blood vessel disease, arthritis, and cataract, has been attributed to tocopherol's ability to function highly efficiently as a lipid-soluble antioxidant [1–3].

Of all compounds with vitamin E activity, α-tocopherol and γ-tocopherol are the principal analogs found in human and animal diets and comprise most of the vitamin E content of tissues. α-tocopherol is the most effective lipid soluble antioxidant *in vivo* and exhibits more bioactivity and reactivity toward reactive oxygen species than any of the other tocopherols. However, γ-tocopherol is a better antioxidant than is α-tocopherol when assessed *in vitro*. In nature, vitamin E is synthesized only by plants, and the richest dietary sources of vitamin E are edible vegetable oils [4].

The SCE technique is thought to be a sensitive indicator of DNA damage in mammalian cells [5]. Markers for DNA damage are of special interest since DNA damage is considered as a crucial mechanism in cancer pathogenesis [6]. Thus, increased cytogenetic damage in vivo, as determined by SCE in human peripheral lymphocytes, is being discussed as a pre-indicator of a potentially increased cancer risk. In a review dietary and blood antioxidants are discussed as playing an important role in preventing cancers by reducing DNA damage [7, 8].

This study was designed to compare the effects of two different plant oils, γ-tocopherol- and polyunsaturated-rich corn oil and a α-tocopherol- and monounsaturated-rich mixture of olive/sunflower oil providing similar α-tocopherol contents, but different α-tocopherol equivalents/diene acid equivalents ratio due to their various fatty acid patterns. The main focus of the study was the determination of the effects of these two oil groups, considering their different tocopherol and fatty acid patterns, on DNA damage by using the sister chromatid exchange assay in healthy, non-smoking young men.

Materials and methods

Subjects

The study population consisted of 20 normal healthy males between the ages of 19 and 31 years living in the area of Vienna, Austria. All participants were non-smokers, free of acute or chronic illness, in the normal range of the body mass index (± 10%), and not taking any medications or vitamin supplements for 2 weeks before the start of the study. They were informed of the purpose, nature, and potential risks of the study and gave consent in writing. The study protocol was approved by the Ethical Committee of the Medical Faculty, University of Vienna.

Experimental design

The design of the study was a cross-over protocol consisting of an adjustment period and two diet periods of 2 weeks (see Fig. 1). The first two weeks were used as an adjustment period (T1) during which all volunteers took the diet containing 12 mg α-tocopherol equivalents/d (ø 11.6 MJ, ø fat intake ~110 g/d, P/S ratio=1, α:γ-tocopherol ratio=1:0.1). Volunteers were then randomized evenly into two groups. During week 3 and 4 (T2), group A took a diet (saturated/monounsaturated/polyunsaturated fatty acids, S/M/P = 29/33/38) with 80 g of γ-tocopherol rich corn oil and group B was given a diet (S/M/P = 29/42/22) with a mixture of 68 g olive oil and 12 g of sunflower oil as the main fat source. At week 5, the cross-over occurred and the trial ended after week 6 (T3). The main characteristics of fats used in the two diets are presented in Table 1. The daily food intake during the respective study periods was prepared in accordance with Recommended Dietary Allowances of the German Nutrition Society, consisting of 50–55% carbohydrates, 15% protein and 30–35% fat of total calories. All food was prepared at the Institute of Nutritional Science, University of Vienna, and offered three times daily in the presence of one of the investigators during all intervention periods. The subjects ate lunch at the institute on work days. Food for the rest of the day and the next morning as well as for weekends were packed and given to the subjects on Fridays. Blood samples (45 ml) were taken from overnight fasted volunteers into heparin-containing vacutainer

Table 1 Composition of corn oil and olive/sunflower oil

	Corn oil	Olive/ Sunflower oil
Daily consumption (g/d)	80	80
SFA:MUFA:PUFA	13:33:54	14:69:17
P/S ratio	4.2	1.2
α-Tocopherol (mg/d)	20	24
γ-Tocopherol (mg/d)	100	2.4
α:γ-Tocopherol ratio	1:5	1:0.1
α-Tocopherol equivalents (mg α-TE/d)[a]	45	24.6
α-TE excretion (mg α-TE /d)	20.8	7.6
α-TE retention (mg α-TE /d)	24.2	17
Diene acid equivalents (g DE/d)[b]	44	15
α-TE/DE ratio (mg/g)[c]	0.55	1.13

[a] TE = α-tocopherol equivalents = mg α-tocopherol + 0.25 x mg γ–tocopherol
[b] DE = Diene acid equivalents = 0.025 x monoene acids + 1 x diene acids + 2 x triene acids + 4 x tetraene acids
[c] α-TE/DE ratio = mg α-tocopherol equivalents retention/ g diene acid equivalents

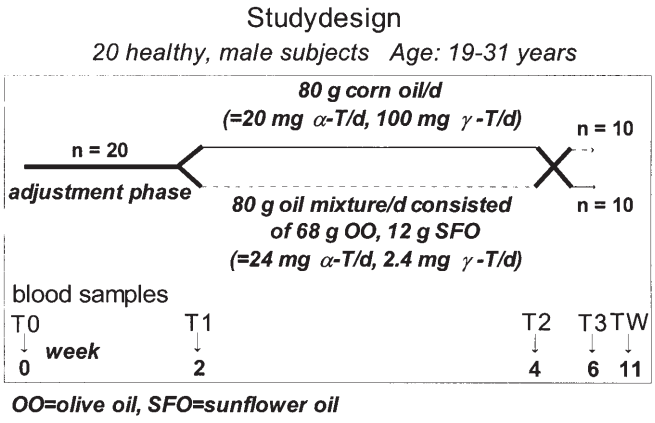

Fig. 1 Design of the intervention; 20 healthy male subjects aged 19–31 years.

tubes by venipuncture five times during the study period: at baseline (T0), at the end of week 2 (T1), at the end of week 4 (T2), at the end of week 6 (T3), and 5 weeks after finishing the intervention (TW).

SCE assay

Heparinized blood for lymphocyte culture was obtained by venipuncture and processed within 2 h. Evaluation of SCE in peripheral lymphocytes was performed as described elsewhere [9]. Briefly, duplicate 0.8 ml volumes of whole blood were added to 9.5 ml EMEM supplemented with 100 units/ml penicillin-streptomycin solution, 2 mM of L-glutamine and 15% v/v heat-inactivated fetal bovine serum; 0.1 ml of phytohemagglutinin, 0.1 ml of lithium heparin and 0.05 ml of 5 mM 5-bromo-2-deoxyuridine, to give 25 μM, were added and incubated at 37 °C in 5% CO_2 for 72 h. Colchicine (10 μg/ml) was added 2 h before harvesting. The cells were hypotonized using 0.075 M KCl solution and fixed with fresh methanol:acetic acid (3:1, v/v). Metaphase slides were prepared and were stained with 5% Giemsa solution in freshly made Sörenson's buffer. For each subject, 50 metaphases were scored to determine the mean SCE frequency. Mean SCE for HFC (high frequency cells) was calculated from the highest five metaphases for each study subjects.

Blood parameters

Plasma was obtained from heparinized blood samples by centrifugation (1000 rpm, for 10 min). An appropriate portion of platelet-rich-plasma was collected for estimation of ascorbic acid on the same day. The rest of the platelet-rich plasma was centrifugated at 3000 rpm for 15 min and the supernatant fraction (platelet-deficient plasma) was separated. The plasma was divided in appropriate aliquots and stored at -80 °C until further analysis. Plasma ascorbic acid was measured photometrically by the method of Denson and Bowers [10]. The HPLC procedure developed by Jakob and Elmadfa [11] was used to measure retinol, β-carotene, and tocopherol concentrations in plasma. Total cholesterol in plasma was measured colorimetrically by the method of Siedel et al. [12], using a test kit from Boehringer-Mannheim GmbH.

Statistical analysis

The statistical significance of differences between the two groups was determined by the non-parametric Mann-Whitney U-test, and comparisons for the different dietary periods within a group were performed using the Wilcoxon matched pairs test. Statistical analysis was conducted using SPSS for Windows. Values were expressed as mean ± SE and differences were considered significant at a value of $P < 0.05$.

Results

The volunteers reported no side effects due to ingestion of the diets with corn oil or olive/sunflower oil as the main fat-source. The subjects maintained their weight throughout the study.

After two weeks of the corn oil diet, the plasma concentration of α-tocopherol decreased significantly, compared to the olive/sunflower oil diet in the two groups, although within the normal range (group A: 18.45 ± 0.86 μmol/L vs 22.99 ± 1.11 μmol/L, $P = 0.03$; group B: 18.51 ± 1.45 μmol/L vs 24.40 ± 1.49 μmol/L, $P = 0.02$) (Fig. 2).

The level of γ-tocopherol showed a highly significant reduction after the adjustment diet (T1) and after the olive/sunflower oil diet, compared to the values at baseline (T0), after the corn oil diet and washout (TW), as caused by the low content of γ-tocopherol in these diets (α:γ-tocopherol ratio = 1:0.1). As expected, the corn oil diet resulted in an increased plasma γ-tocopherol concentration in the two study groups (group A: 4.19 ± 0.29 μmol/L at T2; group B: 2.99 ± 0.25 μmol/L at T3) (Fig. 2).

Essentially, a similar trend was observed if the α- and γ-tocopherol plasma status was expressed as the α- and γ-tocopherol/cholesterol ratio except for the adjustment phase (T1). The two-week adjustment phase was accompanied by a significant decrease of the α- and γ-tocopherol/cholesterol ratio, as there was an increase in plasma cholesterol during this phase. The plasma content of retinol, β-carotene and ascorbic acid remained nearly uninfluenced by the tocopherol-defined diet (Table 2).

The effect of two different oil diets on the frequency of SCE in peripheral lymphocytes is shown in Fig. 3. At T2, intake of the corn oil diet decreased mean SCE significantly, compared to values at baseline ($P = 0.019$), after the adjustment diet ($P = 0.005$), and washout ($P = 0.032$) in group A. A similar trend was observed in group B at T3, but it did not reach statistical significance. The

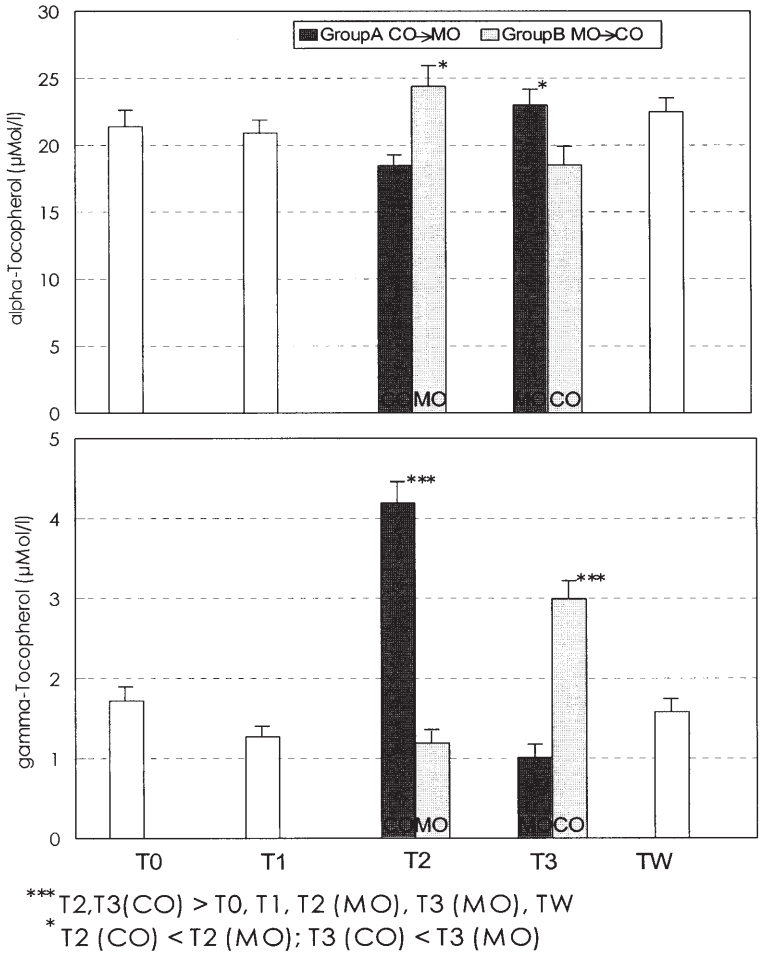

Fig. 2 Mean plasma levels of α-tocopherol and γ-tocopherol for the different diet periods. Error bars represent the standard error of the mean. CO, corn oil diet; MO, mixed oil diet (olive/sunflower oil) (n = 20).

corn oil diets were also associated with a decrease in mean SCE frequency in high frequency cells, compared with the value after the adjustment diet in group A (at T2, $P = 0.04$) and group B (at T3, $P = 0.04$) (Fig. 4). The mean SCE and HFC after the olive/sunflower oil diet were slightly but not significantly higher than after the corn oil diet.

Five weeks after finishing the intervention (washout), α-tocopherol, γ-tocopherol, α- and γ-tocopherol adjusted for cholesterol, mean SCE and HFC returned to basal levels in all volunteers.

Table 2 Plasma level of α- and γ-tocopherol adjusted for plasma cholesterol, plasma levels of retinol, β-carotene and ascorbic acid at baseline (T0), after adjustment diet (T1), corn oil (CO) diet, mixed oil (MO) diet (T2, T3), and 5 weeks after the intervention (TW)

	T0	T1	T2		T3		TW
			GroupA (CO)	Group B (MO)	GroupA (MO)	Group B (CO)	
α-Tocopherol/cholesterol (µmol/mmol)	5.35 ± 0.35	4.50 ± 0.16	4.45 ± 0.20	5.82 ± 0.23	5.19 ± 0.19	4.89 ± 0.25	5.27 ± 0.18
γ-Tocopherol/cholesterol (µmol/mmol)	0.41 ± 0.03	0.27 ± 0.02	1.00 ± 0.05	0.28 ± 0.02	0.23 ± 0.03	0.79 ± 0.04	0.37 ± 0.04
Retinol (µmol/L)	1.48 ± 0.09	1.18 ± 0.07	1.29 ± 0.11	1.25 ± 0.07	1.24 ± 0.07	1.25 ± 0.07	1.34 ± 0.07
β-Carotene (µmol/L)	0.57 ± 0.09	0.57 ± 0.05	0.40 ± 0.05	0.46 ± 0.06	0.44 ± 0.05	0.45 ± 0.05	0.61 ± 0.13
Ascorbic acid (µmol/L)	87.47 ± 3.32	86.99 ± 3.55	84.20 ± 5.57	83.58 ± 2.59	96.19 ± 3.30	100.44 ± 3.87	85.25 ± 2.63

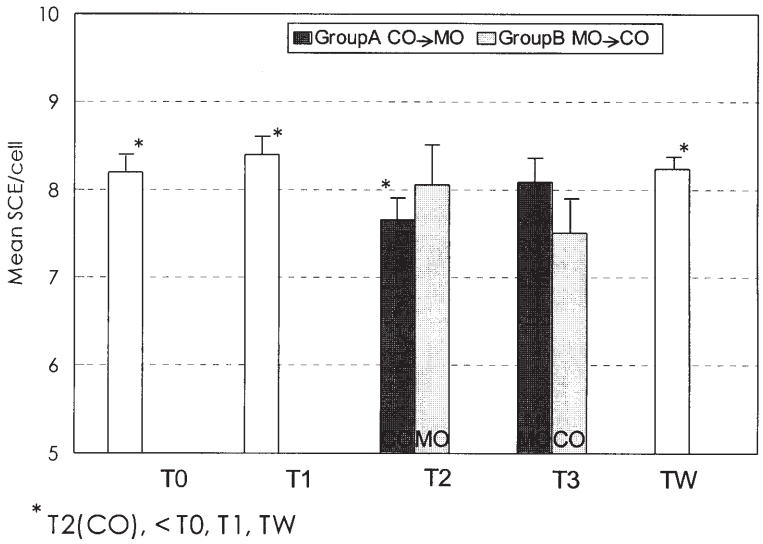

Fig. 3 Mean SCE per cell for the different diet periods. Error bars represent the standard error of the mean. CO, corn oil diet; MO, mixed oil diet (olive/sunflower oil) (n = 20).

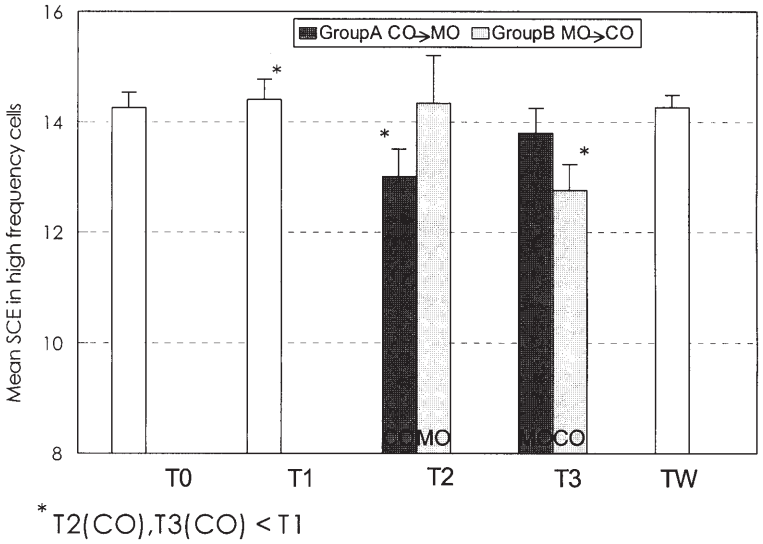

Fig. 4 Mean SCE in high frequency cells for the different diet periods. Error bars represent the standard error of the mean. CO, corn oil diet; MO, mixed oil diet (olive/sunflower oil) (n = 20).

Discussion

This study was carried out in healthy, non-smoking male subjects with the purpose of comparing the effects of γ-tocopherol rich corn oil and α-tocopherol rich olive/sunflower oil, as main dietary fats in normal diets, on DNA damage estimated by SCE in lymphocytes.

The use of SCE as a biomarker for food components in vivo, especially vitamin C, was shown by Rivas-Olmedo et al. [13].

The diets were planned to have the same energy, total fat, and other nutrient content throughout the study periods. The differences between the two diets were content of γ-tocopherol, the percentage of MUFA and PUFA and also the calculated α-tocopherol equivalents/diene acid equivalents ratio. After 2 weeks of the corn oil diet, the plasma α-tocopherol level decreased significantly in the two groups, although the content of α-tocopherol of the corn oil and olive/sunflower oil was nearly identical (20 mg in corn oil and 24 mg in olive/sunflower oil). This result may be due to the high content of polyunsaturated fatty acids (PUFAs) in the corn oil (P/S ratio = 4.2). Increased uptake of PUFAs may also affect vitamin E status, by either impairing the absorption of the antioxidants [14] or by causing increased vitamin E consumption in plasma and tissues due to enhanced lipid peroxidation [15–17].

The decrease of DNA damage in human lymphocytes due to α-tocopherol supplementation in combination with vitamin C and ß-carotene, or high vegetable juice consumption has been shown [18, 19].

In spite of the low concentration of plasma α-tocopherol, the corn oil diet decreased the frequency of SCE in peripheral lymphocytes compared with the values at baseline, after the adjustment period, and after washout. These results strongly suggest that γ-tocopherol in corn oil possesses a high antioxidative potency in preventing DNA damage caused from the ubiquitous exposure of humans to reactive oxygen species (ROS) or reactive nitrogen species (RNS), both endogenously and exogenously in healthy men [7].

SCEs have been shown to be induced by reactive oxygen species or chemical inducers *in vitro*, which were subsequently found to be inhibited by the addition of antioxidants to culture (α-tocopherol, β-carotene, and vitamin C) [20–22]. Although γ-tocopherol has, to the best of our knowledge, not been investigated with respect to a protective effect against SCE-inducing agents, many studies show that the antioxidative potency of γ-tocopherol is at least equal or even better than that of α-tocopherol when assessed *in vitro* [3, 23].

Recently, several investigations have shown γ-tocopherol is superior to α-tocopherol in reducing reactive nitrogen species or to react with them to form a non-nitrosating agent may be particularly important in carcinogenesis, since nitrosating agents can deaminate DNA bases causing mutations [24, 25]. γ-tocopherol is also better than other lipid-soluble antioxidants in preventing NO_2-mediated DNA strand breaks [26].

After the two week olive/sunflower oil diet, the frequency of SCE was slightly but not significantly higher than after the corn oil diet. From this result we can suggest that there are some synergistic effects between γ- and α-tocopherol, because approximately the same content of α-tocopherols in both plant oil diets.

In conclusion, the results from the present study in healthy subjects indicate that the combination of γ-tocopherol with α-tocopherol in the corn oil diet despite the high ω6-PUFA (P/S ratio of the oil = 4.2) gave better protection against DNA damage than α-tocopherol alone in the olive/sunflower oil diet (high in MUFA). Further analyses are necessary to give a clear conclusion about the synergistic interaction between γ-tocopherol and α-tocopherol.

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