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ORIGINAL CONTRIBUTION

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Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods

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Summary *Aim of the study:* The present study was conducted to investigate changes in the plasma concentration of carotenoids and carotenoid oxidation products, vitamin A, α- and γ-tocopherol, and ubiquinone-10 during a dietary intervention trial with 23 male healthy volunteers.
Method: A two week carotenoid depletion period was followed by a daily consumption of 330 mL tomato juice (40 mg lycopene), then by 330 mL carrot juice (15.7 mg α-carotene and 22.3 mg β-carotene), and then by a 10 g spinach powder preparation (11.3 mg lutein and 3.1 mg β-carotene) served with main meals for two weeks, respectively. Blood samples were collected in the morning after an overnight fasting and carotenoids, vitamin A, tocopherols, and ubichinone were analyzed by reversed-phase HPLC.
Results: During the tomato juice intervention, plasma concentrations of trans- and cis-lycopene increased 3-fold compared to the depletion period. Lycopene oxidation products could be demonstrated in plasma and were significantly elevated compared to control ($p < 0.001$). After two weeks of carrot juice consumption, α-carotene and β-carotene concentrations increased 8.6- and 3.2-fold, respectively. Finally, during the spinach consumption period the lutein concentration increased 2-fold, while the β-carotene concentrations were still elevated 2-fold.
Conclusions: The moderate change in dietary habits, e.g., the consumption of 330 mL of carotenoid-rich vegetable juices caused significant changes in the plasma carotenoid concentrations, indicating a high bioavailability of carotenoids from these processed vegetable products. The changes in plasma carotenoid concentrations reflected the carotenoid composition of the consumed foods. However, particularly during the tomato juice intervention period the occurrence of lycopene oxidation products and cis-lycopene isomers in plasma was eminent. The formation may be due to antioxidant reactions of lycopene in the organism.

Key words Carotenoids – lycopene – lutein – α-carotene – β-carotene – oxidation – antioxidant – human

Introduction

There is increasing interest in dietary phytochemicals, since a large number of epidemiologic studies have shown a lower risk of cancer and cardiovascular disease in individuals consuming diets high in vegetables and fruit (2, 4, 23, 41). Plasma carotenoid concentrations are negatively correlated with the risk for these diseases (13) and carotenoids may, therefore, in part mediate the protective effects of vegetables and fruit. However, intervention trials with β-carotene supplements given to male smokers failed to show beneficial effects on cancer risk, but in contrast showed an increased lung cancer risk (1, 31). Therefore, other carotenoids besides β-carotene occuring in human plasma have recently become a major issue in nutrition research, including α-carotene, lycopene, and lutein (40, 44). While vast information on absorption, metabolism, and the action of β-carotene in humans is available (14, 26, 32), much less is known about these other carotenoids occuring in high concentrations in vegetables, such as lycopene in tomato and lutein in spinach or kale. The role of lycopene as an important carotenoid for humans has recently been reviewed, emphasizing the strongest singlet oxygen quenching ability among all carotenoids (40). Its potential health effects in humans include a reduction of prostate cancer risk (17) and myocardial infarction risk (23). The underlying mechanisms of these lycopene effects are still unclear, but there is some evidence that the antioxidant capacity of lycopene and the generation of lycopene metabolites are at least partly involved. The singlet oxygen quenching capacity of carotenoids may form carotenoid isomers in vitro (43). Data on isomerization of carotenoids in vivo as a result of singlet oxygen quenching are not available so far. However, the occurrence of cis-lycopene isomers in human plasma after a single dose of lycopene containing products has been demonstrated in the past (10, 34, 39). Little is known about the metabolism of lycopene in humans, like isomerization and oxidation due to tomato intake for several days (44). Data about the interaction of carotenoids during absorption in man are scarce and not conclusive. For β-carotene it has been shown that it may improve lycopene absorption (19) and reduce (24) as well as increase (16) lutein absorption. In these studies carotenoids were given as single compounds or as natural carotenoid mixtures and not as a food preparation.

In order to obtain more information about the bioavailability of carotenoids from food under physiological conditions, a dietary intervention study with tomato juice (lycopene), carrot juice (β-carotene), and spinach powder (lutein) was conducted. A study design without washout periods between the different vegetable intervention periods has been chosen to mimic more closely the dietary behavior of consumers and to investigate possible interactions in carotenoid absorption between lycopene, β-carotene, and lutein. In addition, the generation of carotenoid isomers and of carotenoid oxidation products during the dietary treatment was quantified. The vegetable products were used as a source for carotenoids to investigate whether minor dietary interventions would increase plasma carotenoids to a concentration, which in other studies were negatively correlated with the risk of cancer and of cardiovascular disease.

Materials and methods

Subjects

Twenty three non-smoking men, 27–40 years old, were recruited from research institutes of the Research Center Karlsruhe. The subjects were in good health as determined by a screening history and medical examination. They refrained from taking vitamin supplements or any medication two months before and during the study. The study was approved by the Medical Ethical Committee of the Landesärztekammer Baden-Württemberg and all participants gave written consent. Height and body weight were determined, and body mass index (BMI) was calculated. Body fat was measured by bioelectrical impedance analysis (Biodynamics 310, Seattle, Washington, USA).

Study design

The study of 8 weeks and was divided in 4 periods of 2 weeks each. During the entire study the volunteers were on their usual diet but were instructed to avoid food with a high carotenoid content. They were given a list of vegetables and fruit and the corresponding products, commonly eaten in Germany, to be excluded from their diet. The fruit and vegetables are given in Table 1. To check compliance, subjects had to document their entire fruit and vegetable consumption on a dietary record. The first two weeks served as a depletion period, during which no additional carotenoid-rich food was given (day -14 to day 0). During the following 14 days, the subjects ingested 330 mL of a commercially available tomato juice daily with their meals: tomato period (day 0 to day +14). During the next two weeks tomato juice was replaced by 330 mL of carrot juice daily: carrot period (day +14 to day +28). Finally, during the last two experimental weeks a liquid spinach powder preparation (10 g of spinach powder) was given with the daily meals: spinach period (day +28 to day +42). Subjects were asked to consume the vegetable products with their main meals in order to assure a high bioavailability of the carotenoids. In this study, washout periods were omitted between the different intervention periods to study possible interactions between carotenoids during absorption. Vegetable juices (Schoenenberger, Magstadt, Germany) and spinach powder (Völpel, Königmoos, Germany) were obtained directly from the producers. For the carotenoid analysis

Table 1 Fruits and vegetables from which the volunteers had to abstain during the study

fruits	vegetables
apricot, dryed	bell pepper
cantaloupe	broccoli
guava	brussels sprouts
mango	carrot
nectarine	chicory leaves
papaya	corn
peach, dryed	cucumber pickle
pink grapefruit	fennel
watermelon	kale
	leek
	lettuce
	mangold
	peas
	pumpkin
	scallion
	spinach
	tomato
	zucchini

10 g of juice and 1 g of spinach powder were used, respectively.

Blood samples

Fasting blood samples were taken at the beginning of the study and at the end of each week between 7 and 9 am. Blood was drawn from an antecubital vein into prechilled tubes containing EDTA (1.6 mg/mL, Monovette-Sarstedt, Nümbrecht, Germany) and immediately placed on ice in the dark. Plasma was collected after centrifugation at 1500 x g for 10 min at 4 °C. For carotenoid analysis BHT 5µg/mL plasma and 1.5% sucrose (final concentration) weres added to the plasma and then the samples were stored at -80 °C until analysis. For serum measurements, blood was collected in a "Serum Z Monovette" (Sarstedt, Nümbrecht, Germany) and separated after complete clotting by centrifugation at 1500 x g for 10 min at room temperature. Serum was stored at -80 °C until analysis.

Serum measurements

Cholesterol concentrations were assayed by measuring total cholesterol with the CHOD-PAP enzymatic test kit (Boehringer Mannheim, Germany). Sodium, potassium, and chloride were measured using ion selective electrodes (AVL Analysentechnik, Bad Homburg, Germany). In blood, hemoglobin and white blood cell count (WBC) were determined with an automated analyser (F-300, Sysmex, Hamburg, Germany).

Chemicals

Instead of the nomenclature of the Union of Pure and Applied Chemistry, the usual common names are used for the carotenoids (e.g., lutein for (3R,3′R,6′R)-β,e-carotene-3,3′-diol, cis-β-carotene for Z-β,β-carotene, and all-trans-lycopene for all-E-ψ,ψ-carotene). The reference substances α-carotene, β-carotene, and lycopene were obtained from Sigma (Deisenhofen, Germany), lutein from Fluka (Neu-Ulm, Germany). Zeaxanthin, β-cryptoxanthin, and echinenone were gifts from Hoffmann-La Roche (Basel, Switzerland). Carotenoids not commercially available were extracted from suitable plants, isolated pure by chromatographic methods (column liquid chromatography, thin layer chromatography), identified according to their VIS spectra and quantified by means of the specific 1% (1 cm) extinction coefficients (6, 12). Violaxanthin was isolated from spinach, and α-cryptoxanthin from carrot leaves. Vitamin A alcohol (retinol), vitamin E (α-tocopherol), γ-tocopherol, and ubiquinone-10 (coenzyme Q_{10}) were likewise obtained from Fluka.

Methanol, ethanol, acetone, acetonitrile, n-hexane, tetrahydrofuran (THF), diethylether, potassium hydroxide, water-free Na_2SO_4 (all of HPLC quality or p.a. grade), and pyrogallol were obtained from Merck (Darmstadt, Germany), butylhydroxytoluol (BHT) from Sigma.

Equipment

The HPLC systems consisted of two injectors (Waters, type U6K; Rheodyne, type 7725i), two pumps (Waters, type 501), photodiode array detector, PAD (Shimadzu, type SPD-M10AV), UV-VIS detector (Gamma-Analysen-Technik, GAT, type LCD 501), integrator (Merck-Hitachi, type D-2000), and separating columns (VYDAC 201TP54, Separation Group, Hesperia, USA, for carotenoids and µ-Bondapak-Phenyl, Waters, for ubiquinones). Other apparatus used were an Ultra-Turrax T25 (Janke & Kunkel, Germany) and a Rotavapor RE 120 (Büchi, Switzerland).

Preparation of food samples

Carotenoids from food were extracted in an Ultra-Turrax by 50 mL of acetone/ethanol (1:1), to which 1 mL of 5% aqueous pyrogallol solution as antioxidant had been added (modified method (11)). The spinach powder was further saponified for removal of chlorophyll and for hydrolysis of xanthophyll esters by addition of 5 mL of 20% methanolic potassium hydroxide and allowed to rest for 18 h at room temperature in the absence of light. The insoluble residue was separated by filtration. Then 40 mL of 0.5% Na_2SO_4 solution and 40 mL of n-hexane were added and shaken for a few min. After phase separation, the lower phase was removed and shaken again with 40 mL n-hexane. The recombined n-hexane phases were dried by 20 g of water-free Na_2SO_4 and reduced in the rotation evaporator at 35 °C and 150 mbar (15 kPa). Residual n-hexane was removed under nitrogen stream and the residue was added to 5 mL of methanol/THF (1:1). THF had been stabilized by 0.01% BHT. Depending on the color intensity, the sample was diluted by the metha-

nol/THF mixture for determinations by HPLC (additional information see 29).

Preparation of blood plasma samples

To isolate blood carotenoids, vitamins A, E, and ubiquinones, 2 mL plasma were extracted using 4 mL diethylether following the protein precipitation by addition of 2 mL ethanol. The sample was centrifuged for 15 min (3000 rpm, 20 °C) and the upper layer collected. This procedure was repeated twice. The combined ether phase was washed using 10 mL 5% NaCl, separated, and dried with 4 g water-free Na_2SO_4. The ether extract was evaporated in the water bath at 35 °C. Residual ether was evaporated with nitrogen. The residue was dissolved in 1 mL of methanol/THF (1:1) for HPLC analysis, stabilized by 0.01% BHT. Storage of solutions was at 4 °C. Recovery rates of the reference carotenoids were above 80%.

HPLC

For the analysis of carotenoids, tocopherols, and retinol by HPLC, a temperature regulated (20 °C) VYDAC 5 µ-C18 column (250x4.6 mm), plus a precolumn consisting of a Waters Guard-PAK module equipped with a µ-Bondapak C18 insert were used. Eluent for isocratic separation was a mixture of methanol, acetonitrile, and ammonium acetate (85:15:0.01). The prepared blood plasma sample (50 µl) was injected into the HPLC. The flow rate was 1 mL/min. The PAD was used to record chromatograms simultaneously at wavelengths of 450, 325, and 295 nm to quantify the carotenoids, retinol, and tocopherols and to determine peak spectra for carotenoid identification (from 280 to 520 nm) and for the checking of peak purity. Phytofluene and phytoene were separately determined at λ = 347 nm and 286 nm, respectively. Ubiquinones were determined by HPLC using a Waters µ-Bondapak-phenyl column (300x3.9 mm), plus a precolumn consisting of above described module equipped with a CN insert, a mixture of methanol, acetonitrile, and water (71:20:9) as the eluent, and a UV-VIS detector. Detection wavelength was 275 nm. Figure 1 shows a chromatogram of the separation of carotenoids and vitamins A and E (α-tocopherol) in human plasma by HPLC.

For quantification according to the external standard method, a "one-point calibration" was performed for each carotenoid available. In the measured range results were linear. Carotenoids, for which reference substances were not available (phytoene, phytofluene), were quantified by their 1% (1 cm) extinction coefficients E (7, 12). A fictitious concentration, c* = detector signal/E x 100, which had been determined as a function of the size and geometry of the flow-through cell, was related to the c* value of β-carotene (its actual concentration was known), which had been determined in the same way. Cis-derivatives and epoxides were quantified by using determination factors of the initial substances (e.g., lycopene for cis-lycopene and for lycopene-epoxides). Peak 1 in Fig. 1 was quantified as being lutein only. The "carotinoid oxidation products" of peak 3 were quantified using the factor of the zeaxanthin determination. Limits of detection depended on retention times (bottom width of peaks), e.g., 0.02 nMol for β-cryptoxanthin, 0.03 nMol for lycopene, and 0.06 nMol for β-carotene. The internal standard echinenone was used only in the phase of the method developement (it may interfere with α-carotene determination).

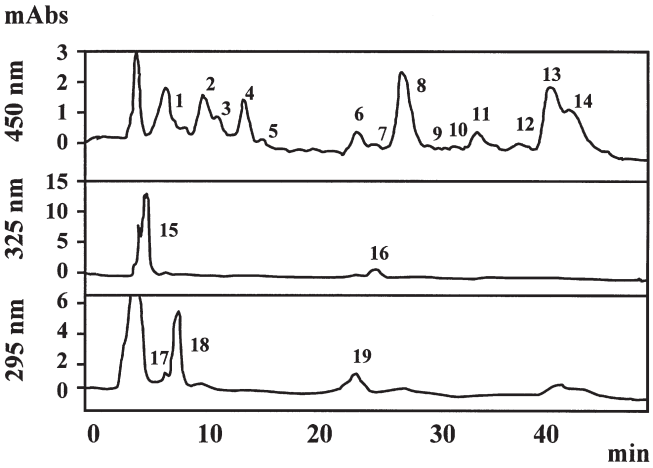

Fig. 1 HPLC chromatogram with consecutive photodiode array detection of single representative plasma sample from day 14. Simultaneous detection at 450nm (upper panel), 325nm (mid panel), and 295nm (lower panel) is given. The denoted peak numbers represent the following carotenoids, vitamin A, and tocopheroles. Upper panel, λ=450nm: peak #1, lutein/zeaxanthin/carotenoid oxidation products (retention time = 5.9 min); #2, carotenoid oxidation products (9.2 min); #3, α-cryptoxanthin (10.6 min); #4, β-cryptoxanthin (12.9 min); #5, β-carotene-5,6,5´,6´-diepoxide (14.6 min); #6, α-carotene (22.8min); #7, cis-α-carotene (24.4min); #8, β-carotene (26.9 min); #9, cis-β-carotene (28.9 min); #10, lycopene-x,x-epoxide (31.6 min); #11, lycopene 5,6-epoxide (33.5 min); #12, lycopene 1,2-epoxide (37.2 min); #13, lycopene (39.9 min); #14, cis-lycopene (41.5 min). Middle panel, λ=325nm: #15, retinol (3.8 min); #16, phytofluene (23.4 min), but detected at λ=347 nm. Lower panel, λ=295nm: # 17, γ-tocopherol (6.1 min), #18, α-tocopherol (7.4 min). Phytoene is denoted as #19, but detected at λ=286nm.

Statistics

Results are given as mean ± SD. Analysis of variance (ANOVA) was used to compare the depletion period and intervention periods. Comparisons of means were performed using the appropiate ANOVA post-test (Tukey-Kramer or Dunn's multiple comparison test). Differences

were considered to be significant at $p < 0.05$. Regression analysis was performed and coefficient of correlation (r) was calculated for the relative plasma lycopene increase after tomato juice consumption versus plasma lycopene concentration before tomato juice intervention. Statistical calculations were done by using the InStat 2.02 statistical program (Graph Pad Software Inc, San Diego, CA, USA) and EXCEL 5.0 (Microsoft Corp., Unterschleissheim, Germany).

Table 2 Anthropometric data

	mean	range
age [years]	34	27 - 40
height [cm]	181	168 - 200
weight [kg]	76	59 - 100
body fat [%]	17	10.7 - 23.6
BMI [kg/m^2]	23	19.6 - 28.1

Table 3 Carotenoid content of tomato juice, carrot juice, and spinach powder

	tomato juice	carrot juice	spinach powder
	mg/100 ml		mg/100 g
% dry matter	6.0	8.7	99.7
all-trans-β-carotene	0.45	6.56	31.1
cis-β-carotene	0.06	0.19	4.83
α-carotene	n.d.	4.76	0.89
β-cryptoxanthin	n.d.	0.01	1.47
α-cryptoxanthin	n.d.	0.007	n.d.
lutein (zeaxanthin)	n.d.	0.154 (0.019)	113.5[1]
violaxanthin	n.d.	n.d.	22.6
lycopene	11.84	n.d.	n.d.
cis-lycopene	0.28	n.d.	n.d.
lycopene-epoxides	0.3	n.d.	n.d.
phytofluene	0.71	0.96	n.d.
phytoene	2.23	2.1	n.d.

n.d.: not detected, [1] see (20)

Results

Anthropometric data of the volunteers are given in Table 2. All subjects tolerated the vegetable intervention well and none of them had to be excluded from analysis due to illness or noncompliance. Blood hemoglobin concentration, white blood cells, and serum electrolytes were within the normal range and did not change significantly during the entire study (data not shown). No significant changes in serum cholesterol concentrations were observed. Mean serum cholesterol during the study was 200 ± 26 mg/dl (mean ± SD). A trend ($p = 0.13$) for a decrease in serum cholesterol was observed after two weeks of tomato juice consumption compared to the depletion period (195.3 ± 27.8 vs. 201.5 ± 31.9 mg/dl, n = 23).

The carotenoid content of the selected vegetable juices and spinach powder are shown in Table 3. With the consumption of 330 mL of tomato juice subjects were supplied with 40 mg of lycopene. Carrot juice provided 22.3 mg β-carotene and 15.7 mg of α-carotene daily, and the spinach powder 11.3 mg of lutein and 3.6 mg β-carotene daily. The consumption of these vegetable products resulted in plasma concentrations of carotenoids, vitamins, and ubiquinone as summarized in Table 4.

The term “carotenoid oxidation products” summarizes ketones, and mono- and dihydroxy-carotenoids which occur in plasma only and seem to be specific plasma carotenoid metabolites (20, 21). The analyzed cis-carotenoid isomers in plasma are 1 or 2 cis-β-carotene isomers (9-cis- and 13-cis-β-carotene according to (35), and 2–3 different geometric cis-lycopene isomers (9-cis-lycopene and 13-cis-lycopene according to (39)). Besides the all-trans and cis isomers, the appearence of several carotenoid oxidation products in human plasma could also be demonstrated. The term “lycopene epoxides” summarizes lycopene-5,6-epoxide and lycopene-1,2-epoxide as determined by their VIS spectra. In some samples a third unidentified peak was found near the two mentioned lycopene epoxides. Other lycopene/cis-lycopene oxidation products such as lycophyll (lycopene-16-16´-diol) and 5,6-dihydroxy-5,6-dihydro-lycopene have been described in the literature (20, 22). Additionally, in some samples mono-epoxides of β-carotene , phytofluene, and phytoene as well as a diepoxide of β-cryptoxanthin were determined by given HPLC retention times and spectra (6, 12).

Two weeks of tomato juice consumption (day 0–14) resulted in a 2.4-fold increase in lycopene and cis-lycopene in plasma, respectively. After two weeks of a tomato-free, but carrot juice-rich diet, plasma lycopene concentration declined to the initial concentration at day 0 (Table 4). Lycopene epoxides increased 3.5-fold after tomato juice consumption and were still elevated on day 28 and 42 (211% and 132%, respectively).

The percentage distribution among the lycopenes in plasma and tomato juice is given in Table 5. Lycopene is predominant in the tomato juice (95.3%) with only small amounts of cis-lycopene and lycopene epoxides. However, in plasma 40% of total lycopene appeared as cis-lycopene and increased to almost 48% at the end of the study. Simultaneously, lycopene decreased from 45% to 38%. Lycopene epoxides in plasma increased up to 18% of the total lycopene on day 28, when tomato juice was already been omitted for 14 days. The relative increase of plasma lycopene from day 0 to 14 depended on the plasma concentration of the total lycopene before tomato juice was given ($r = -0.749$) but not on the total carotenoids ($r = -0.233$) (Fig. 2).

Table 4 Concentrations[1] (nmol/mL) of carotenoids, vitamins, and ubichinone in human plasma

		depletion		tomato	carrot	spinach
	day -14	day -7	day 0	day 14	day 28	day 42
lutein (incl. zeaxanthin)	0.37±0.14 d	0.40±0.13 d	0.35±0.12 d	0.33±0.12 d	0.36±0.11 d	0.71±0.17 a,b,c
carotenoid oxidation products[2]						
cis-lutein	0.02±0.01 c,d	0.02±0.01	0.014±0.004	0.02±0.01 c,d	0.02±0.01 b	0.012±0.004 b
carotenoid oxidation products[2] (+lycopene-16,16'-diol)	0.13±0.04 b,d	0.12±0.04 b,d	0.13±0.04 b,d	0.20±0.05 a,c,d	0.14±0.03 b	0.16±0.05 a,b
α-cryptoxanthin	0.05±0.02 c,d	0.05±0.02 c,d	0.05±0.02 c,d	0.06±0.03 c,d	0.04±0.02 a,b	0.04±0.02 a,b
β-cryptoxanthin	0.16±0.10 c,d	0.16±0.10 c,d	0.18±0.11 d	0.20±0.12	0.21±0.12	0.22±0.13 a
α-carotene	0.24±0.16 c,d	0.22±0.14 c,d	0.20±0.11 c,d	0.17±0.09 c,d	1.47±0.40 a,b	0.79±0.28 a,b
all-trans-β-carotene	0.74±0.44 c,d	0.71±0.41 c,d	0.60±0.36 c,d	0.65±0.25 c,d	2.05±0.72 a,b	1.21±0.52 a,b
cis-β-carotene	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01	0.02±0.01
β-carotene-5,6,6',6'-diepoxide	0.01±0.01 d	0.02±0.01 d	0.014±0.005 d	0.018±0.004	0.02±0.01	0.03±0.01 a
all-trans-lycopene	0.16±0.07 b	0.16±0.08 b	0.16±0.09 b	0.38±0.13 a,c,d	0.15±0.05 b	0.14±0.06 b
cis-lycopene	0.14±0.06 b	0.14±0.07 b	0.15±0.08 b	0.34±0.08 a,c,d	0.19±0.05 b	0.18±0.05 b
lycopene epoxides	0.04±0.02 b,c	0.04±0.01 b,c	0.05±0.03 b,c	0.14±0.04 a,d	0.08±0.02 a	0.05±0.01 b
phytofluene	0.14±0.08 b,c,d	0.13±0.08 b,c,d	0.12±0.06 b,c,d	0.41±0.19 a	0.44±0.15 a	0.29±0.10 a
phytoene	0.14±0.08 b,c	0.12±0.07 b,c,d	0.09±0.05 b,c,d	0.46±0.17 a	0.55±0.16 a,d	0.26±0.08 a,c
vitamin A (retinol)	2.40±0.39	2.32±0.40	2.43±0.49	2.50±0.44	2.28±0.45	2.34±0.46
vitamin E (α-tocopherol)	22.3±5.2 a,b	22.3±5.0 b	24.4±3.8	25.1±3.7 c	22.5±3.6 b	24.1±4.2
γ-tocopherol	1.72±0.64 a,b	1.70±0.67 a,b	2.30±0.75	2.19±0.73	2.20±1.14	2.08±0.73
ubiquinone-10	0.38±0.11 c,d	0.36±0.08 c	0.35±0.08	0.40±0.10 c,d	0.29±0.07 b	0.32±0.09 b

[1]Mean ± SD, n = 23; [2] see (20 and 21); a, b, c, d: $p<0.05$, significantly different from day 0 (a), day 14 (b), day 28 (c), and day 42 (d)

Carrot juice consumption increased plasma concentrations of α-carotene 8.6-fold and β-carotene 3.2-fold and did not decline to basal concentrations within the following 14 days (Table 4). There was only an increase of approximately 50% cis-β-carotene and β-carotene-5,6, 5´,6´-diepoxide. The increase of β-carotene-5,6,5´,6´-diepoxide, however, was not observed at the end of the carrot juice period, but at the end the spinach period with a delay of 14 days.

Spinach powder mixed with water, milk, yoghurt, or soup, given as 10 g portions daily, resulted in a two-fold increase in plasma concentrations of lutein (incl. zeaxanthin) after 14 days (Table 4). The analytical methods used in this study were not sufficient to discriminate lutein oxidation products, possibly hidden in peak 1 in Fig. 1. Lutein epoxide (taraxanthin), zeaxanthin epoxide (antheraxanthin), and zeaxanthin diepoxide (violaxanthin) which are present in high concentrations in fruit and vegetables could not be discriminated from oxidation products in human plasma. Spinach powder contains approximately 13% of violaxanthin, which, however, was not detectable in human plasma.

Throughout the whole intervention period (day 0 to day 42), no significant changes were observed in plasma vitamin A, γ-tocopherol, and α-tocopherol except for a small decrease in α-tocopherol on day 28, which returned

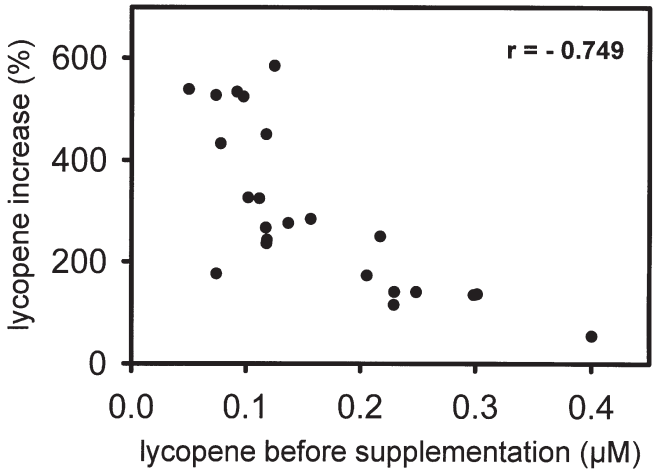

Fig. 2 Correlation plot of plasma lycopene concentration before tomato juice consumption (day 0) and the relative increase in lycopene plasma concentration (%) after tomato juice consumption (day 0 to day 14). n = 23, r: coefficient of correlation.

to basal concentration on day 42. Ubiquinone 10 plasma concentrations did not change until day 14 but then decreased significantly on day 28 and 42.

The carotenoid precursor phytoene and phytofluene which are present in tomato and carrot juice but not in spinach powder could also be determined in human plasma. There was a 6.3-fold, 8-fold, and 3.5-fold increase of phytoene (day 14, 28, 42) and a 4-fold, 4.4-fold, and 2.8-fold increase of phytofluene (day 14, 28, 42) in plasma, respectively.

Discussion

In this study, daily consumption of vegetable products over 14 days increased carotenoid plasma concentrations up to 8-fold, indicating a high bioavailability of carotenoids in these processed products. Furthermore, a predominant cis-isomerization of lycopene, but not of β-carotene or lutein, was observed. In addition, a substantial increase in lycopene oxidation products after vegetable juice consumption was demonstrated in human plasma, suggesting antioxidant activity of lycopene.

The results of this study therefore show that, on the basis of a diet low in carotenoids, the consumption of food given as vegetable juice will increase carotenoid plasma concentrations significantly (8, 9, 27, 44). The baseline carotenoid plasma concentrations reported in this study are in a range which has been described in carotenoid intervention studies before (5, 9, 27, 34, 36, 39, 44). In this study an intervention with vegetable products was chosen to mimic the physiological situation resulting from the ingestion of carotenoid-rich foods instead of isolated carotenoids. It was the aim to investigate the bioavailability of a large dose of carotenoids provided by vegetables. The daily amount of ingested lycopene from 330 mL tomato juice was 40 mg which is 40 times more than the average daily lycopene intake in Germany and the UK (30, 36). The daily intake of 330 mL carrot juice provided 22.3 mg of β-carotene, which is 10 times of the average daily β-carotene intake in Germany and the UK (30, 36). Since spinach juice was not commercially available, 10 g of spinach powder (11.3 mg lutein) were chosen, which delivered again about 10 times of the average daily lutein intake in Germany and the UK (30, 36).

The different amounts of carotenoids provided by the vegetables may partly explain the different increases in carotenoid plasma concentrations, which we observed after the consumption of the various vegetable preparations (lycopene 2.4-fold, α-carotene 8.6-fold, β-carotene 3.2-fold, and lutein 2-fold). However, the small increase in plasma lutein might not only be due to the lower amount of ingested lutein (11.3 mg/d), but also to the preceeding carrot juice period. α-Carotene and β-carotene were still elevated at the end of the spinach period and might, therefore, interact with lutein absorption, which has been proposed by others (24, 27). Additionally, the elevated plasma concentrations of α-carotene and β-carotene might also result from the consumption of the spinach powder preparation (Table 3). However, recently it has been shown that at least at the chylomicron level, lutein and zeaxanthin are taken up preferentially even in the

Table 5 Distribution of lycopene isomers and epoxides in tomato juice and human plasma

		depletion		tomato	carrot	spinach
	day -14	day -7	day 0	day 14	day 28	day 42
	tomato juice		plasma			
			(%)			
all-trans-lycopene	95.3	48.1	45.3	45.0	43.0	36.3	37.9
cis-lycopene	2.3	40.9	42.2	41.6	40.3	45.4	47.9
lycopene epoxides	2.4	11.0	12.5	13.4	16.7	18.3	14.2

presence of high amounts of β-carotene (16). From these contradictory findings, it could be concluded that the β-carotene plasma concentrations in this study do not substantially influence lutein absorption from spinach powder in the volunteers. This conclusion is supported by the fact that the relative increase in lutein plasma concentration seems to depend on lutein plasma concentrations before supplementation ($r = -0.584$), but not on the total plasma carotenoid concentration (TC) ($r = -0.189$). Similar findings were observed for lycopene and β-carotene, showing a great variability among the individuals in the response to the plasma carotenoids present in the vegetable juices. The relative increase in lycopene plasma concentration, seems to depend on lycopene plasma concentrations before supplementation ($r = -0.749$) but not on TC ($r = -0.233$). Similar results were obtained for the relative increase in α-carotene ($r = -0.747$ for α-carotene before supplementation, and $r = -0.326$ for TC), β-carotene ($r = -0.477$ for β-carotene before supplementation, and $r = -0.220$ for TC), and for lutein, as already mentioned. This might indicate that the absorption of carotenoids and their appearence in blood is regulated selectively for each carotenoid and that interactions between carotenoids may play a minor role only.

It has been reported in the past that uptake of lycopene from tomato juice is low (8, 39). In the present study, however, lycopene is well absorbed after tomato juice consumption, since mean plasma lycopene concentration increases 2.4-fold. The findings are in accordance with the results from Micozzi et al. (27), who showed that based on a controlled low-carotenoid diet additional tomato juice consumption prevented the decline of the plasma lycopene concentration, while plasma concentrations of other carotenoids, which were not supplemented, were reduced in plasma (27). The discrepancies found in the different studies might result from the duration of juice consumption and different application forms of the juice. The studies mentioned above (8, 39) were single dose studies. The juice intervention in this study lasted 2 weeks and the volunteers were asked to take the juices with their main dishes, providing enough fat with the meal for carotenoid absorption. At least in one study (39), where lycopene uptake from tomato juice was low, no additional fat was given with the juice, which might be responsible for the low lycopene absorption.

The slight reduction in serum cholesterol concentrations on day 14 after tomato juice consumption ($p = 0.13$) might be a result of increased lycopene plasma concentrations. Recently, it was shown that dietary supplementation with lycopene (60 mg daily for 3 months) reduced plasma LDL cholesterol in healthy volunteers by 14% (15). The authors attributed the cholesterol lowering effect of lycopene to the inhibition of macrophage 3-hydroxy-3-methyl glutaryl coenzyme A reductase. The lower lycopene intake by the volunteers in this study and the much shorter intervention period may in part explain the discrepancy between the two studies.

Carotenoids are acceptors of the energy that is transferred from singlet oxygen to the quencher (25). Therefore, carotenoids do possess a remarkable antioxidant potential in the sense of an extended antioxidant definition: biological antioxidant (18). When looking at the plasma concentrations of the main representative carotenoids in the vegetable products of this study (lycopene, α- and β-carotene, and lutein), an outstanding position of lycopene is obvious. Compared to other carotenoids, cis-isomers and oxidation products of lycopene are measurable in relatively high quantities in human plasma. The question arises, what is behind the different metabolism of this single carotenoid. The importance of lycopene for humans has recently been reviewed (40), but the role of lycopene oxidation products and cis-isomers in human plasma is still unclear. Research on carotenoid isomers in human plasma has been focused on 9-cis-β-carotene (3, 35, 37, 38, 42, 45). Only trace amounts of 9-cis-β-carotene could be detected in these studies and it has been demonstrated that 9-cis-β-carotene is poorly absorbed (3, 38) and that an isomerization to all-trans-β-carotene occurs in the human gut after oral supplementation with 9-cis-β-carotene (45). Little is known about the uptake and appearence of cis-lycopene in human plasma (10, 34, 39, 44). Information on the time course of cis-lycopene for several weeks is so far not available. Here, we report on the plasma concentrations of cis-lycopene over a period of 8 weeks, with two weeks of tomato juice consumption included. The results show that the consumption of tomato juice containing 95% trans-lycopene leads to a substantial increase of cis-lycopene in plasma. Cis-lycopene accounts for about 40–50% of the lycopene isomers which is in accordance with previous observations (39). The molar concentrations of lycopene rapidly decreased after tomato juice intake was stopped. However, cis-lycopene concentrations remained elevated and did not reach baseline values even 4 weeks after tomato juice intervention. The relative distribution of lycopenes (trans-lycopene, cis-lycopene, lycopene epoxides) in plasma show a decrease of trans-lycopene and a delayed relative increase of cis-isomers in plasma during the study, which points toward a different metabolism for trans-lycopene and cis-lycopene isomers in humans. The occurrence of high amounts of cis-lycopene in plasma could be due to a preferential uptake of cis-isomers, a conversion of trans-lycopene to cis-lycopene, or a higher degradation rate of trans-lycopene compared to cis-lycopene. Heat-treated lycopene (24 h at 37 °C, dissolved in n-hexane) and canned tomato products preferentially show a cis-lycopene with a delayed retention time to 45–50 min in the HPLC chromatogram (Fig. 1). This cis-lycopene isomer was also detected in the LDL fraction of day 14 and represents 13% of the total cis-lycopene isomers (data to be published elsewhere). In contrast to other cis-lycopene isomers, its spectrum clearly shows the typical cis-peak (at 360 nm). Delayed retention time and typical cis-peak in the spectrum may indicate a trans-cis isomerization in the center

of the lycopene molecule, which might be possible only in the presence of heat. It has previously been shown that lycopene is the most efficient singlet oxygen quencher (see 40) and phenoxyl radical scavenger (28) among the naturally occuring carotenoids. Since we have found high plasma concentrations of cis-lycopene, we might speculate of a quenching reaction of lycopene with singlet oxygen, as it is described for β-carotene (25), resulting in triplet oxygen and an activated (rotating and vibrating) lycopene molecule switching from the all-trans to the cis-isomer. However, further studies are needed to answer this question.

Another feature of lycopene in human blood, and a second possible antioxidant action of lycopene, is the chemical reaction with peroxides or peroxyradicals, thereby forming lycopene epoxides. Recently, lycopene has been shown to be the most efficient scavanger of phenoxyl radicals followed by β-carotene, zeaxanthin, and lutein (28). In the present study, lycopene epoxides appear in rather high concentrations in plasma, compared to other carotenoid epoxides, and increase even more after tomato juice consumption. The predominant generation of lycopene epoxides in human plasma indicates a preferential oxidation of lycopene and might point to a distinct antioxidant function of lycopene. The fate of lycopene epoxides in plasma is unclear. However, like the cis-isomers, plasma concentrations of lycopene epoxides were also elevated after tomato juice consumption has been terminated, although the trans-isomers had already reached baseline values. Again, in human plasma for lycopene epoxides a different metabolism has to be considered compared to the all-trans-isomer. A further reaction mechanism of lycopene epoxides could be the hydrolysis leading to diols. One example is the lycopene oxidation product 5,6-dihydroxy-5,6-dihydro-lycopene (20) which should be the result of lycopene-5,6-epoxide hydrolysis. Additionally, lycoxanthin (lycopene-16-ol) and lycophyll (lycopene-16,16´-diol) detected in human plasma, with their methyl-based hydroxy-groups, could either be generated enzymatically, or by reaction with hydroxy-radicals, indicating another hypothetical antioxidant mechanism of lycopene.

Oxidation products from other carotenoids were also detectable in human plasma. A β-carotene-5,6-5´,6´-diepoxide could be demonstrated in plasma. It increased after carrot juice consumption, but reached its maximum on day 42, where carrot juice consumption was stopped already for 14 days. However, the relative contribution of β-carotene-5,6-5´,6´-diepoxide to total carotenes accounts for less than 2%. In contrast, lycopene-epoxides account for 11–18% of total lycopenes.

Phytofluene with 5 conjugated double bonds and phytoene with 3 conjugated double bonds in the chromophor are acyclic carotenoids with the same chain length as lycopene (11 conjugated double bonds). In some plasma samples up to 20% of epoxides of these two carotenoids could be detected, which is similar to the 18% epoxides from lycopene. Presumably the length of the chromophore (the number of conjugated double bonds) is important for the reactivity and, therefore, the antioxidative capacity of a carotenoid. Oxidation products previously described in the literature (20) such as ketones and hydroxy-ketones from α-carotene and β-carotene, as well as oxidation products from lutein and zeaxanthin could not be detected because of lacking mass spectrometry facilities.

In conclusion, moderate changes in diet, e.g., intervention with carotenoid-rich vegetables under physiological conditions, increase plasma carotenoid concentrations in healthy volunteers substantially. Uptake of carotenoids seems to depend on the preexisting plasma concentration of the corresponding carotenoid mainly, and not on total plasma carotenoid concentration. Of the three main carotenoids (lycopene, β-carotene, and lutein) studied in this trial, lycopene shows striking differences concerning its metabolism in plasma compared to other carotenoids under investigation. Lycopene cis-isomers and epoxides increase in plasma after tomato juice consumption reflecting its different metabolism and/or a massive cis-isomerization and epoxidation *in vivo*. Results about the relative distribution of carotenoids in lipoproteins, functional antioxidant measurements, and immunological data are under preparation and will be published elsewhere. Results on DNA-protecting effects from this trial have recently been published (33).

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