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ORIGINAL CONTRIBUTION

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Plasma concentration response to drinks containing β-carotene as carrot juice or formulated as a water dispersible powder

Summary *Background* Bioavailability of β-carotene is highly variable and depends on the source, the formulation and other nutritional factors. *Objective* It was the aim of the study to compare β-carotene plasma response to β-carotene dosing with two commercially available drinks, containing β-carotene from carrot juice or as water dispersible β-carotene powder. *Design* In a randomized, parallel group study design, 4 volunteers per group received daily β-carotene doses of 6–7 or 18–22 mg of either drink over 6 weeks. Blood samples for determination of carotenoid and vitamin A plasma concentrations were collected before supplementation and over the dosing period. *Results* Apparent steady-state β-carotene concentrations were attained after 40 days of supplementation. Consumption of the beverage containing β-carotene as a water dispersible powder resulted in a higher response of β-carotene plasma concentrations with increments of 3.84 ± 0.60 µmol/L ($p < 0.05$, dose: 7.2 mg/d) and 5.04 ± 0.72 µmol/L ($p < 0.05$, dose: 21.6 mg/d), respectively, in comparison to the carrot juice-based drink with increments of 0.42 ± 0.33 µmol/L (dose: 6 mg/d) and 1.71 ± 0.55 µmol/L (dose: 18 mg/d), respectively. β-carotene was cleared from the plasma with an apparent half-life of 6–11 days. Plasma concentrations of α-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene remained almost unchanged, whereas retinol plasma concentrations increased slightly. By contrast, with the exception of elevated 13-cis-retinoic acid in one group (21.6 mg/d, water dispersible powder), the concentrations of all-trans-retinoic acid, and the oxo-derivatives or retinoic acid were not significantly affected by β-carotene supplementation. *Conclusions* The results confirm that the relative bioavailability of β-carotene depends largely on the source of β-carotene and demonstrate the superior bioavailability of β-carotene powder in comparison to that in carrot juice.

Key words β-carotene – bioavailability – carrot juice – β-carotene powder – healthy volunteers

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Introduction

Intestinal absorption of dietary β-carotene from fruits and green leafy vegetables varies widely [1–3]. Factors known to affect carotenoid bioavailability have recently been critically reviewed [4]. Mainly due to effects of the cellular matrix of the plant material and of the size of the crystals within the chromo- and chloroplasts, the carotenoid is only poorly available for solubilization in the lipid phase and for incorporation into mixed bile salt micelles [5–8], resulting in low systemic availability. In contrast, administration of pure β-carotene in supplements has generally produced higher blood concentrations in comparison to intake of β-carotene from natural sources. Such β-carotene supplements have been used as

reference material to determine relative bioavailabilities from food sources.

Most of these supplements contain the carotene in a water dispersible form, which is obtained by microencapsulation in gelatine and embedding it in a starch matrix. Such formulations are referred to as β-carotene powder. β-carotene plasma concentrations resulting from dietary intake correspond to only 5–30 % of those from equal doses in water dispersible powder formulations [9]. The observed differences in plasma concentration response are thought to reflect differences in the molecular environment and the physical state of the carotene [10].

The relevance of those differences in bioavailability for commercially available foods has not been investigated yet. Therefore, in the present study we directly compared the effect of repeated administration of two different commercially available drinks on plasma concentrations of β-carotene and most common plasma carotenoids. These fruit juice-based drinks contain β-carotene either added with carrot juice or as β-carotene powder in an otherwise very similar environment.

Material and methods

Subjects

For this study, healthy, male, caucasian non-smoking volunteers, 20–40 years old, having a body mass index of between 22 and 28, with a moderate intake of fat and β-carotene were included. For screening 10 days prior to the start of the study, fat intake was assessed by nutritional records, and β-carotene intake by measuring total carotene in plasma. Subjects were eligible, if their daily fat intake was between 75 and 160 g and their β-carotene plasma concentration, as calculated from the measured total plasma carotene, between 0.3 and 2.0 µmol/L. Because gender differences have been described for β-carotene plasma concentrations [11, 12], only male volunteers were chosen for this investigation in order to reduce variability.

Drinks and treatment groups

Two different commercially available fruit juice-based breakfast drinks were used for the investigation. Except for the source of β-carotene the drinks were of comparable composition (Table 1). Drink 1 contained 80 % fruit juices and 20 % carrot juice as the β-carotene source, whereas in drink 2 β-carotene was present as β-carotene 10 % CWS (10 % synthetic cold water soluble β-carotene, Hoffmann-La Roche Ltd., Switzerland). In the following, this 10 % CWS β-carotene formulation will be referred to as β-carotene water dispersible powder. Per 100 ml drink, 2.4 mg (drink 1) or 3.6 mg β-carotene (drink 2), respectively, were provided.

Table 1 Nutritional content of drinks (1 L). In drink 1, β-carotene was contained as a component of carrot juice, whereas in drink 2 synthetic β-carotene was present in the form of 10 % CWS β-carotene (Hoffmann-La Roche Ltd., Switzerland), a water dispersible powder formulation. The relative amounts of cis β-carotene are given in parentheses

Nutrient	Drink 1	Drink 2
Energy (kJ)	1,790	1,460
Fat (g)	1.0	1.6
Protein (g)	6.0	9.0
Carbohydrates (g)	93	69
Fiber (g)	6.0	7.5
Ascorbic acid (mg)	300	450
α-tocopherol (mg)	50	75
β-carotene (mg)	24 (11 %)	36 (22 %)
α-carotene (mg)	8.1	0.1
Lutein (mg)	1.6	0.3
Zeaxanthin (mg)	0.6	0.1

Study design and conduct

The local ethics committee approved the study protocol and the trial was conducted according to the Declaration of Helsinki in its most recent version and GCP-ICH-guidelines. All study participants provided their written informed consent.

The study was conducted in a randomized, open-label, parallel-group study design. Each of 4 volunteers per group received daily volumes of either 250 ml (group 1a) or 750 ml (group 1b) of drink 1, or 200 ml (group 2a) or 600 ml (group 2b) of drink 2 resulting in daily β-carotene doses as shown in Table 2. These drink volumes did not provide for exactly comparable β-carotene doses; however, these volumes were easy for the volunteers to measure at home. Assuming a half-life for β-carotene of about 7 to 14 days [2], a treatment period of 6 weeks was considered adequate to achieve steady state of β-carotene plasma concentrations.

For screening, volunteers were subjected to a physical examination, which included an electrocardiogram, blood chemistry, and serology (hepatitis B and C, HIV–1/2). Furthermore, β-carotene plasma concentration and documentation of nutritional habits were assessed. Volunteers were instructed to avoid products with high β-carotene content and they had to document all meals and snacks during the study three times weekly in a nutritional diary. For the low dose groups of both drinks, the drink had to be taken together with breakfast, the high dose of both drinks was taken in three daily doses together with breakfast, lunch and dinner, respectively. An adhesive label was attached at the bottles and these had to be stripped of and taped into the nutritional diary in order to record compliance. At every visit, di-

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Table 2 Demographic data of volunteers, calculated initial β-carotene plasma concentration and fat intake at screening (10 days prior to start of the study). Differences were statistically not significant at the 5 % level

Group	Group 1a	Group 1b	Group 2a	Group 2b
Number of subjects	4	4	4	4
Source of β-carotene	carrot juice	carrot juice	powder	powder
Daily β-carotene dose in mg (μmol)	6 (11.2)	18 (33.6)	7.2 (13.4)	21.6 (40.2)
Age (years)	29.5±5.8	29.5±5.8	32.0±5.0	29.3±2.9
Height (m)	1.79±0.06	1.81±0.06	1.79±0.10	1.86±0.06
Weight (kg)	75.5±8.7	82.2±8.5	73.7±10.3	83.5±3.9
BMI (kg/m^2)	23.4±1.2	25.0±1.2	22.9±1.5	24.3±1.9
β-carotene in plasma (μmol/L)[1]	1.07±0.52	0.70±0.07	1.08±0.53	0.53±0.25
Fat intake (g/day)	91.5±8.4	107.5±17.3	99.5±23.7	92.3±11.5

[1] calculated from total carotene plasma concentrations

aries were checked for compliance and adherence to dietary restrictions, and blood specimens of 7 ml were collected after an over night fast before breakfast. During the baseline assessment phase (day 1–5) blood samples were collected daily. During the early dosing phase (day 6–46), the weekly visits included supply with fruit drink, control of nutritional diaries and the collection of one blood sample. From day 47–51 (plateau phase) blood samples were again drawn every day.

Fasting blood samples were collected in sodium heparin-containing tubes at predetermined time-points (see above) and centrifuged immediately at 4 °C at 2000 g over 10 min. The plasma was separated and stored at –35 °C.

Determination of carotenoids, vitamin A related compounds, and tocopherol in plasma

For logistical reasons, at screening total carotene (the sum of α- and β-carotene) was determined according to Vuilleumier [13]. The β-carotene plasma concentration was then calculated using a β-carotene/α-carotene ratio of 3.21 [14]. All-trans-β-carotene, cis-β-carotene, β-cryptoxanthin, lycopene, retinol and α-tocopherol were measured according to Aebischer [15]. Briefly, human plasma was analyzed by a dilution step with water and precipitation of proteins with ethanol, followed by extraction of β-carotene and other lipophilic molecules with n-hexane in presence of the antioxidant butylhydroxytoluene (BHT). Separation was achieved on a reversed phase column (250x4.6 mm, Primesphere 5-C18, Phenomenex), detection and quantification was performed with visible light at wavelengths of 450 nm or 470 nm for lycopene. Retinol and tocopherols were determined fluorimetrically (retinol: Ex 330 nm/Em 470 nm; tocopherols: Ex 298 nm/Em 328 nm).

All calibrations were done with photometrically adjusted concentrations (molar extinction coefficients see ref. 15). The applied analytical methods were specific for all-trans and cis-β-carotene and selective versus other carotenoids, which are commonly present in plasma. During a period of two years the reproducibility (inter-day, different conditions), represented by the relative standard deviation, was 4.4 %. Lutein and zeaxanthin in human plasma were determined by HPLC with photometric detection at 472 nm. Specification of analytical accuracy is provided in Table 3.

For every volunteer, in one sample collected during the baseline phase (study day 1) and one sample of the β-carotene steady-state phase (study day 51), plasma concentrations of all-trans-retinoic acid, 9-cis-retinoic acid, 13-cis-retinoic acid, all-trans–4-oxo-retinoic acid, 9-cis–4-oxo-retinoic acid, and 13-cis–4-oxo-retinoic acid were assayed according to Wyss and Bucheli [16].

Table 3 Characteristics of analytical procedures for determination of carotenoids, retinol, retinyl palmitate and α-tocopherol

Analyte	Recovery after 1st extraction (%)	LOD[1] nmol/L	ULD[2] μmol/L	CV (%) intra-day	inter-day
β-cryptoxanthin	97.7	18	24	1.8	3.7
Lycopene	97.2	1	22	2.1	2.6
β-carotene	99.3	1	37	1.9	2.5
All-trans-β-carotene	97.2	1	40	1.5	4.4
cis/trans-β-carotene	100	1	40	1.5	4.4
Retinol	93.5	70	10	3.0	5.7
Retinyl palmitate	96.6	38	20	3.9	10.4
α-tocopherol	95.7	46	46	2.0	1.9
γ-tocopherol	100	47	24	5.4	2.8
Lutein	98.0	2	18	3.0	5.4
Zeaxanthin	97.0	2	18	3.2	4.9

[1] *LOD* lower limit of detection; [2] *ULD* upper limit of detection

Data evaluation and statistics

Randomization was performed using the custom-made software "Random, BZT". Data are given as mean ± 1 SD. For exploratory group comparisons, plasma concentration increments of total β-carotene (= sum of all-trans-β-carotene and cis-β-carotenes) were defined as the difference of the mean plasma concentrations at "steady state" (study days 47, 48, 49, 50, and 51) and "baseline" (study days 1, 2, 3, 4, and 5). Dose-normalized increments were obtained by dividing these increments through the respective daily β-carotene dose in μmol. In spite of the small group size, statistical analysis was attempted. Significance of differences between groups was calculated by two-sided Wilcoxon tests ($p = 0.05$) using SAS 6.12. Comparisons of $t_{1/2}$ resulting for the three treatments have been based on unpaired t-tests obtained with StatView (SAS Institute Inc©). Retinol, retinoic acids, their oxo derivatives, and carotenoids other than β-carotene were tested for significant differences between baseline and steady-state concentrations using paired t-tests (StatView, SAS Institute Inc©). Data in tables and figures are mean ± 1SD, unless stated otherwise. Differences have been examined by testing for statistical significance at the 5 % significance level.

Estimation of β-carotene plasma half-life

Estimation of β-carotene plasma half-life was based on empirical modeling of the minimal plasma concentration $C_{min}(t)$ (i. e., concentrations at the end of a dosing interval τ) time profiles in the dosing phase. Individual β-carotene plasma concentration time-curves were subjected to nonlinear regression based on weighted least squares analysis, using the numerical module of the software SAAM II (SAAM Institute Seattle). The approach to plateau was modeled by fitting plasma concentrations according to:

$$C_{min}(t) = C_{ss,\,min} * (1 - e^{-\alpha 1 * N * \tau}) + C_{BL} \quad (1)$$

In Eq. 1, C_{BL} is the initial (basal) β-carotene concentration at time t = 0 days, which was calculated as the average concentration of pre-dose samples collected in the run-in phase. $C_{ss,min}$ represents the minimal steady-state concentration, N the number of doses administered and τ the dosing interval (τ = 1 day) and α1 the elimination rate constant. The fitting procedure yielded in estimates of parameters $C_{ss,min}$ and α1. Half-life was calculated as:

$$t_{1/2} = \frac{\ln(2)}{\alpha 1} \quad (2)$$

This procedure provides a rough estimate of the elimination half-life of β-carotene under the assumption that the amount of β-carotene rises on multiple dosing just as it does following a constant-rate intravenous infusion. This implies that the approach to plateau is solely dependent on the retention of the carotenoid in the body as determined by the elimination half-life of β-carotene. This approach for estimating the elimination half-life is valid only under the following assumptions: i) the rate of β-carotene absorption exceeds that of elimination by at least an order of magnitude and ii) systemic availability and clearance are constants from dose to dose [17].

Results

Characteristics of study population

In agreement with the inclusion criteria for the study, three out of a total of 20 volunteers screened had to be excluded because of low β-carotene plasma levels, while one volunteer was excluded for other reasons. Table 2 shows the demographic variables, initial β-carotene plasma concentrations and intake of fat of the 16 volunteers included in the study. Observing the inclusion/exclusion criteria and randomization procedures resulted in comparable group characteristics (Table 2). Compliance was excellent and drinks were well tolerated.

Time-course of β-carotene plasma concentration

There were no statistically significant differences in the mean β-carotene plasma concentrations across all groups and a mean β-carotene baseline concentration of 0.81 ± 0.53 μmol/L was calculated for the entire (n = 16 subjects) study population. The increase in β-carotene plasma concentrations is shown in Fig. 1. Study days 1–5, and 47–51 represent apparent baseline and steady state periods, respectively. Within subject variations at baseline and steady state were small (CV ≤ 6.6 %, data not shown). Consumption of drink 2 resulted in substantially higher dose-normalized increments than drink 1 (Table 4). Furthermore, for groups 1a and 1b (low and high dose) the dose-normalized increments are comparable. For groups 2a and 2b, the mean dose-normalized increment for the high dose was approximately half of that achieved after supplementation with the low dose.

Irrespective of cis-β-carotene content in the two drinks, cis isomers in plasma remained unchanged from baseline to the end of the study, contributing 5–6 % of total β-carotene (Table 4).

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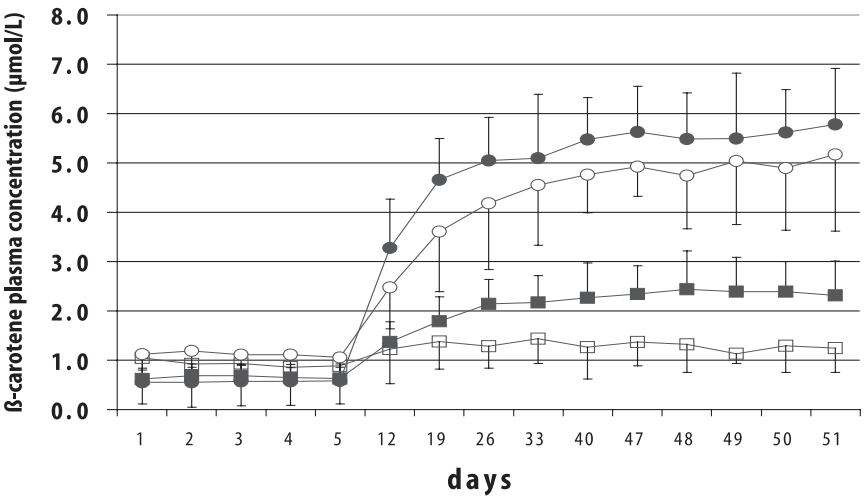

Fig. 1 Plasma concentration/time profile of β-carotene during the study. Group 1a: □; group 1b: ■, group 2a: ○, group 2b: ●. Start of supplementation was at day 5.

Table 4 Increments in β-carotene plasma concentrations after 6 weeks of regular consumption of drink 1 or drink 2 at 2 different dose levels (difference between mean values of β-carotene plasma concentrations measured on days 1–5 and on days 47–51). Figures in parentheses denote percentage of β-carotene cis-isomers

Group	Baseline (µmol/L)	Steady-state (µmol/L)	Increment (µmol/L)	DN increment[1] (µmol/L/µmol)
1a (n = 4)	0.90 ± 0.82 (5.5 %)	1.32 ± 0.52 (5.2 %)	0.42 ± 0.33	0.07 ± 0.03
1b (n = 4)	0.66 ± 0.21 (6.3 %)	2.36 ± 0.64 (5.6 %)	1.71 ± 0.55[a, b]	0.05 ± 0.02[a, b]
2a (n = 4)	1.12 ± 0.57 (5.6 %)	4.95 ± 1.15 (5.8 %)	3.84 ± 0.60[a]	0.29 ± 0.05[a, b]
2b (n = 4)	0.57 ± 0.33 (6.2 %)	5.60 ± 0.99 (6.3 %)	5.04 ± 0.72	0.13 ± 0.02

[1] *DN* dose-normalized
[a] $p \leq 0.05$ versus group 1a (paired t-test)
[b] $p \leq 0.05$ versus group 2b (paired t-test)

Plasma concentration of other carotenoids and vitamin A-related compounds

In groups 1a and 1b, in which 6.5 or 19.5 µmoles of α-carotene were ingested, we found increments in α-carotene plasma concentrations of 0.68 and 1.33 µmol/L (Table 5), which correspond to dose-normalized increments of 0.18 and 0.12 µM/µmol respectively. In the β-carotene powder groups we were not able to detect any increments in α-carotene plasma concentrations (Table 5). Furthermore, for group 1a, the α-carotene plasma concentration increment tended to be larger than that for β-carotene, although the amount of α-carotene in drink 1 was only one third of that of β-carotene. Neither dose of the β-carotene powder drink exerted a consistent influence on plasma concentrations of other carotenoids. Lutein concentrations decreased slightly, while β-cryptoxanthin concentrations were increased by 26 % (Table 5).

Retinol plasma concentrations increased in the high-dose groups 1b and 2b by almost 30 % (n. s.), while the increase by 18 % in group 2a was significant (Table 6). Regular consumption of the two drinks had no significant effect on the plasma concentrations of all-trans-retinoic acid and the metabolites, 9-cis-, and 13-cis- 4-oxy-retinoic acid (Table 6). The 13-cis-retinoic acid concentration was significantly increased by 40 %, but only in group 2b. 9-cis-Retinoic acid and all-trans 4-oxo-retinoic acid did not reach measurable plasma concentrations.

Kinetic evaluation of ß-carotene plasma concentrations

For estimation of half-life of β-carotene in plasma only data from groups 1b, 2a and 2b could be used because the increments in group 1a were too small to be evaluated (Fig. 2). Results of half-life assessment were 9.2 ± 2.6 days in group 1b, 6.4 ± 0.9 days in group 2a and 11.4 ± 3.7 days in group 2b, respectively. Based on apparent half-life, the percent reaching steady state within the dosing period was calculated and found to range between 95 % and 99 % for the three groups.

Discussion

In the present investigation in healthy volunteers, the bioavailability of β-carotene from a water dispersible synthetic powder in a commercially available drink was found to be higher than from carrot juice.

The β-carotene plasma concentrations achieved after 6 weeks of supplementation were comparable to those

Table 5 Baseline, steady-state concentrations and increments (differences between mean plasma concentrations measured at baseline and steady state) for various plasma carotenoids

	α-carotene (μmol/L)	β-cryptoxanthin (μmol/L)	Lutein (μmol/L)	Zeaxanthin (μmol/L)	Lycopene (μmol/L)
Group 1a					
Baseline	0.27±0.23	0.35±0.24	0.24±0.14	0.07±0.04	0.62±0.23
Steady-state	0.95±0.21	0.40±0.26	0.26±0.14	0.08±0.05	0.75±0.21
Increment	0.68±0.12[a]	0.05±0.14	0.02±0.01[a]	0.01±0.01	0.13±0.28
Group 1b					
Baseline	0.12±0.02	0.30±0.18	0.22±0.08	0.05±0.01	0.61±0.31
Steady-state	1.44±0.24	0.35±0.16	0.24±0.09	0.07±0.03	0.72±0.12
Increment	1.33±0.25[a]	0.06±0.09	0.02±0.03	0.02±0.02	0.10±0.30
Group 2a					
Baseline	0.24±0.21	0.32±0.16	0.22±0.03	0.05±0.01	0.61±0.25
Steady-state	0.25±0.09	0.28±0.10	0.19±0.03	0.05±0.01	0.58±0.12
Increment	0.01±0.11	−0.05±0.08	−0.03±0.02[a]	−0.01±0.01	−0.03±0.15
Group 2b					
Baseline	0.10±0.06	0.19±0.07	0.24±0.04	0.05±0.01	0.67±0.18
Steady-state	0.09±0.04	0.24±0.06	0.20±0.06	0.05±0.02	0.61±0.22
Increment	−0.02±0.02	0.05±0.03[a]	−0.04±0.02[a]	−0.00±0.02	−0.06±0.04

[a] $p \leq 0.05$ baseline versus steady-state concentrations (paired t-test)

Table 6 Increments in plasma concentrations of retinol, all-trans-retinoic acid (all-trans-RA), 13-cis-retinoic acid (13-cis-RA), 9-cis-oxo-retinoic acid (9-cis 4-o-RA), and 13-cis-4-oxy-retinoic acid (13-cis-4-o-RA) before and after 6 weeks of regular consumption of drink 1 or drink 2 at 2 different dose levels

	Retinol μmol/L	all-trans RA nmol/L	13-cis-RA nmol/L	9-cis 4-o-RA nmol/L	13-cis 4-o-RA nmol/L
Group 1a					
Before	2.19±0.38	4.26±1.03	4.53±0.23	3.59±3.05	11.07±5.31
After	2.28±0.48	4.33±1.50	4.79±0.97	2.39±1.78	9.67±4.20
Group 1b					
Before	1.84±0.23	3.89±0.90	4.56±0.73	4.83±3.15	11.10±5.72
After	2.34±0.23	4.33±0.70	5.39±0.83	6.30±5.98	13.99±7.54
Group 2a					
Before	1.53±0.25	3.79±0.80	4.59±0.90	3.37±1.02	10.56±1.34
After	1.81[a]±0.30	4.16±1.00	4.96±0.47	3.82±2.42	12.15±2.67
Group 2b					
Before	1.86±0.57	3.63±1.63	4.23±1.33	12.98±19.37	25.60±32.76
After	2.41±0.80	4.96±2.00	5.99[a]±2.10	13.83±12.44	29.39±24.93

[a] $p \leq 0.05$ before versus after (paired t-test)

reported in the ATBC (5.6 μmol/L; [18]) and CARET trials (3.9 μmol/L; [19]) conducted over several years with daily doses of 20 mg/dL or 30 mg plus 25,000 IU vitamin A, respectively. In these trials in heavy chronic smokers and asbestos workers supplementation with β-carotene was associated with an apparently increased risk of lung cancer. These findings are in contrast to the results of other intervention trials, where supplementation had resulted in a risk reduction of cancer [20, 21] as well as to epidemiological studies [22] which indicate a lower cancer risk with higher dietary intake of β-carotene.

The mean baseline concentration of 0.81 μmol/L β-carotene observed in our subjects was comparable to the median β-carotene plasma concentration of 0.64 μmol/L reported by the VERA nutritional survey conducted in Germany [23] and showed very little within-subject variation over 7 days. Thus, dose response to β-carotene intake was thought to be superimposed over a basal β-carotene plasma concentration remaining almost constant during the dosing period. Since absorption of β-carotene is virtually complete within less than 12 hours [24] and disposition rates are on the order of 10 days [2, 25, 26], the pharmacokinetic approach chosen [17] as well as the trial duration were adequate. The β-carotene half-lives estimated ranged between 6.5 and 11 days, in agreement with others [2, 25, 26]. In a long-term pharmacokinetic study with one adult volunteer, Dueker [27] reported two half-lifes for β-carotene disposition of 13 and 40 days, respectively. The estimates of our study characterise rather the faster disposition process and not terminal elimination.

As disposition kinetics between synthetic and natural carotenoid were comparable, differences in plasma response were entirely related to differences in β-carotene application forms. A carrot juice-based drink, similar to that used in the present study, was administered to 23 volunteers for two weeks [14] and resulted in a dose-normalized β-carotene increment of 0.035μM/

μmol. Törrönen et al. [28] reported a dose-normalized increment of 0.024 μM/μmol for a group of 13 volunteers after consumption of carrot juice for 6 weeks. Both results are quite similar to ours (Table 4). Törrönen and co-workers [28] also studied β-carotene supplements at an identical dose level and reported a 2.6-fold greater plasma response to the capsules than to the carrot juice. This ratio is comparable to that observed in the current study, where dose-normalized increments of plasma β-carotene in the β-carotene powder groups exceeded that of carrot juice by factors of 2.6 and 4.1, respectively.

The crucial role of β-carotene formulation as a determinant of its bioavailability has long been recognized for supplements in capsule form but to our knowledge not for drinks fortified with β-carotene. Several factors known to influence bioavailability of carotenoids such as fat and fiber were comparable for the two beverages tested in the current study and therefore could not account for the observed differences. However, it should be considered that concurrent fat intake varied considerably, since drinks in the low dose groups were only taken together with breakfast, whereas in the high dose groups, drinks were taken together also with lunch and dinner, i. e., meals containing probably more fat and thus allowing for a higher degree of absorption. Other variables such as particle size distribution known to affect β-carotene bioavailability have not been controlled in the present investigation. Synthetic and palm oil-derived β-carotene added to the same food matrix exerted comparable relative bioavailabilities [29].

Administration of the β-carotene powder drink did not result in a dose-proportional plasma concentration response. A similar delayed and less efficient absorption has already been reported for high β-carotene doses [30], suggesting a saturation mechanism for regulating β-carotene plasma concentrations [31]. Limited plasma transport capacity and induction of metabolism may also be involved.

Despite the differences in the relative content of cis-β-carotene in the drinks given with 11% (drink 1) and 22% (drink 2), respectively, the percentage of cis-β-carotene in plasma with about 5.5% to 6.2% remained constant throughout the study. A preferential absorption of the all-trans-isomer of β-carotene was consistently shown [9]. Moreover, a large amount of 9-cis-β-carotene will become isomerized in the enterocytes before entering the blood stream [6].

We observed a tendency towards higher plasma concentrations of retinol (Table 6), especially after administration of the higher doses of both drinks demonstrating the role of β-carotene as precursor of vitamin A. Our subjects were on a diet avoiding high β-carotene intake, but vitamin A consumption was not restricted. Accordingly, retinol plasma concentrations were in the 25–75 percentile of the normal range before and after supplementation [23]. This indicated that retinol homeostasis was maintained in spite of β-carotene dosing. Moreover, retinoic acid concentrations remained unchanged, except for 13-cis-retinoic acid (in group 2b), which is known to exhibit a longer half-life as compared to other retinoic acids [32, 33].

In contrast to previous publications, in this study β-carotene ingestion did not consistently decrease plasma concentrations of other carotenoids. It had been suggested that α-carotene plasma concentrations may increase after ingestion of β-carotene powder [34, 35]. Our results indicate that the uptake of α- and β-carotene from the fruit juice-based beverage was at least comparable, if not better for α-carotene.

In conclusion, despite the small sample size, our results demonstrate the superior bioavailability of β-carotene provided as a powder in a commercially available drink when compared with a carrot-juice fortified drink. Moreover, the half-life of β-carotene was independent of the formulation used and ranged between 6 and 11 days. Our data illustrate the dependence of β-carotene bioavailability from the matrix it is embedded.

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