Attenuation of Blood Parameters in Smokers and Non-Smokers after Intake of a Complex Food Additive

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This report describes an intervention study with healthy volunteers (20 smokers, 28 non-smokers) taking a food additive mainly containing vitamin C (ascorbic acid), vitamin E (α-tocopherol), ubiquinone (Q10), vitamin A and zinkoxide for four weeks in a double blind, randomized and placebo controlled manner. Before and after the intervention blood was withdrawn and general blood parameters were analyzed. In addition, lipid soluble antioxidants were analyzed in blood plasma by HPLC and the water soluble antioxidative properties were testet with the enzymic xanthin/xanthinoxidase-reaction. In summary the results show that the smoker-verum group exhibit a significant down regulation of the leukocyte counts. The test for antioxidants show the following significant differences after intervention:
Smokers exhibit an increase of both vitamin E and coenzyme Q10 and an attenuation of their (before intervention) clearly increased water soluble – antioxidative potential, non-smokers showed only an increase of vitamin E and trends of an increase of Q10 and water soluble-antioxidative potential. These results may contribute to the discussion of the intrinsic deficiency brought about by smoking and the possible attenuation of part of these deficiency by increasing the intake of certain vitamins or food additives.

Key words: Antioxidants, Reactive Oxygen Species, Food Additives

Introduction

Molecular atmospheric oxygen contains two unpaired electrons in the unreactive triplet state and has thus to be activated in order to react with other biological molecules in the singlet ground state. All aerobic cells produce reactive oxygen species (ROS) and are thus subject to oxygen "stress" (Sies, 1991). In order to counteract potential damage, a well-balanced antioxidative strategy has been elaborated during some hundred million years during the coevolution between plants and animals. Under certain circumstances, however, intrinsic defense systems in humans are not sufficient to completely eliminate free radical-driven damage to important biomolecules such as lipids in membranes, structural proteins, enzymes or nucleic acids. Additional support of these intrinsic systems comes from food ingredients: antioxidants such as the vitamins A, C and E or ubiquinol cooperate with a wealth of compounds not directly being addressed as vitamins in defense against ROS. Antioxidants, acting as free radical scavengers or quenchers of activated states in addition comprise a wealth of classes of organic molecules including phenolics, terpenoids and flavonoids as the most prominent ones. In animals and humans, oxidative stress is provoked by infections (inflammation) or intoxication such as smoking where ROS may accumulate. Depending on the strength of these impact(s), several symptoms indicate the deviation from normal, steady-state-metabolism accompanied by a decrease of endogenous antioxidants such as ascorbic acid, glutathione or α-tocopherol (Elstner, 1993; Halliwell, 1996). Biochemical model reactions contribute to our knowledge about potential dangers and increase the understanding of

corresponding mechanisms and dose-response effects of these food ingredients, food additives or drugs acting as antioxidants (Schempp *et al.*, 2000; Schneider and Elstner, 2000; Janisch *et al.*, 2002).

In this report we describe the results of a double blind, randomized and placebo controlled study on administration of a food additive containing such antioxidants, conducted with 48 persons, selecting 20 smokers and 28 non-smokers. We investigated on:

- clinical parameters such as leukocytes counts, hemoglobin and hematocrit,
- plasma content of vitamins E and A and ubiquinone Q10 as determined by HPLC and
- antioxidative capacity of blood plasma as determined by the inhibition of radical production by the X/XOD (xanthine/xanthine oxidase)-reaction.

Materials and Methods

Conduction of the study and food additive

After a pre-examination on 2nd and 3d April 2002 48 healthy volunteers, 20 smokers and 28 non-smokers, had to take the food additive or a placebo of identical shape until 2nd and 3d May 2002. After blood withdrawal before and at the end of intervention, routine medical blood parameters as well as biochemical properties such as antioxidative potential or lipophilic antioxidant contents of the corresponding plasma samples were determined (see below).

The food additive taken for the indicated time intervall by the test persons (2 capsules of OXANO® produced and distributed by "formula Müller-Wohlfahrt" Health & Fitness AG,) contained in one capsule: 225 mg Vitamin C, 275.2 μg Vitamin A, 36 mg Vitamin E, 10 mg Ubiquinone, 5 mg Zink). The responsibilities in selecting the volunteers and for conduction of the whole study was by "formula Müller-Wohlfahrt" Health & Fitness AG.

Reagents

KMB (α-keto-γ-methiol-butyric acid), all-trans retinol (vitamin A), α-tocopherol, α-tocopherol acetate, ubiquinone (Q10) and xanthine (X) were obtained from Sigma, München, Germany; Xanthine oxidase (XOD) was purchased from Roche, Mannheim, Germany; EDTA (ethylenediaminetetraacetic acid) was from Merck, Darmstadt, Germany. All other chemicals were of the highest grade of purity available (Merck). The gases for gas chromatography were from Messer, Griesheim (N_2: type 5.0; H_2: type 5.0; synth. air; ethylene calibration gas).

Plasma preparation

Shortly after receiving 0.8 ml EDTA stock solution (25 mg/ml) was added to 20 ml blood and the blood was immediately centrifuged at 10 °C for 20 min at 1600 × *g*. After recovering the plasma (supernatant) 100 μl saccharose solution (60%) per 10 ml plasma were added and the stabilized plasma was stored at – 70 °C in N_2-atmosphere.

Extraction and quantification of antioxidants

To 250 μl of the plasma samples 250 μl of ethanol were added. The ethanol contained α-tocopherol acetate as internal standard. Plasma and ethanol were mixed thoroughly in order to precipitate the proteins. Subsequently 500 μl hexane were added and the sample was vortexed for 1 min. After centrifugation (4000 × *g*) the hexane phase was removed and the ethanolic phase was again extracted with hexane as described above. The hexane phases were combined and hexane was evaporated in a vacuum concentrator (Christ, Osterode, Germany). The pellet was resolved in 50 μl methanol and analysed by HPLC (Beckmann, München, Germany) on a reversed phase column (Nucleosil 300, ODS, 7 μm, 125 × 4.6 mm) isocratically (methanol/acetonitrile/dichloromethane 45:45:10) with UV-detection (Beckmann), at 280 nm (Graßmann *et al.*, 2001).

Vitamin A-, Vitamin E- and ubiquinone-contents were calculated with the aid of corresponding calibration curves.

Antioxidant activity of plasma

The antioxidant activity was evaluated by the following biochemical model system:

The xanthine/xanthine oxidase (X/XOD)-reaction

Using xanthine as substrate, XOD produces $O_2^{\cdot -}$ and H_2O_2 and OH-radicals via the Haber-Weiss reaction (Elstner, 1993; Halliwell and Gut-

teridge, 1999). This reaction is characteristic for reperfusion injury after ischemic events. It is detectable as ethene release from α-keto-γ-methiolbutyric acid (KMB). Ethene formation from KMB was analysed by gas chromatography as described previously (v. Kruedener *et al.*, 1995; Hippeli *et al.*, 1997). The values for ethylene production refer to picomoles per total reaction and were calculated with the aid of an ethene calibration gas: 1 ml = 235.15 pmol, 1 bar. The reaction mixtures contained in a total volume of 2 ml: 100 mM phosphate buffer (pH 7.4), 0.5 mM xanthine, 0.016 U XOD, 1 mM KMB and 300 µl of the plasma samples. After incubation of 30 min at 37 °C in sealed gas-tight reaction tubes 1 ml gas of the headspace has been retained with a gas-tight syringe and analysed gas chromatographically.

To compare the antioxidant activities of the different plasma samples, the ethene formation of the control reaction (X/XOD-reaction without plasma) was set as 100%. The ethene formations of the reactions in the presence of plasma-samples were calculated as% inhibition of the control reaction.

Statistics

Results are expressed as minimum and maximum values and means ± S. E. M. Differences between smokers and non-smokers were analysed using unpaired two tailed t-test; the statistical significance of changes of blood parameters after intervention was determined by a matched pair *t*-test (two tailed). Results were considered significant for $p < 0.05$.

Results

Clinical blood parameters

Smokers as compared to non-smokers exhibit significantly increased hemoglobin and hematocrit values and show also a clear trend for increased total leukocyte counts ($p = 0.088$), mainly caused by an increase of the number of neutrophils (Table I). Dividing into the forthcoming placebo- and verum-groups, however (Table II), these differences between smokers and non-smokers only show up in the placebo group and are retained for hemoglobin and hematocrit during the period of four weeks after intervention. These in the placebo group significantly increased neutrophil counts before intervention are due to both, very high neutrophil count in one test person (see maximal value in Table I: 11.8×10^3 cells/µl blood) and the low number of test persons in this group ($n = 7$). Eliminating the data of this test person allows no significant difference in the neutrophil counts between smokers and non-smokers.

A "before-after" comparison between the four groups (non-smokers-verum; smokers-verum; non-smokers-placebo; smokers-placebo) shows that in the smoker group the intake of verum yields a significant reduction of the total leukocyte counts due to a (non-significant) decrease of lymphocytes, and especially neutrophils ($p = 0.058$) (Table III). The non-smoker placebo group after the intervention exhibits a significant reduction in lymphocytes.

Table I. Comparison of non-smokers with smokers before intervention.

Parameter	Unit	Non-smokers (n = 28)			Smokers (n = 20)			Difference	Significance ($p < 0.05$)
		Min.	Max.	Mean ± S. E. M.	Min.	Max.	Mean ± S. E. M.		
Erythrocytes[a]	n^6/µl	3.66	5.49	4.68 ± 0.46	4.23	5.45	4.78 ± 0.38	0.10	n.s
Haemoglobin[a]	g/dl	11.0	15.9	13.7 ± 1.3	11.9	17.0	14.5 ± 1.4	0.8	**0.035**
Haematocrit[a]	%	34.1	46.6	40.8 ± 3.1	36.7	49.4	43.0 ± 3.6	2.2	**0.034**
Thrombocytes[a]	n^3/µl	171	415	249 ± 53	176	424	268 ± 65	19	n.s.
Leukocytes[a]	n^3/µl	4.0	9.3	6.66 ± 1.68	5.2	15.3	7.82 ± 2.55	1.16	(0.088)
Lymphocytes[a]	n^3/µl	1.3	3.5	2.19 ± 0.65	1.8	3.7	2.34 ± 0.47	0.15	n.s.
Neutrophiles[a]	n^3/µl	1.6	6.0	3.73 ± 1.28	2.3	11.8	4.85 ± 2.46	1.12	(0.088)

Note: n^3, n^6: one thousand, one million cells per µl; Min., Max.: minimal or maximal value within a sample; Difference: difference in the means between smokers and non-smokers.

Table II. Comparison of non-smokers with smokers: clinical data.

Parameter	Unit	Non-smokers verum (n = 12)			Smokers verum (n = 12)			Difference	Significance ($p < 0.05$)
		Min.	Max.	Mean ± S. E. M.	Min.	Max.	Mean ± S. E. M.		
Erythrocytes **b. i.**	$n^6/\mu l$	3.66	5.49	4.72 ± 0.56	4.24	5.2	4.68 ± 0.3	−0.04	n. s.
Erythrocytes **a. i.**	$n^6/\mu l$	3.5	5.55	4.7 ± 0.6	4.04	5.29	4.65 ± 0.41	−0.05	n. s.
Haemoglobin **b. i.**	g/dl	11	15.8	13.5 ± 1.58	11.9	15.7	14.13 ± 1.07	0.63	n. s.
Haemoglobin **a. i.**	g/dl	10.4	15.8	13.42 ± 1.55	11.6	15.8	13.98 ± 1.37	0.56	n. s.
Haematocrit **b. i.**	%	34.1	46.5	40.45 ± 3.9	36.7	46.6	41.85 ± 2.81	1.4	n. s.
Haematocrit **a. i.**	%	32.4	46.2	40.34 ± 3.98	35.6	47	41.73 ± 3.82	1.39	n. s.
Thrombocytes **b. i.**	$n^3/\mu l$	182	295	241.83 ± 37.08	176	424	257.67 ± 67.33	15.84	n. s.
Thrombocytes **a. i.**	$n^3/\mu l$	164	309	239.17 ± 43.5	176	408	250.42 ± 76.46	11.25	n. s.
Leukocytes **b. i.**	$n^3/\mu l$	4	9	6.87 ± 1.94	5.2	13	7.42 ± 2.2	0.55	n. s.
Leukocytes **a. i.**	$n^3/\mu l$	4.2	9.8	6.7 ± 1.71	4.1	13.1	6.65 ± 2.46	−0.05	n. s.
Lymphocytes **b. i.**	$n^3/\mu l$	1.3	3.5	2.2 ± 0.68	1.8	3.7	2.42 ± 0.52	0.22	n. s.
Lymphocytes **a. i.**	$n^3/\mu l$	1.4	2.8	2.05 ± 0.43	1.4	3.5	2.2 ± 0.73	0.15	n. s.
Neutrophiles **b. i.**	$n^3/\mu l$	1.9	6	4.12 ± 1.38	2.3	6.3	3.86 ± 1.18	−0.26	n. s.
Neutrophiles **a. i.**	$n^3/\mu l$	2.1	6.4	3.9 ± 1.46	2	5	3.38 ± 1.10	−0.52	n. s.
		Non-smokers placebo (n = 16)			Smokers placebo (n = 7)				
Erythrocytes **b. i.**	$n^6/\mu l$	4.06	5.36	4.66 ± 0.38	4.23	5.45	4.97 ± 0.47	0.31	n. s.
Erythrocytes **a. i.**	$n^6/\mu l$	4.11	5.54	4.71 ± 0.43	4.37	5.37	4.96 ± 0.36	0.25	n. s.
Haemoglobin **b. i.**	g/dl	12.4	15.9	13.88 ± 0.93	13.1	17	15.26 ± 1.56	1.38	**0.015**
Haemoglobin **a. i.**	g/dl	12.7	15.8	13.63 ± 1.07	13.2	16.8	15.03 ± 1.41	1.1	(0.051)
Haematocrit **b. i.**	%	37.5	46.6	41.11 ± 2.31	38.7	49.4	44.93 ± 4.3	3.82	**0.011**
Haematocrit **a. i.**	%	38.1	46.5	41.47 ± 2.63	39.5	49.9	44.7 ± 4.21	3.23	**0.035**
Thrombocytes **b. i.**	$n^3/\mu l$	171	415	255.06 ± 62.54	211	341	268.71 ± 50.25	13.65	n. s.
Thrombocytes **a. i.**	$n^3/\mu l$	179	356	247.25 ± 54.16	170	336	256.14 ± 71.91	8.89	n. s.
Leukocytes **b. i.**	$n^3/\mu l$	4	9.3	6.51 ± 1.51	5.7	15.3	8.26 ± 3.27	1.75	(0.090)
Leukocytes **a. i.**	$n^3/\mu l$	4.1	11.1	6.54 ± 2.06	5.2	9.1	7.23 ± 1.34	0.69	n. s.
Lymphocytes **b. i.**	$n^3/\mu l$	1.3	3.3	2.18 ± 0.64	1.8	3	2.23 ± 0.4	0.08	n. s.
Lymphocytes **a. i.**	$n^3/\mu l$	1.2	3.1	2.01 ± 0.6	1.8	2.6	2.26 ± 0.29	0.25	n. s.
Neutrophiles **b. i.**	$n^3/\mu l$	1.6	5.5	3.51 ± 1.14	3.5	11.8	5.9 ± 3.35	2.39	**0.033**
Neutrophiles **a. i.**	$n^3/\mu l$	2	6	3.41 ± 1.09	3	5.7	4.4 ± 1.16	0.99	n. s.

Note: n^3, n^6: one thousand, one million cells per µl; Min., Max.: minimal or maximal value within a sample; Difference: difference in the means between smokers and non-smokers; b. i.: before intervention, a. i.: after intervention; Verum: volunteers received 2 capsules/day containing the complex food additive (Oxano®), Placebo: volunteers received 2 capsules/day identical to "verum" not containing the complex food additive.

Contents of antioxidants

There are no detectable differences in the plasma contents of vitamin A, vitamin E and coenzyme Q10 between the smoker and non-smoker groups, before or after the intervention (Table IV).

Comparison of the corresponding group-pairs (Table V), however, shows that both verum groups exhibited significant increases of both vitamin E and Q10, where a siginificant increase of vitamin E independent on smoking and a significant increase of Q10 dependent on smoking could be differentiated. In the non-smoker group Q10 increase showed a clear, close to significant trend ($p = 0.057$).

Antioxidative potential of blood plasma: the X/XOD-reaction

The antioxidative capacities of blood plasma as determined by means of the enzymic X/XOD-test system allows reflections on the presence and activities of water soluble antioxidants against free radical attack since this reaction generates simultaneously superoxide, hydrogen peroxide and OH-radicals. The antioxidative capacity before intervention in smokers is significantly increased as compared to the non-smoker group (Table VI): This fact is interpreted as an increase in internal defense against continuous free radical attack by components of cigarette smoke, *e. g.* NO_2 (Handel-

Table III. Comparison of correponding pairs: clinical data.

Parameter	Unit	Before Intervention			After Intervention			Difference ± S. E. M.	Significance ($p < 0.05$)
		Min.	Max.	Mean ± S. E. M.	Min.	Max.	Mean ± S. E. M.		
Non-smokers verum (n = 12)									
Erythrocytes	$n^6/\mu l$	3.66	5.49	4.72 ± 0.57	3.50	5.55	4.70 ± 0.61	−0.02 ± 0.15	n. s.
Haemoglobin	g/dl	11.0	15.8	13.5 ± 1.6	10.4	15.8	13.4 ± 1.6	−0.1 ± 0.5	n. s.
Haematocrit	%	34.1	46.5	40.4 ± 3.9	32.4	46.2	40.3 ± 4.0	−0.1 ± 1.4	n. s.
Thrombocytes	$n^3/\mu l$	182	295	242 ± 37	164	309	239 ± 44	−3 ± 19	n. s.
Leukocytes	$n^3/\mu l$	4.0	9.0	6.9 ± 1.9	4.2	9.8	6.7 ± 1.7	−0.2 ± 1.4	n. s.
Lymphocytes	$n^3/\mu l$	1.3	3.5	2.2 ± 0.7	1.4	2.8	2.1 ± 0.4	−0.1 ± 0.4	n. s.
Neutrophiles	$n^3/\mu l$	1.9	6.0	4.1 ± 1.4	2.1	6.4	3.9 ± 1.5	−0.2 ± 0.9	n. s.
Smokers verum (n = 12)									
Erythrocytes	$n^6/\mu l$	4.24	5.20	4.68 ± 0.31	4.04	5.29	4.65 ± 0.42	−0.03 ± 0.17	n. s.
Haemoglobin	g/dl	11.9	15.7	14.1 ± 1.1	11.6	15.8	14.0 ± 1.4	−0.1 ± 0.6	n. s.
Haematocrit	%	36.7	46.6	41.8 ± 2.9	35.6	47.0	41.7 ± 3.9	−0.1 ± 1.6	n. s.
Thrombocytes	$n^3/\mu l$	176	424	258 ± 68	176	408	250 ± 77	−8 ± 33	n. s.
Leukocytes	$n^3/\mu l$	5.2	13.0	7.4 ± 2.2	4.1	13.1	6.7 ± 2.5	−0.7 ± 1.0	**0.022**
Lymphocytes	$n^3/\mu l$	1.8	3.7	2.4 ± 0.5	1.4	3.5	2.2 ± 0.7	−0.2 ± 0.5	n. s.
Neutrophiles	$n^3/\mu l$	2.3	6.3	3.9 ± 1.2	2.0	5.0	3.4 ± 1.1	−0.5 ± 0.8	(0.058)
Non-smokers placebo (n = 16)									
Erythrocytes	$n^6/\mu l$	4.06	5.36	4.66 ± 0.39	4.11	5.54	4.71 ± 0.44	0.05 ± 0.24	n. s.
Haemoglobin	g/dl	12.4	15.9	13.9 ± 1.0	12.7	15.8	13.9 ± 1.1	0.05 ± 0.59	n. s.
Haematocrit	%	37.5	46.6	41.1 ± 2.4	38.1	46.5	41.5 ± 2.7	0.36 ± 2.03	n. s.
Thrombocytes	$n^3/\mu l$	171	415	255 ± 63	179	356	247 ± 55	−8 ± 29	n. s.
Leukocytes	$n^3/\mu l$	4.0	9.3	6.5 ± 1.5	4.1	11.1	6.5 ± 2.1	0.0 ± 1.6	n. s.
Lymphocytes	$n^3/\mu l$	1.3	3.3	2.2 ± 0.6	1.2	3.1	2.0 ± 0.6	−0.2 ± 0.3	**0.027**
Neutrophiles	$n^3/\mu l$	1.6	5.5	3.5 ± 1.2	2.0	6.0	3.4 ± 1.1	−0.1 ± 0.8	n. s.
Smokers placebo (n = 7)									
Erythrocytes	$n^6/\mu l$	4.23	5.45	4.97 ± 0.48	4.37	5.37	4.96 ± 0.36	−0.01 ± 0.31	n. s.
Haemoglobin	g/dl	13.1	17.0	15.3 ± 1.6	13.2	16.8	15.0 ± 1.4	−0.3 ± 0.7	n. s.
Haematocrit	%	38.7	49.4	44.9 ± 4.3	39.5	49.9	44.7 ± 4.2	−0.2 ± 1.9	n. s.
Thrombocytes	$n^3/\mu l$	211	341	269 ± 51	170	336	256 ± 72	−13 ± 74	n. s.
Leukocytes	$n^3/\mu l$	5.7	15.3	8.3 ± 3.3	5.2	9.1	7.2 ± 1.3	−1.1 ± 3.3	n. s.
Lymphocytes	$n^3/\mu l$	1.8	3.0	2.2 ± 0.4	1.8	2.6	2.3 ± 0.3	0.1 ± 0.3	n. s.
Neutrophiles	$n^3/\mu l$	3.5	11.8	5.9 ± 3.4	3.0	5.7	4.4 ± 1.2	−1.5 ± 3.9	n. s.

Note: n^3, n^6: one thousand, one million cells per µl; Min., Max.: minimal or maximal value within a sample; Difference: difference in the means after and before intervention; Verum: volunteers received 2 capsules/day containing the complex food additive (Oxano®), Placebo: volunteers received 2 capsules/day identical to "verum" not containing the complex food additive.

mann *et al.*, 1996). After intervention, this effect is no longer observed, however. The reason for this might be that there is a strong trend ($p = 0.067$) of an increase (ca. 17%) of the antioxidative potential in the verum non-smoker group after intervention. In contrast in the smoker group there is a trend for a (ca. 6%) small decrease (Table VII).

In the placebo non-smoker group the antioxidative potential after intervention remains unchanged, as expected. In the smoker group, however, there is a significant decrease by ca. 13% within a time frame of four weeks after intervention ($p = 0.018$).

Discussion

Since NO_2 is a free radical, smokers inhale several (approximately 16×10^6) million free radicals per puff (Pryor *et al.*, 1983) and their antioxidative capacities seem to be continuously under "repair stress" since they show endothelial dysfunctions (Heitzer *et al.*, 1996), higher plasma contents of

Table IV. Comparison of non-smokers with smokers: lipophilic antioxidant content.

Antioxidant (μM in plasma)	Non-smokers verum (n = 12)			Smokers verum (n = 12)			Difference	Significance ($p < 0.05$)
	Min.	Max.	Mean ± S. E. M.	Min.	Max.	Mean ± S. E. M.		
Vitamin A before intervention	0.96	3.66	1.72 ± 0.75	0.86	2.35	1.57 ± 0.42	−0.15	n.s.
Vitamin A after intervention	0.98	4.02	1.71 ± 0.80	0.69	4.39	1.81 ± 0.99	0.10	n.s.
Vitamin E before intervention	12.69	22.87	16.78 ± 2.94	10.46	19.41	15.15 ± 3.08	−1.63	n.s
Vitamin E after intervention	16.58	35.21	20.84 ± 5.31	12.24	28.35	19.99 ± 5.01	−0.85	n.s
Q10 before intervention	1.04	2.8	2.1 ± 0.61	0.97	3.44	1.71 ± 0.69	−0.39	n.s
Q10 after intervention	0.86	4.18	2.88 ± 1.23	0.96	6.18	2.8 ± 1.42	−0.08	n.s
	Non-smokers placebo (n = 16)			Smokers placebo (n = 7)				
Vitamin A before intervention	1.06	2.24	1.75 ± 0.34	1.15	2.8	1.79 ± 0.57	0.04	n.s
Vitamin A after intervention	0.98	3.27	1.66 ± 0.52	1.26	3.3	1.93 ± 0.67	0.27	n.s
Vitamin E before intervention	10.03	23.97	17.54 ± 4.17	12.08	29.49	18.01 ± 6.56	0.47	n.s
Vitamin E after intervention	10.94	30.92	18.38 ± 4.94	11.72	26.74	17.54 ± 7.64	−0.84	n.s
Q10 before intervention	1.02	3.5	2.16 ± 0.57	1.17	4.12	2.17 ±1.01	0.01	n.s
Q10 after intervention	1.02	4.47	2.39 ± 1	1.15	3.57	2.41 ± 0.88	0.02	n.s

Note: Min., Max.: minimal or maximal value within a sample; Difference: difference in the means between smokers and non-smokers; Verum: volunteers received 2 capsules/day containing the complex food additive (Oxano®), Placebo: volunteers received 2 capsules/day identical to "verum" not containing the complex food additive.

lipid peroxidation products such as 8-epi-prostaglandin-F2-alpha (Reilly *et al.*, 1996) and lipid peroxides as well as lower contents of antioxidants such as carotene, retinal, α- and γ-tocopherol; significance could only be shown for β-carotene, however (Al-Senaidy *et al.*, 1997). Using the lipid peroxidation marker F2-isoprostane Dietrich and colleagues (2002) could only observe protective effects of the antioxidants ascorbate, lipoic acid and tocopherol in smokers with a clearly increased body mass index. Recently Biesalski's group (Schneider *et al.*, 2001) reported on the effects of smoking using the formation of micronuclei in lymphocytes and the ascorbyl free radical (EPR-measurements) as stress indicators. They found after short term supplemtation of vitamins C and E (7 days), that the ascorbyl radical increased and micronuclei in blood lymphocytes decreased as an indication of the protective effects of this antioxidant intervention.

This and other parameters of smoking are taken as basis for the epidemiologically clear picture that smokers envisage a dramatically increased risk of cancer and atherosclerosis where antioxidants under discussion are supposed to partially attenuate this risk (Kacmaz *et al.*, 1997).

In our intervention study with 48 healthy volunteers (20 smokers, 28 non-smokers) taking a food additive mainly containing vitamin C (ascorbic acid), vitamin E (α-tocopherol), ubiquinone (Q10), vitamin A and zinkoxide for four weeks in a double blind, randomized and placebo controlled manner, blood parameters such as haematocrit and haemoglobin are shown to be significantly increased in smokers before the intervention. Leukocytes-, thrombocytes- and erythrocytes-counts showed no significant differences. There is a clear but not significant trend for an increased total leukocytes- and especially neutrophils-count in smokers, however. As also reported by others (Al-

Table V. Comparison of correponding pairs: lipophilic antioxidant content.

Antioxidant (μM in plasma)	Before Intervention			After Intervention			Difference ± S. E. M.	Significance ($p < 0.05$)
	Min.	Max.	Mean ± S. E. M.	Min.	Max.	Mean ± S. E. M.		
Non-smokers verum (n = 12)								
Vitamin A	0.96	3.66	1.72 ± 0.75	0.98	4.02	1.71 ± 0.80	−0.01 ± 0.25	n.s.
Vitamin E	12.69	22.87	16.78 ± 2.94	16.58	35.21	20.84 ± 5.31	4.06 ± 3.81	**0.004**
Q10	1.04	2.73	2.10 ± 0.61	0.86	4.18	2.88 ± 1.23	0.78 ± 1.27	(0.057)
Smokers verum (n = 12)								
Vitamin A	0.86	2.35	1.57 ± 0.42	0.69	4.39	1.81 ± 0.99	0.24 ± 0.85	n.s.
Vitamin E	10.46	19.41	15.15 ± 3.08	12.24	28.35	19.99 ± 5.01	4.83 ± 4.88	**0.006**
Q10	0.97	3.44	1.71 ± 0.69	0.96	6.18	2.80 ± 1.42	1.09 ± 1.10	**0.006**
Non-smokers placebo (n = 16)								
Vitamin A	1.06	2.24	1.75 ± 0.34	0.98	3.27	1.66 ± 0.52	−0.09 ± 0.44	n.s
Vitamin E	10.03	23.97	17.54 ± 4.17	10.94	30.92	18.38 ± 4.94	0.84 ± 3.66	n.s.
Q10	1.02	2.74	2.16 ± 0.57	1.02	4.47	2.39 ± 1.00	0.23 ± 0.82	n.s.
Smokers placebo (n = 7)								
Vitamin A	1.15	2.80	1.79 ± 0.57	1.26	3.30	1.93 ± 0.67	0.15 ± 0.22	n.s.
Vitamin E	12.08	29.49	18.01 ± 6.56	11.72	26.74	17.54 ± 4.64	−0.47 ± 3.19	n.s.
Q10	1.17	4.12	2.17 ± 1.01	1.15	3.57	2.41 ± 0.88	0.24 ± 0.77	n.s.

Note: Min., Max.: minimal or maximal value within a sample; Difference: difference in the means after and before intervention; Verum: volunteers received 2 capsules/day containing the complex food additive (Oxano®), Placebo: volunteers received 2 capsules/day identical to "verum" not containing the complex food additive.

Table VI. Comparison of non-smokers with smokers: antioxidant capacity of blood plasma.

% Inhibition of the X/XOD-reaction	Non-smokers verum (n = 12)			Smokers verum (n = 12)			Difference	Significance ($p < 0.05$)
	Min.	Max.	Mean ± S. E. M.	Min.	Max.	Mean ± S. E. M.		
Before intervention	12.5	77.5	34.9 ± 18.4	17	79	56.5 ± 19.5	21.6	**0.011**
After intervention	14	95.5	52 ± 22.7	6	84.5	50.7 ± 23.5	0.7	n.s.
	Non-smokers placebo (n = 16)			Smokers placebo (n = 7)				
Before intervention	12	62.5	42.4 ± 12.4	36.5	72.5	54.4 ± 12.7	12	**0.046**
After intervention	19.5	76.5	44.7 ± 18.2	29	62	41.6 ± 12.6	−3.1	n.s.

Note: Min., Max.: minimal or maximal value within a sample; Difference: difference in the means between smokers and non-smokers; Verum: volunteers received 2 capsules/day containing the complex food additive (Oxano®), Placebo: volunteers received 2 capsules/day identical to "verum" not containing the complex food additive.

Senaidy *et al.*, 1997) there is a trend for a lower content of the lipid soluble antioxidants vitamin A and Q10 in the plasma of smokers.

After the intervention with the food additive, a significant ($p < 0.05$) reduction of total leukocyte number in the smoker verum group as compared to the smoker placebo group was observed.

Other blood properties such as antioxidative status, as far as the fat soluble and water soluble fractions in the blood plasma are concerned, showed certain significant or close to significant differences between the four groups before and after intervention:

Smokers after intervention exhibit a significant

Table VII. Comparison of correponding pairs: antioxidant capacity of blood plasma.

% Inhibition of the X/XOD-reaction	Before Intervention			After Intervention			Difference ± S. E. M.	Significance ($p < 0.05$)
	Min.	Max.	Mean ± S. E. M.	Min.	Max.	Mean ± S. E. M.		
Non-smokers verum (n = 12)	12.5	77.5	34.9 ± 18.4	14	95.5	52 ± 22.7	17.1 ± 29.2	(0.067)
Smokers verum (n = 12)	17	79	56.5 ± 19.5	6	84.5	50.7 ± 23.5	−5.8 ± 50.5	n.s.
Non-smokers placebo (n = 16)	12	62.5	42.4 ± 12.4	19.5	76.5	44.7 ± 18.2	2.3 ± 19.1	(0.05)
Smokers placebo (n = 7)	36.5	72.5	54.4 ± 12.7	29	62	41.6 ± 12.6	−12.7 ± 7.4	**0.018**

Note: Min., Max.: minimal or maximal value within a sample; Difference: difference in the means between smokers and non-smokers; Verum: volunteers received 2 capsules/day containing the complex food additive (Oxano®), Placebo: volunteers received 2 capsules/day identical to "verum" not containing the complex food additive.

i) increase of both vitamin E and coenzyme Q10,
ii) decrease of leukocyte counts and
iii) attenuation of their clearly increased water soluble – antioxidative potential

Non-smokers after intervention exhibit a significant

i) increase only of vitamin E and
ii) trends of an increase of Q10 and water soluble – antioxidative potential.

Intrinsic antioxidative plasma substances such as albumin and uric acid are by far the most potent radical scavengers present in a high concentration (Halliwell, 1996). We can assume that this plethora of antioxidative power is outranging any minor food additions. A different picture can be seen if we look at the lipophilic antioxidants vitamin E and Q10 which are clearly influenced by the intervention: whereas vitamin A is untouched in all test groups by the intervention, vitamin E and ubiquinone are increased after the intervention. Vitamin E is increased in both smokers and non-smokers, where Q10 is only significantly increased in the smoker verum group as compared to the corresponding placebo group or the non-smokers.

During the atherosclerotic process, which seems to be enhanced in smokers, LDL oxidation is assumed to represent one basic pathogenic reaction and lipophilic antioxidants are assumed to play a key role in protection. Stocker's group in Australia (Neuzil and Stocker, 1994; Thomas *et al.*, 1996) discussed a cooperation of vitamin E and Q10 in this context. We recently reported on this cooperative redox protection (Schneider and Elstner, 2000) showing that the following sequence of electron flow might play a key role in the LDL particle:

$$\alpha\text{-lipoic acid} \rightarrow Q10 \rightarrow \text{vitamin E} \rightarrow LOO^{\cdot}$$

In smokers the initiating peroxyl radical $LOO^{\cdot}$ might be produced by reaction of an unsaturated fatty acid with the free radical NO_2. The peroxyl radical is "repaired" by vitamin E, and the emanating vitamin E alkoxyl radical in turn is reduced by reduced Q10, ubiquinole, yielding ubiquinone, or is reduced by ascorbate (Buettner, 1993). Ubiquinone in turn is supposed to be rapidly reduced in the plasma (Mohr *et al.*, 1992; Kaikkonen *et al.*, 2001), most likely by thioctic (lipoic) acid (Kozlov *et al.*, 1999; Schneider and Elstner, 2000). Since in smokers the main target of radical attack seems to be the lipophilic phase, lipophilic antioxidants thus represent the first aid. Since these substances must be taken with the food and cannot be synthesized de novo in the body, only the water soluble antioxidative system can be turned on as support for the repair of vitamin E. This seems to be the case in smokers before the intervention. After intervention when the pools of vitamin E and Q10 are visibly "filled", the water soluble pool of the antioxidative potential is decreased as an indication of attenuation of the antioxidative scenario since smoking has not been given up during the time post intervention. We take these results as a valuable support of several *in vitro* findings and in vivo reports as well as clinical studies: Deficiencies in antioxidants in smokers and measurable effects of smoking on products of lipid peroxidation may

be attenuated by certain antioxidant food additives. Due to the increase of intrinsic water soluble antioxiants defence systems in smokers, the dominating effects thus seem to be achieved by the intake of vitamin E and Q10.

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