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Intestinal absorption of lycopene from different matrices and interactions to other carotenoids, the lipid status, and the antioxidant capacity of human plasma

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Abstract *Background:* The bio-availability of carotenoids has been investigated in animal studies as well as in human studies, so far mostly for β -carotene. Only few results exist for lycopene. In recent studies, lycopene was significantly better available from processed tomatoes compared to raw tomatoes, when using daily intakes between 16.5 mg and 75 mg lycopene.

Aim of the study In a comparative study the availability of a low oral lycopene dosage of 5 mg/d from different food matrices versus soft gel capsules containing tomato oleoresin was assessed. In addition to the plasma carotenoid content, the effect of lycopene ingestion on other plasma carotenoids, the lipid status parameters, and the antioxidant activity was estimated.

Methods Twenty-two female adults (20 – 27 y) were randomized in three groups and were advised to minimize their carotenoid intake for two weeks. After this initial period, two groups received a portion of tomatoes or tomato juice adjusted to a lycopene dose of 5 mg/d, the third group ingested the same dose comprized in soft gel capsules containing tomato oleoresin. During the test period of 6 weeks, the participants continued reducing the intake of carotenoids from food. Fasting blood samples were withdrawn prior to the study, before supplementation started, and then weekly while supplemented. Seven-day dietary records were pre-

pared before the study started and after one week of supplementation. Carotenoids were analyzed by reversed phase HPLC with diode array detection. Dietary records were evaluated using the computer software EBIS 2.1. The plasma total cholesterol, HDL cholesterol, and triglycerides were determined enzymatically. In addition, the antioxidant activity of plasma was estimated by using the TEAC and the TRAP assays.

Results The basal levels of lycopene in plasma were comparable for all groups (0.2 – 0.3 $\mu\text{mol/l}$) and decreased significantly during the two weeks of depletion to approximately 50 % of the basal values. Other plasma carotenoids such as β -carotene and β -cryptoxanthin decreased significantly, too, whereas lutein and zeaxanthin remained unchanged. After supplementation with tomato oleoresin capsules or tomato juice, the plasma lycopene increased significantly, while it remained unchanged during intake of tomatoes.

Normal dietary habits were practiced of all volunteers before and during the study except vitamin C whose intake was significantly lower during the study period, because the probands were recommended to reduce the intake of fruits and vegetables. Lycopene supplementation did not affect the lipid status parameters of the three groups. After ingestion of lycopene the antioxidant activity of the

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plasma was not altered. Mean TEAC values were estimated to 0.33 ± 0.05 mmol/l and TRAP values to 1.0 ± 0.1 mmol/l and showed no significant differences in all groups during the whole study period.

Conclusions The bioavailability of lycopene varied significantly depending on the administered matrix. Lycopene from tomato oleoresin capsules and tomato juice (processed tomatoes) was better absorbed from the intestine than lycopene from raw tomatoes.

The daily intake of 5 mg lycopene, an intake comparable to

the usual daily carotenoid intake, did not affect cholesterol and triglycerides in plasma or its antioxidant capacity.

Key words Intestinal absorption – lycopene – tomato matrices – tomato oleoresin capsules – antioxidant capacity

Background

The bioavailability of carotenoids has been investigated in animal studies as well as in human studies, so far mostly for β -carotene. In ferrets, β -carotene uptake from enriched fruit juice (25) or from preparations dispersed in water (26) enhanced the serum β -carotene concentrations significantly more than carrot juice. In preruminant calves, serum β -carotene levels were significantly higher after supplementation with water-soluble β -carotene supplements compared to β -carotene in oil (3). Interactions between lycopene and canthaxanthin were observed in rats (5). After lycopene intake from tomatoes the canthaxanthin content in liver and plasma was reduced compared to control rats without lycopene intake. In rats, a reduction in the intestinal microflora after treatment with an antibiotic mixture resulted in an increased liver storage of α - and β -carotene, possibly caused by the enhancement of the intestinal transit time (12).

In a human study, after lutein intake the absorption of similarly given β -carotene was significantly reduced, and vice versa (14). After intake of a carotenoid mixture containing large amounts of β -carotene and minor amounts of lutein and zeaxanthin, these two xanthophylls were preferentially transported from the intestinal lumen into chylomicrons (8). Regarding interactions between carotenoids, it has been argued recently that interferences are only effective between carotenes and xanthophylls and not among carotenes (24). Synthetic β -carotene was better absorbed than β -carotene from carrots, tomato juice, and broccoli (15). Comparably, β -carotene was better available from supplements than from carrots and carrot juice. (23). Pectin intake, on the other hand, significantly reduced the absorption of β -carotene (21).

After a single lycopene intake of 23 mg from tomato paste, the content in chylomicrons rose 2.5 times higher than after an intake from tomatoes (9). Similarly, 16.5 mg/d (one week) lycopene were significantly better available from tomato paste than from tomatoes (19). In a recent study with lycopene supplements and tomato juice, a comparable absorption in the dosage range 70 - 75 mg/d lycopene (four weeks) was demonstrated for all matrices (17).

Aim of the study

The objective of this study was to assess the long-term bioavailability of lycopene from different matrices in a lower dosage range of 5 mg/d, corresponding to the usual total carotenoid intake per day which was recently estimated to be 5.33 mg (18). These authors used analytical data and the German National Food Consumption Survey (NVS) for their estimation. In contrast, recent studies often used unusually high doses of lycopene to assess the availability: 16.5 mg/d in form of 300 g tomatoes/d (19), 23 mg as single dose (400 g tomatoes/d) (9), or 70–75 mg/d comprized in 476 g tomato juice/d (17). In addition, the effects of lycopene ingestions on lipid status parameters and on the antioxidant capacity of plasma were investigated.

Methods

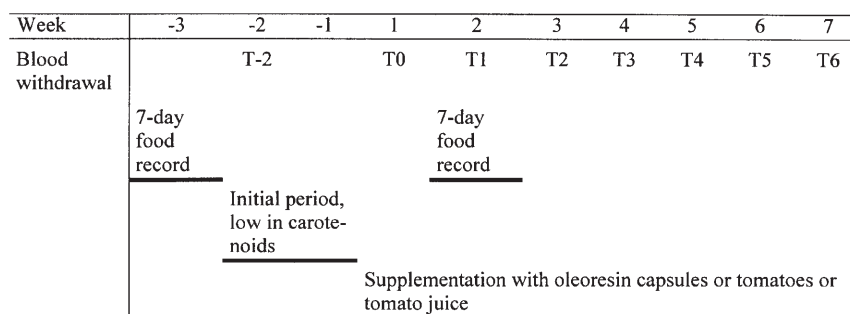
Participants and study protocol

Twenty-two female adults participated in this study. They were non-smokers and did not take β -carotene and vitamin A supplements. The participants were randomly divided into three groups. Their main characteristics are shown in Table 1. All participants gave their informed written consent. The study protocol was approved by the Local Ethical Committee.

All persons were advized to minimize their carotenoid intake from food for two weeks by avoiding food rich in carotenoids like tomatoes and tomato products, carrots and carrot products, red pepper, yellow and orange fruits. After this period, they ingested 5 mg lycopene daily for

Table 1 Main characteristics of all participant groups

Group	age (y)	weight (kg)	BMI (kg/m ²)
1 "capsules" (n=8)	21.0±0.8	58.1± 5.8	20.3±1.7
2 "tomatoes" (n=6)	21.8±2.6	62.0±11.8	22.0±4.4
3 "tomato juice" (n=8)	21.4±1.6	61.4± 6.4	22.0±2.0

Fig. 1 Scheme of the study design.

Blood withdrawal: after overnight fasting, between 7.30 and 8.30 o'clock
T: Blood withdrawal day

six weeks from soft gel capsules containing tomato oleoresin or the analogous amount of tomatoes or tomato juice, and continued reducing their carotenoid intake from food. The tomato oleoresin was prepared from raw tomatoes by removal of water and extraction to produce a concentrated oleoresin containing 6 % lycopene in the natural oils of tomatoes, which was encapsulated. These soft gel capsules (Lyc-O-Mato®) were a gift from LycoRed, Beer-Sheva, Israel. Tomatoes and tomato juice were purchased in a local store. Each group ingested the lycopene supplement with dinner. The lycopene content of the tomato juice was analyzed prior to the start of the study, the lycopene content of the tomatoes after each purchase, in order to calculate the equivalent amount of juice and tomatoes for the participants.

Fasting blood samples were withdrawn in EDTA tubes prior to the study, after the two weeks of depletion, and thereafter weekly while supplemented. The whole study design is shown in Fig. 1.

Assessment of nutritional status

The nutrient intake was estimated by 7-day food records prior to the study and one week after start of the supplementation period. The food records were evaluated with the aid of the computer software ebis 2.1 (E+D Partner, Neu-Anspach, Germany).

Sample preparation

Immediately after withdrawing, plasma was separated by centrifugation and the amount of total cholesterol, HDL cholesterol, and triglycerides were analyzed. Separate plasma samples were stored at -80 °C for carotenoid analysis and antioxidant activity measurements.

Analytical methods

Total cholesterol, HDL cholesterol, and triglycerides were analyzed enzymatically, using test kits from Roche Diagnostics (formerly Boehringer Mannheim), Mann-

heim, Germany. Carotenoids were extracted according to Bieri et al. (2), slightly modified. Briefly, 500 µl plasma and 500 µl ethanol with the internal standard were vortexed and then extracted twice with 250 µl n-hexane each. The combined organic phases were evaporated to dryness using a gentle stream of nitrogen. The residue was dissolved in 250 µl of the mobile phase for the HPLC analysis at 9 ± 2 °C on a VYDAC 201TP54 column with diode array detection (4). A standard chromatogram (A) and a chromatogram of a plasma sample (B) after six weeks ingestion of oleoresin capsules are shown in Fig. 2. The detection limits of this HPLC method were between 0.011 and 0.025 µmol/l for the carotenoids investigated. The antioxidant activity of the plasma was estimated by using the TEAC assay (16) and the TRAP assay (11).

Statistical analysis

All results are given as mean values \pm standard deviation. Differences between variables were tested for significance by one way ANOVA (SPSS for Windows, version 6.1.2, SPSS, München, Germany), using a level of significance of $p < 0.05$. In the case of inhomogeneous variances, the paired t-test for independent samples (level of significance $p < 0.05$) was used. The results were defined as "comparable" if $p > 0.05$.

Results

Lycopene

Baseline plasma carotenoid contents of the three groups are listed in Table 2. All carotenoids were not significantly different between the groups ($p > 0.05$).

After the two week diet with low carotenoid intake, the plasma lycopene concentrations decreased (group 1, $p > 0.05$; group 2-3, $p < 0.05$) to 44-59 % of the basal values (group 1, 0.17 ± 0.09 µmol/l; group 2, 0.22 ± 0.08 µmol/l; group 3, 0.27 ± 0.08 µmol/l).

Table 2 Basal carotenoid levels in plasma of the three study groups

Carotenoid \ Group		Group 1 (n=8) "capsules"	Group 2 (n=6) "tomatoes"	Group 3 (n=8) "tomato juice"
Lutein	($\mu\text{mol/l}$)	0.33 ± 0.16	0.34 ± 0.12	0.29 ± 0.14
Zeaxanthin	($\mu\text{mol/l}$)	0.26 ± 0.16	0.23 ± 0.09	0.22 ± 0.13
β -Cryptoxanthin	($\mu\text{mol/l}$)	0.30 ± 0.15	0.22 ± 0.09	0.31 ± 0.14
β -Carotene	($\mu\text{mol/l}$)	0.57 ± 0.66	0.40 ± 0.14	0.53 ± 0.27
Lycopene	($\mu\text{mol/l}$)	0.17 ± 0.09	0.22 ± 0.08	0.27 ± 0.08

The plasma lycopene levels were significantly enhanced from $0.10 \pm 0.05 \mu\text{mol/l}$ to $0.25 \pm 0.08 \mu\text{mol/l}$ after two weeks of supplementation with lycopene soft gel capsules (Fig. 3) and from $0.12 \pm 0.05 \mu\text{mol/l}$ to $0.22 \pm 0.08 \mu\text{mol/l}$ after supplementation with tomato juice (Fig. 4) and maintained these levels during the next four weeks of supplementation. On the other hand, no significant change of the plasma level could be observed after intake of tomatoes (Fig. 5).

Other carotenoids

β -carotene was found as the main carotenoid in all plasma samples and its contents did not vary significantly between the three groups (group 1, $0.57 \pm 0.66 \mu\text{mol/l}$; group 2, $0.40 \pm 0.14 \mu\text{mol/l}$; group 3, $0.53 \pm 0.27 \mu\text{mol/l}$). Besides (E)- β -carotene, some plasma samples contained 13(Z)-carotene, too.

The low carotenoid diet during the first two weeks significantly reduced the plasma β -carotene concentration to $0.26 \pm 0.10 \mu\text{mol/l}$ (group 2, "tomatoes") or to $0.36 \pm 0.19 \mu\text{mol/l}$ (group 3, "tomato juice"). Supplementation with lycopene did not significantly affect plasma β -carotene concentrations in either group.

The plasma contents of the two xanthophylls lutein (group 1, $0.33 \pm 0.16 \mu\text{mol/l}$; group 2, $0.34 \pm 0.12 \mu\text{mol/l}$; group 3, $0.29 \pm 0.14 \mu\text{mol/l}$) and zeaxanthin (group 1, $0.26 \pm 0.16 \mu\text{mol/l}$; group 2, $0.23 \pm 0.09 \mu\text{mol/l}$; group 3, $0.22 \pm 0.13 \mu\text{mol/l}$) were comparable ($p > 0.05$) in all groups and did not change significantly during the supplementation period with lycopene.

The provitamin A-active xanthophyll β -cryptoxanthin showed comparable ($p > 0.05$) plasma concentrations in all groups (group 1, $0.30 \pm 0.15 \mu\text{mol/l}$; group 2, $0.22 \pm 0.09 \mu\text{mol/l}$; group 3, $0.31 \pm 0.14 \mu\text{mol/l}$). The low ca-

Fig. 2 HPLC chromatograms of a carotenoid standard mixture (A) and a plasma sample after six weeks ingestion of oleoresin capsules (B), chromatographic conditions: VYDAC 201TP54 (250 x 4.6 mm, 5 μm) preceded by a 10 x 4.0 mm ProntoSil 120-3-C18 H guard column, 2.0 ml/min methanol/acetonitrile/isopropanol (55 + 44 + 2, m/m/m), column temperature 7.0 $^{\circ}\text{C}$, detection UV 450 nm, 1 = capsanthin, 2 = lutein, 3 = zeaxanthin, 4 = canthaxanthin, 5 = β -apo-8'-carotenal (internal standard), 6 = β -cryptoxanthin, 7 = echinenone (internal standard), 8 = α -carotene, 9 = (E)- β -carotene, 10 = 15(Z)- β -carotene, 11 = 13(Z)- β -carotene, 12 = 9(Z)- β -carotene, 13 = (E)-lycopene, 14 = 9(Z)-lycopene,

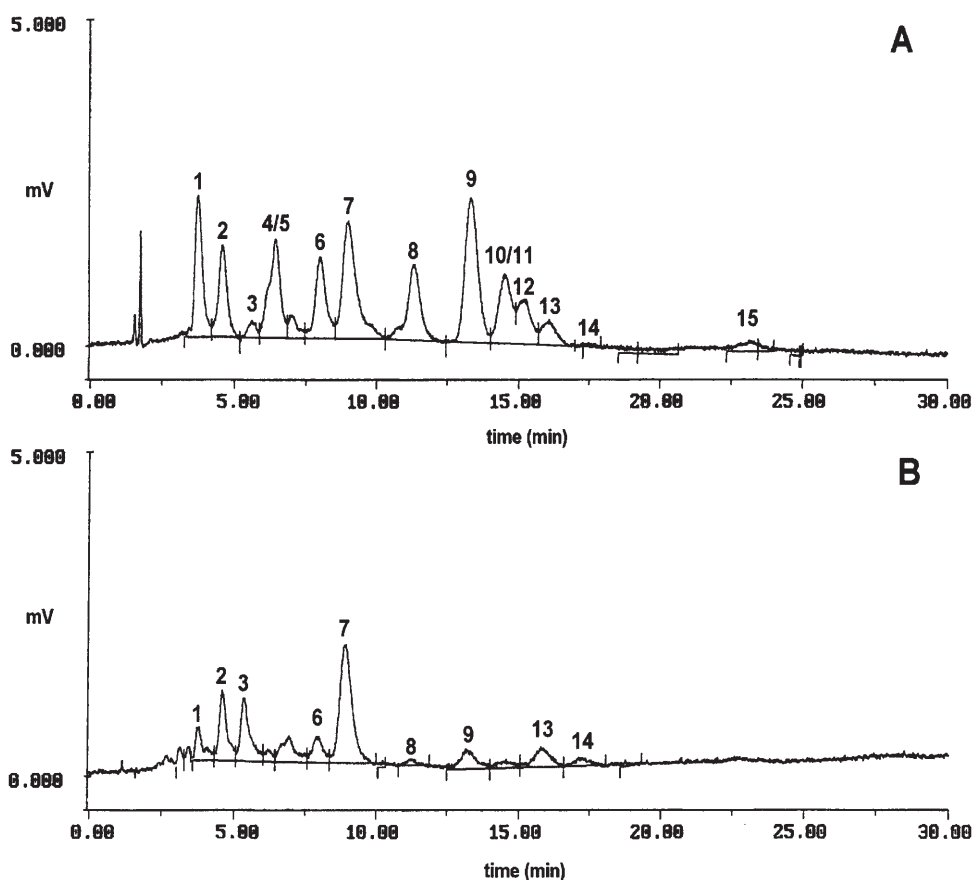


Table 3 Daily intakes of dietary energy and selected nutrients prior to the study (food record 1)

Nutrient \ Group		Group 1 (n=8) "capsules"	Group 2 (n=6) "tomatoes"	Group 3 (n=8) "tomato juice"
Energy	(MJ/d)	8.2 ± 2.1	7.2 ± 0.9	8.2 ± 1.9
Protein	(g/d)	73 ± 7	63 ± 10	66 ± 20
Fat	(g/d)	73 ± 23	68 ± 13	70 ± 16
Carbohydrates	(g/d)	238 ± 69	205 ± 46	247 ± 58
Dietary fiber	(g/d)	20 ± 7	20 ± 9	24 ± 5
Retinolequ.	(mg/d)	1.0 ± 0.6	1.1 ± 0.6	1.2 ± 0.5
Carotene	(mg/d)	3.0 ± 3.1	2.7 ± 2.7	3.4 ± 2.5
Tocopherolequ.	(mg/d)	9.2 ± 3.8	9.2 ± 2.4	11.3 ± 3.2
Thiamine	(mg/d)	1.4 ± 0.4	1.2 ± 0.1	1.3 ± 0.3
Riboflavin	(mg/d)	1.6 ± 0.5	1.6 ± 0.3	1.5 ± 0.3
Folic acid	(µg/d)	290 ± 136	220 ± 160	309 ± 68
Vitamin B ₆	(mg/d)	1.9 ± 0.8	1.4 ± 0.3	1.6 ± 0.4
Vitamin B ₁₂	(µg/d)	4.4 ± 1.4	4.5 ± 1.1	4.5 ± 2.3
Vitamin C	(mg/d)	118 ± 54	99 ± 47	162 ± 80
Calcium	(mg/d)	1001 ± 314	891 ± 290	1162 ± 912
Magnesium	(mg/d)	327 ± 104	323 ± 124	367 ± 64
Iron	(mg/d)	13 ± 3	12 ± 3	13 ± 2
Iodine	(µg/d)	103 ± 33	94 ± 20	111 ± 41

rotenoid diet reduced these contents significantly to 47-64 % of the basal values within three (group 3) or four weeks (groups 1 and 2). The plasma concentrations remained at these levels during the next 4-5 week diet low in carotenoids.

Nutritional status

The nutrient intake of the volunteers prior to the study (food record 1) is shown in Table 3 with special regard to critical vitamins and minerals. The mean carotenoid intake declined from 3 mg/d to 1 mg/d during the low carotenoid diet, showing a significant difference only for group 3 ("tomato juice"). Additionally, the evaluation of the second 7 day-food record (week 2) showed significantly reduced vitamin C intakes, while the other parameters were comparable ($p > 0.05$) to food record 1.

Parameters of lipid status

The plasma concentrations of total cholesterol, HDL cholesterol, and triglycerides were in a comparable range ($p > 0.05$) in all groups prior to the study and did not change significantly during the supplementation period. Total cholesterol concentrations ranged between 168 ± 26 mg/100 ml (group 1) and 181 ± 25 mg/100 ml (group 3) prior to the study, those of HDL cholesterol between

51 ± 13 (group 2) and 65 ± 17 mg/100 ml (group 1). Triglycerides were determined between 83 ± 25 mg/100 ml (group 1) and 99 ± 39 mg/100 ml (group 3).

Antioxidant capacity

The TEAC values of all groups did not vary significantly prior to the study and were analyzed to 0.33 ± 0.05 mmol/l. The ingestion of lycopene did not affect the TEAC value significantly. The TRAP value (1.0 ± 0.1 mmol/l) was in a comparable range ($p > 0.05$) in all groups and also not affected by the lycopene intake.

Discussion

The absorption of lycopene from either tomato juice or oleoresin capsules was not significantly different in contrast to tomatoes showing a significantly lower intestinal absorption.

In a recent study, a single dose of lycopene (23 mg) from tomato paste increased the lycopene concentrations in chylomicrons 2.5 times higher than a dose equivalent intake from tomatoes (9). 16.5 mg/d lycopene from tomato puree resulted in significantly higher plasma concentrations compared to tomatoes (19). Another study compared the uptake of lycopene into plasma from sup-

Fig. 3 Plasma lycopene concentrations (mean \pm s) over time in subjects consuming daily portions of **oleoresin capsules** for six weeks after a two week initial period, *⁰ significant higher than prior to supplementation (T0).

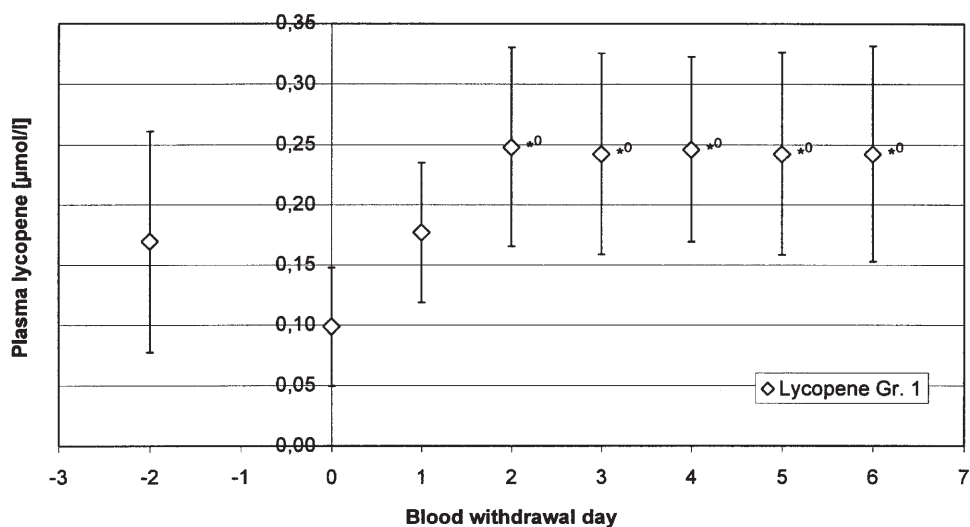


Fig. 4 Plasma lycopene concentrations (mean \pm s) over time in subjects consuming daily portions of **tomato juice** for six weeks after a two week initial period, *⁻² significant lower than basal value (T-2), *⁰ significant higher than prior to supplementation (T0).

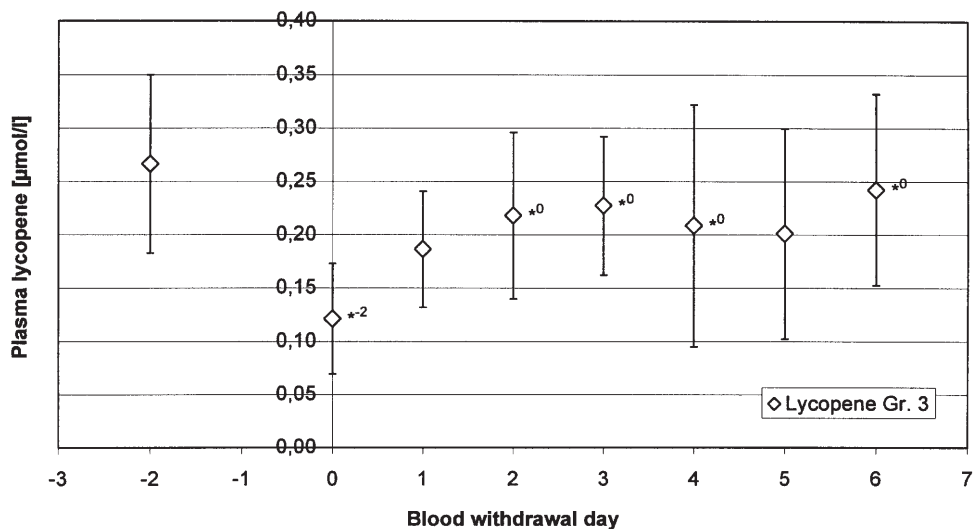
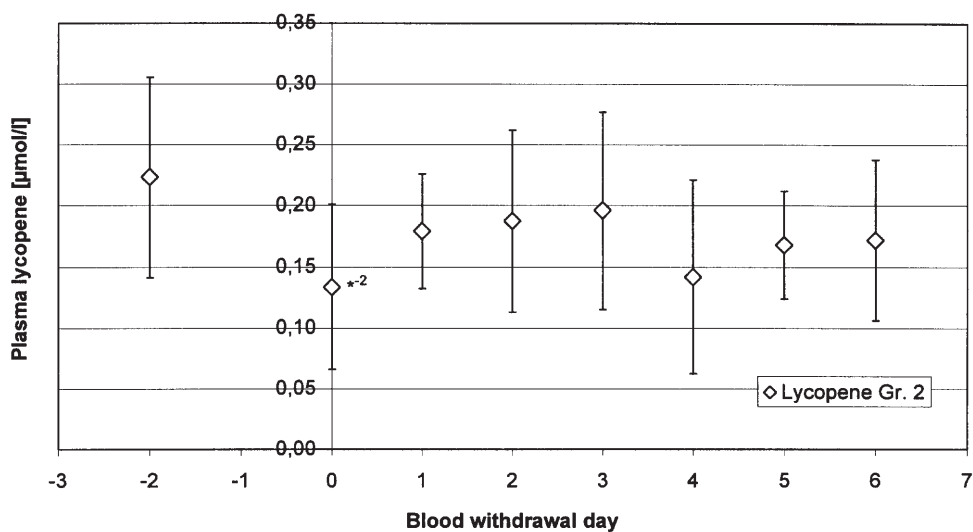


Fig. 5 Plasma lycopene concentrations (mean \pm s) over time in subjects consuming daily portions of **tomatoes** for six weeks after a two week initial period, *⁻² significant lower than basal value (T-2).



plements and from tomato juice. Lycopene plasma concentrations were significantly enhanced after a four week daily ingestion of 70 - 75 mg (17). No significant differences were shown in lycopene absorption between supplements and tomato juice. In all studies, lycopene was significantly better absorbed from processed tomato products compared to raw tomatoes, independent of the doses. Lycopene serum concentrations increased significantly after ingestion of 39 - 75 mg/d lycopene from spaghetti sauce, tomato juice, and lycopene capsules (21), and did not indicate any significant differences between the matrices. Resuming these studies, lycopene absorption from supplements and from processed tomato products were comparable.

In contrast, β -carotene is better available from a supplement than from carrot juice (23), showing no significant differences between carrot juice and carrots. Until now, to our knowledge all lycopene studies have used higher daily lycopene doses compared to the one used in our investigation. The intestinal absorption of lycopene varied significantly depending on the matrix, having supplemented a wide range of daily doses.

In contrast, a single dose tomato juice, containing 12 mg lycopene (6) as well as a daily intake (6 weeks) of 12 mg lycopene from tomato juice (15) was not able to enhance the lycopene plasma concentrations. These contradictory results have possibly been caused by the much higher initial lycopene plasma concentrations (0.8 - 0.9 $\mu\text{mol/l}$) compared to our investigation (0.2 - 0.3 $\mu\text{mol/l}$).

The six week supplementation with lycopene from different matrices did not affect the plasma concentrations of β -carotene, lutein, and zeaxanthin. Plasma β -cryptoxanthin contents decreased significantly in all groups during the low carotenoid diet, caused by the reduced intake of yellow and orange fruits which are the main source of this xanthophyll.

The dietary intake of carotenoids prior to the study was comparable ($p > 0.05$) for all volunteers and declined from 3 mg/d to 1 mg/d, caused by the diet low in carotenoids. Only the vitamin C intake decreased significantly from food record 1 to food record 2, also caused by the low carotenoid diet, which means a diet low in fruits and vegetables. Concluding these results, the participants did not change their eating behavior during the study participation.

Contents of total cholesterol, HDL cholesterol, and triglycerides in plasma were in an identical range for all

groups and comparable to results from a large German study, the VERA study (13). These parameters of the lipid status did not change significantly after ingestion of lycopene and were comparable to recent results after ingestion of tomato products and lycopene capsules (1, 22). In contrast, a three month intake of 60 mg/d lycopene significantly reduced the LDL cholesterol concentration without any effect on HDL cholesterol (7). The daily intake of 20 mg β -carotene for two years significantly enhanced HDL cholesterol concentrations, whereas the contents of total cholesterol and triglycerides were not affected (10). These contradictory results need further research for factors influencing these lipid parameters.

The daily intake of 5 mg lycopene over a 6 week period did not alter the antioxidant capacity of plasma. Both assays used in our study are able to estimate the delay on radical formation and/or the possibility to scavenge radicals. In contrast to our results, the daily intake of 39 - 75 mg lycopene for one week significantly reduced the oxidizability of LDL (1), whereas the daily intake of 60 mg β -carotene over a period of three month did not reduce the LDL oxidizability (20). It can be concluded that there are differences in the antioxidant capacity of plasma after taking carotenoids, depending on the type of carotenoid ingested and the dosage.

Conclusions

In summary, from our results it was shown that 5 mg/d lycopene was absorbed from oleoresin capsules and tomato juice (processed tomatoes) in identical magnitudes. Both matrices resulted in a significantly improved intestinal absorption of lycopene compared to raw tomatoes. The daily intake of 5 mg lycopene, a dosage corresponding to usual total carotenoid intakes, did not influence other plasma carotenoids or the plasma lipid parameters (total cholesterol, HDL cholesterol, and triglycerides). Additionally, the antioxidant capacity of plasma was not affected by these multiple doses of lycopene.

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