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Plasma responses in human subjects after ingestions of multiple doses of natural α-cryptoxanthin: a pilot study

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Xanthophylls have attracted a lot of interest since their health benefits were documented. Unfortunately, studying their intestinal absorption is often affected by high baseline levels present in the fasting plasma. As α-cryptoxanthin is rarely found in the traditional European diet, its concentration in human plasma is extremely low. A pilot human intervention study was designed using α-cryptoxanthin for the first time as a marker xanthophyll in a minimally formulated cellulose-based supplement. α-Cryptoxanthin was administered in gelatin soft-gel capsules in multiple doses of 156 μg/d to three male volunteers (age 27·3 (SD 4·7) years; BMI 21·6 (SD 0·3) kg/m^2) for 16 d after a 2-week carotenoid depletion period. Fasting blood samples were taken before the intervention and after 3, 6, 9, 13 and 16 d. Plasma HPLC analyses allowed for determination of the concentration; liquid chromatography–MS in the single ion monitoring mode was used to confirm peak assignment. The concentrations of α-cryptoxanthin increased significantly after only 3 d of supplementation. The concentration-time plots showed a characteristic shape with a first maximum after day 6, a decline until day 9 and a gradual second rise until the end of the study. Standardisation of plasma α-cryptoxanthin concentrations to triacylglycerol or total cholesterol did not influence the characteristics. The maximum concentrations reached at the end of the intervention period ranged from 0·077 to 0·160 μmol/l. These results suggest a high intestinal absorption and an enrichment of α-cryptoxanthin in the plasma even from a minimally formulated cellulose-based supplement.

α-Cryptoxanthin: Plasma xanthophyll response: Liquid chromatography–mass spectroscopy in the single ion monitoring mode: Pilot studies

Xanthophylls have attracted a lot of interest since it was presumed that an increased nutritional uptake might provide protection against certain diseases. In particular, the health benefits of lutein, zeaxanthin and β-cryptoxanthin have been well documented in the last decade. For example, the non-provitamin A xanthophylls lutein and zeaxanthin may protect the human eye from age-related macula degeneration and cataracts (Beatty *et al.* 1999; Mares-Perlman *et al.* 2002). In accordance with their high nutritional value, plants rich in lutein such as *Tagetes erecta* (Hadden *et al.* 1999; Breithaupt *et al.* 2002) are actually cultivated on a large scale. β-Cryptoxanthin was recently associated with an increased protein formation and/or prevention of loss of proteins in man and animals (Eichinger *et al.* 2005). A high plasma level of β-cryptoxanthin has also been linked to a protective effect against rheumatoid arthritis (Cerhan *et al.* 2003; Pattison *et al.* 2005) and – together with Zn at low concentrations – to a positive effect on bone components *in vitro* (Uchiyama *et al.* 2005). Craft *et al.* (2004) found that β-cryptoxanthin belongs to the major xanthophylls in the human brain, where particularly the frontal cortex, which is generally susceptible to Alzheimer's disease, exhibited higher concentrations than other parts. However, a possible mode of action still remains to be elucidated.

Looking into more detail, β-cryptoxanthin is the most popular representative of a group of three structural isomers (Fig. 1). In accordance with the isoprenoid biosynthetic pathway in plants (van den Berg *et al.* 2000), enzymic hydroxylation of symmetric β-carotene leads to the formation of β-cryptoxanthin, whereas the same reaction starting from asymmetric α-carotene (β,ε-carotene) gives rise to two reaction products: β,ε-carotene-3′-ol (α-cryptoxanthin) and β,ε-carotene-3-ol (zeinoxanthin). This designation is in accordance with modern xanthophyll nomenclature. However, it must be highlighted that the structural cryptoxanthin isomers α-cryptoxanthin and zeinoxanthin were often confused in older literature, causing misinterpretation of the carotenoid content of food (Schlatterer & Breithaupt, 2005).

Due to the presence of unsubstituted β-ionone rings, α- and β-cryptoxanthin act as provitamin-A precursors. β-Cryptoxanthin and zeinoxanthin are found in numerous food plants. β-Cryptoxanthin is present in high amounts in red paprika, citrus fruits, persimmon and in tropical fruits such as papaya and mango (Chandrika *et al.* 2003; Meléndez-Martínez *et al.* 2003; Collera-Zuniga *et al.* 2004), whereas zeinoxanthin forms a typical component of oranges and maize (Meléndez-Martínez *et al.* 2003, 2005; Cortés *et al.* 2004; Schlatterer & Breithaupt, 2005). Mercadante & Rodriguez-Amaya (2001)

Abbreviations: LC, liquid chromatography; MTBE, methyl *tert*-butyl ether; SIM, single ion monitoring.

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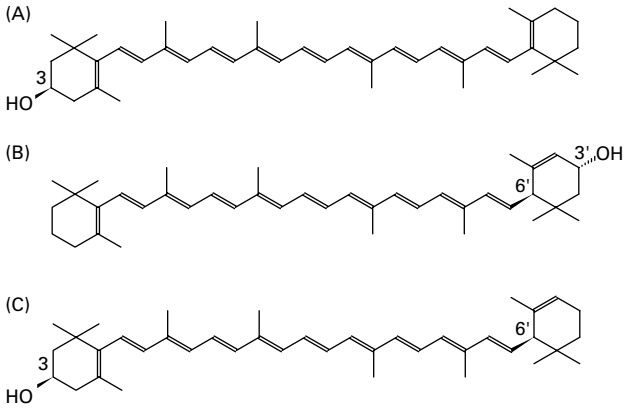

Fig. 1. Chemical structures of cryptoxanthin isomers: (A) β-cryptoxanthin (β, β-carotene-3-ol); (B) α-cryptoxanthin (β,ε-carotene-3′-ol); (C) zeinoxanthin (β, ε-carotene-3-ol).

pointed out that Brazilian green and leafy vegetables such as watercress, lettuce, chicory, endive and parsley contain trace amounts of α-cryptoxanthin, but not β-cryptoxanthin as often reported. The α-cryptoxanthin standard used by these researchers was isolated from *Amaranthus viridis*. However, as far as we know, the fresh green leaves of carrots serve as the only important α-cryptoxanthin source (Müller, 1997). Health functions of α-cryptoxanthin are essentially not known.

Since α-cryptoxanthin is rarely found in food plants, the respective concentration in human plasma is expected to be extremely low (no reliable data available). Consequently, the use of α-cryptoxanthin as a marker xanthophyll allows the determination of the intestinal absorption without interference caused by minor xanthophylls in HPLC analyses. Thus, for the first time, a pilot human intervention study was designed using a cellulose-based α-cryptoxanthin formulation without further additives. The supplement was administered in gelatin soft-gel capsules in multiple doses of 156 μg/d to three male volunteers for 16 d and fasting blood samples were analysed before and after 3, 6, 9, 13 and 16 d.

Experimental design

Materials

Light petroleum (boiling fraction 40–60°C), methanol, acetone, ethyl acetate, ethanol, diethyl ether, *n*-hexane, silica gel 60 (0·063–0·200 mm) and 2,6-di-*tert*-butyl-p-cresol were purchased from Merck (Darmstadt, Germany). Methyl *tert*-butyl ether (MTBE) and KOH (pellets >85 %) were from Sigma-Aldrich (Taufkirchen, Germany). All solvents were distilled before use. Ultra-pure water was obtained from a Milli-Q 185 apparatus (Millipore, Eschborn, Germany). β-Apo-12′-carotenal (standard) was generously provided by BASF (Ludwigshafen, Germany), β-cryptoxanthin was a gift from DSM (Kaiseraugst, Switzerland), anhydrolutein I (3′,4′-didehydro-β,γ-carotene-3-ol) was obtained from CaroteNature (Lupsingen, Switzerland) and zeinoxanthin was isolated from canned maize (*Zea mays*) as described previously (Schlatterer & Breithaupt, 2005). Cellulose powder (Elcema P100®) was obtained from Synopharm (Barsbüttel, Germany). The test kits for *in vitro* determination of plasma triacylglycerol (method TR210) or cholesterol (method CH200) were purchased from Randox Laboratories GmbH (Krefeld, Germany). Carrot leaves from ecological production were obtained from the Research Station for Husbandry and Organic Farming, University of Hohenheim (Stuttgart, Germany).

Subjects

Three male individuals aged 22, 29 and 31 years (age 27·3 (SD 4·7) years; BMI 21·6 (SD 0·3) kg/m^2) were recruited from staff and students of the Institute of Food Science (Hannover, Germany). The characteristics of the participants are summarised in Table 1. None of them suffered from gastrointestinal diseases or took laxatives or drugs lowering plasma triacylglycerol or cholesterol concentrations. Participants were requested to keep a normal diet but to avoid xanthophyll-rich food such as oranges, peaches, red paprika, papaya, fruit juices, xanthophyll-containing dietary supplements and vitamin-fortified beverages during the depletion period and during the intervention study. The protocol was approved by the Medical Ethics Committee of the Medizinische Hochschule (Hannover, Germany). All participants provided written informed consent.

Study design

The study was designed as a human pilot intervention study and consisted of a 2-week depletion period followed by a 16 d intervention phase. After taking the first fasting plasma sample, participants received a breakfast and took the first supplement under supervision afterwards. Before leaving, each participant received fifteen capsules in a closed plastic box and was advised to take one of them after the individual breakfast each morning together with some water. All

Table 1. Characteristics of the three participants
(Mean values and standard deviations)

					Triacylglycerol (mmol/l)*		Cholesterol (mmol/l)*	
Participant	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)	Mean	SD	Mean	SD
1	31	70·0	1·79	21·8	0·62	0·12	4·83	0·76
2	29	60·4	1·69	21·2	0·95	0·19	4·70	0·55
3	22	59·8	1·66	21·7	0·80	0·11	5·28	1·56

*Six determinations per participant.
For details of subjects and procedures, see this page.

participants were instructed to store the box in a refrigerator at 4°C. Further fasting blood samples were taken in the morning after 3, 6, 9, 13 and 16 d.

Preparation of supplements

Isolation of α-cryptoxanthin from carrot leaves. Fresh green carrot leaves (2·6 kg, without thick stems) from ecological production were homogenised in samples using a bowl cutter SL11 (cutter spindle 1400 rpm, bowl 19 rpm; 1 min; ADE, Hamburg, Germany). The resulting mush was filled in samples of 200 g into glass columns (550 × 30 mm) and the carotenoids were extracted by a mixture of methanol–ethyl acetate–light petroleum (1:1:1, by vol.; 1 litre per column). To allow for phase separation, the extract was mixed with water (100 ml). All organic phases were combined, the solvent was evaporated in vacuum (30°C; 50 mbar) and the residue dissolved in diethyl ether (600 ml).

Saponification. For removal of lipids and chlorophylls, the oily residue was dissolved in diethyl ether and saponified overnight using methanolic KOH (30 %, w/v; 10 ml per 100 ml ether). To remove alkali, the ether phase was washed and evaporated again (30°C; 50 mbar). The residue was dissolved in light petroleum and subjected to flash chromatography (glass column 400 × 20 mm) on silica gel (10 g) suspended in light petroleum. Samples of 10 ml were poured onto the column head. The first band, obtained by elution with light petroleum, consisted mainly of β-carotene and was discarded. The second band obtained by elution with light petroleum–acetone (95:5, v/v) was collected, the solvent completely removed (30°C; 50 mbar) and the residue dissolved in ethanol (210 ml). Liquid chromatography (LC)–MS analyses using an atmospheric pressure chemical ionisation interface operated in the positive mode proved the main carotenoid to be α-cryptoxanthin (Schlatterer & Breithaupt, 2005). Thus, 10 mg α-cryptoxanthin/210 ml ethanol were obtained.

Preparation of capsules. Cellulose powder (5·5 g) was suspended in 200 ml of the ethanolic α-cryptoxanthin stock solution in a round-bottomed flask. The solvent was evaporated slowly (30°C; 50 mbar) until a dry free-flowing powder was obtained. Samples of exactly 100 mg were filled manually in gelatin capsules. Thus, fifty-four capsules were prepared.

Quantification of α-cryptoxanthin. The α-cryptoxanthin concentration of the capsules was determined after solvent extraction with methanol–ethyl acetate–light petroleum (1:1:1, by vol.) by HPLC. Due to similar molar extinction coefficients, a β-cryptoxanthin calibration graph was applied for quantification of α-cryptoxanthin. The graph was generated by plotting β-cryptoxanthin peak areas (milli absorption units) *v.* the respective concentrations (range 1·08–21·52 mg/l). An α-cryptoxanthin concentration of 156·3(SD 4·9)μg/capsule (*n* 3) was determined. The stability of α-cryptoxanthin was verified at the end of the study with additional capsules. A loss of 2·8 % after 16 d storage at 4°C (*n* 3) was observed. As this stability was regarded as sufficient for the intervention period no further antioxidants (for example, α-tocopherol) were added.

Plasma sample preparation

Conventional procedure. At any blood withdrawal, at least 10 ml were sampled from each participant. Immediately after collection, the plasma was obtained by centrifugation (2000 ***g***; 19°C; 10 min) and stored at −20°C in plastic caps. The work-up procedure was based on a method of Khachik *et al.* (1997). In brief, 1·5 ml plasma were precipitated with ethanol (1 ml containing 1 % 2,6-di-*tert*-butyl-p-cresol (w/v)) and mixed on a shaker (1 min). An aliquot (0·1 ml) of an ethanolic β-apo-12′-carotenal standard (20 μg/ml) was added and the solution was mixed again. To extract the carotenoids, 3 ml *n*-hexane were added. The mixture was stirred, the precipitate was spun down using a centrifuge (644 ***g***; 10°C; 5 min), the organic layer was transferred into a 10 ml brown glass vial, and the extraction procedure was repeated. The combined organic phases were evaporated, the residue dissolved in 0·8 ml MTBE–methanol (1:1, v/v), passed through a 0·45 μm membrane filter and subjected to HPLC–variable wavelength detector analysis. To determine possible losses during the work-up procedure, the HPLC peak areas of the standard were monitored. The recovery of β-apo-12′-carotenal from extractions of all plasma samples accounted for 103 (SD 6)% (*n* 18). Plasma triacylglycerol and total cholesterol were measured manually by using commercial *in vitro* enzymic test kits.

Extract for liquid chromatography–mass spectroscopy in the single ion monitoring mode analysis. To verify α-cryptoxanthin identification, one large plasma sample of participant 3 (5 ml) was extracted three times with a ternary mixture of methanol, ethyl acetate and light petroleum (1:1:1, by vol.; 30 ml each). The solvent was removed (30°C, 50 mbar) and the dry residue was saponified as described earlier (1 ml methanolic KOH (30 %, w/v)–10 ml diethyl ether) to remove interfering lipids. The residue was dissolved in 1·0 ml MTBE–methanol (1:1, v/v), passed through a 0·45 μm membrane filter and subjected to LC–MS analysis in the single ion monitoring (SIM) mode.

Analysis and chromatography

The HPLC consisted of a modular system HP1100 (Hewlett-Packard GmbH, Waldbronn, Germany) with the variable wavelength detector set to 450 nm. For carotenoid separation, a C30 column (250 × 4·6 mm internal diameter; 5 μm; YMC Europe, Schermbeck, Germany) including a pre-column (10 × 4·0 mm internal diameter; 5 μm) was used and kept at 35°C. LC–MS was performed on an HP1100 modular HPLC system, coupled to a Micromass (Manchester, UK) VG platform II quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionisation interface, operating in the positive mode (scan range *m/z* 300–700; data processing by MassLynx 3.2). The mobile phase consisted of mixtures of methanol–MTBE–water (81:15:4, by vol. (A) and 6:90:4, by vol. (B)), starting with 99 % A, followed by a gradient to obtain 44 % A at 39 min, 0 % A at 45 min, 99 % A at 50 min and isocratic 99 % A from 50–55 min at a flow rate of 1 ml/min. For LC–MS(SIM) analysis, the following settings were used: selected mass, 535·4 Da; dwell time, 0·2 s; inter-channel delay, 0·02 s; span, 0·2 Da; injection volume, 30 μl. Further MS parameters have been detailed previously (Breithaupt *et al.* 2002).

Results

Representative HPLC analyses of samples of the fractionated extract obtained from carrot leaves proved that α-cryptoxanthin was the main xanthophyll, accompanied by small amounts of α- and β-carotene, which were co-extracted. Due to their low concentration (35 μg β-carotene/capsule; *n* 3) it was anticipated that both would probably not compete with excess α-cryptoxanthin and thus should not affect absorption kinetics remarkably. Identification of α-cryptoxantin was based on its characteristic absorption spectrum (424(sh)/446/474 nm, determined online in the HPLC solvents), the retention time and the typical fragmentation pattern found in LC–MS experiments: the respective mass spectrum showed a quasimolecular ion at *m/z* 553·4 ($M + H^+$) with low relative abundance (9 %) and one daughter ion at *m/z* 535·4 ($M + H-H_2O^+$) with high intensity (100 %). The loss of water from the quasimolecular ion occurs readily, a phenomenon typical for xanthophylls bearing at least one hydroxylated ε-ionone ring (Mercadante & Rodriguez-Amaya, 2001; Schlatterer & Breithaupt, 2005). Thus, LC–MS studies clearly supported the assignment of α-cryptoxanthin.

Figure 2 depicts extended sections of HPLC chromatograms of representative plasma samples (participant 3; Table 1) before (trace A) and after supplementation for 16 d (trace B). An additional trace C shows the same extract as trace B spiked with α-cryptoxanthin. Trace A proves that the fasting plasma sample extract contains neither α-cryptoxanthin nor another xanthophyll co-eluting at the expected retention time. In particular, the retention time difference between α- and β-cryptoxanthin (1·5 min) was definitely high enough to avoid peak overlapping (*R* 5·0). Although other common plasma carotenes and xanthophylls appeared in the chromatograms, they did not interfere with the separation of cryptoxanthin isomers. Their assignment was based on the respective absorption spectra and the quasimolecular ions. Particularly, zeinoxanthin and anhydrolutein I (3′,4′-didehydro-β,γ-carotene-3-ol), a metabolite of lutein usually found in human plasma (Khachik *et al.* 1995), did not co-elute. Thus, quantification of α-cryptoxanthin could be accomplished without baseline correction. Peak assignment was further assured by LC–MS analyses of a concentrated and saponified plasma sample of participant 3 (Table 1); to enhance sensitivity, the interface was operated in the SIM mode, using *m/z* 535·4 to scan for the typical α-cryptoxanthin fragment ion. The respective trace is depicted in insert D (Fig. 2), unequivocally proving correct assignment of α-cryptoxanthin (2). The high sensitivity of the LC–MS(SIM) analysis applied is obvious if the α-cryptoxanthin concentration in the concentrated sample (0·34 μmol/l) and a SIM-signal:noise level of 3:1 are considered; the SIM technique allows for unequivocal determination of concentrations up to 0·1 μmol/l (55 μg/l), corresponding to a plasma amount of 20 nmol/l (11 μg/l).

For quantitative assessment, plasma concentration-time curves (μmol/l *v.* d) of all three participants were constructed (Fig. 3(A)). In each case, the plasma concentrations increased significantly after 3 d of supplementation. The concentration-time plots of each individual showed an early maximum after 6 d, a decline in concentration until day 9 and a gradual second rise until the end of the study. Maximum concentrations reached at the end of the intervention period were 0·077, 0·160 and 0·157 μmol/l, respectively.

To estimate the intestinal absorption of α-cryptoxanthin from the supplement, two assumptions were considered: (i) 4 % of the body weight is plasma (Barua, 1999); (ii) after an initial phase of about 13 d, a final steady state can be reached. Such a plateau was recently ascertained by Hartmann *et al.* (2004) after giving multiple doses of zeaxanthin (1 mg/d for 42 d) after a booster period of about 2 weeks. Taking into account that a plasma xanthophyll plateau is in function not only of the time of supplementation but also of the amount supplied (which is rather low in the present study), the time required to reach a plateau is probably more that 16 d. For example, Olmedilla *et al.* (2002) described the plateau formation after 4 weeks upon supplementation with several carotenoids. However, based on a mean concentration of 0·131 μmol/l and a mean plasma mass of 2·54 litres (three

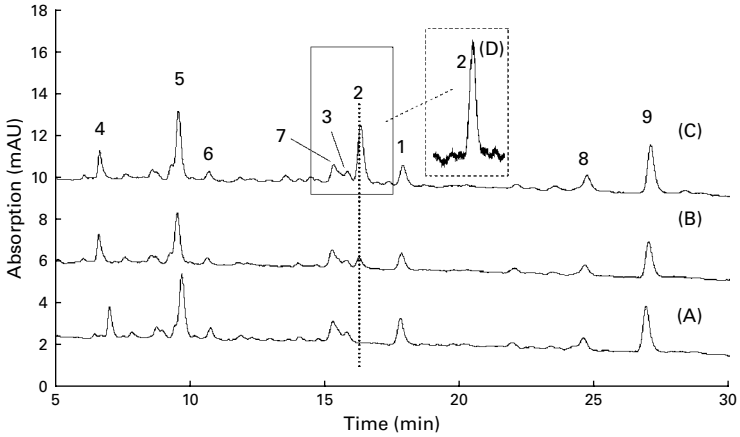

Fig. 2. HPLC chromatograms (extended sections; variable wavelength detector, 450 nm) of representative plasma samples originating from participant 3 (Table 1) (milli absorption units; mAU): (A) before supplementation; (B) after the intervention (16 d). Trace (C) corresponds to the plasma sample spiked with α-cryptoxanthin. The insert (D) represents the relevant section of the mass trace (*m/z* 535·4), suitable for identification of α-cryptoxanthin in liquid chromatography–MS in single ion monitoring mode analysis, determined in a concentrated and saponified sample extract (5 ml plasma/1 ml final volume) of participant 3. Peak assignment: 1, β-cryptoxanthin; 2, α-cryptoxanthin; 3, zeinoxanthin; 4, β-apo-12′-carotenal (standard); 5, lutein; 6, zeaxanthin; 7, anhydrolutein I (3′,4′-didehydro-β,γ-carotene-3-ol); 8, α-carotene; 9, β-carotene. For details of subjects and procedures, see p. 372.

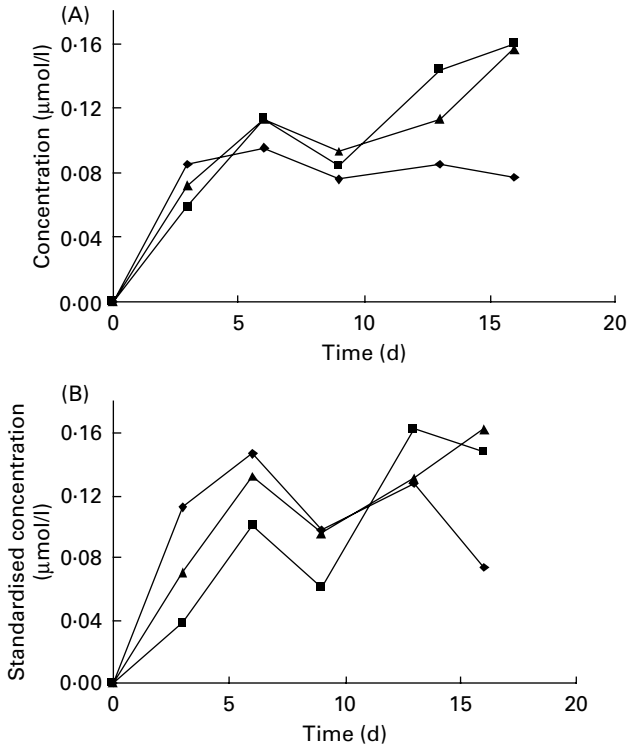

Fig. 3. Plasma concentrations of α-cryptoxanthin studied in dependence of the sampling time (μmol/l *v.* d) after taking sixteen capsules containing 156 μg α-cryptoxanthin each by three male individuals (■, ▲, ◆): (A) without standardisation; (B) standardised to triacylglycerol plasma concentrations. For details of subjects and procedures, see p. 372.

each), the participants had a mean total amount of 0·333 μmol (184 μg) α-cryptoxanthin in their circulating plasma after sixteen doses of the supplement. As only 156 μg were administered per d, this amount points to both an efficient absorption of the xanthophyll and even to an enrichment of α-cryptoxanthin in the human plasma (the clearance period of a single dose may be 2–3 weeks; Novotny *et al.* 2005; Tang *et al.* 2005). Thus, the results of the present pilot study suggest a high intestinal absorption of α-cryptoxanthin even from a minimally formulated cellulose-based supplement.

To account for potential variations in the triacylglycerol or total cholesterol levels, α-cryptoxanthin concentrations were corrected; each α-cryptoxanthin value was divided by the corresponding triacylglycerol or total cholesterol value and multiplied by the mean triacylglycerol or total cholesterol concentration of all participants. Both triacylglycerol and total cholesterol concentrations were in the normal range for all human subjects (desirable concentrations: triacylglycerol (< 1·25 mmol/l; total cholesterol (< 5·18 mmol/l; Jordan *et al.* 1995; see Table 1 for mean values). α-Cryptoxanthin plasma responses adjusted for triacylglycerol are shown in Fig. 3 (B). The time course of triacylglycerol- and total cholesterol-corrected α-cryptoxanthin concentrations resembled that of the uncorrected plots. Thus, standardisation did not influence the shape of the curves.

Areas under the curve (0–16 d; μmol × d/l) were determined by the trapezoidal rule without baseline area correction (SigmaPlot). The respective areas under the curve (0–16 d) values, calculated from the adjusted concentration-time plots, were as follows (for participant 1, 2 and 3 respectively): 1·676, 1·423 and 1·644 (standardised to triacylglycerol); 1·329, 1·691 and 1·305 (standardised to total cholesterol). Since area under the curve values are regarded as proportional to the bioavailability (Yao *et al.* 2000), comparison of the triacylglycerol- or cholesterol-adjusted values indicated that there were only small variations among subjects in the intestinal absorption of α-cryptoxanthin given in multiple doses.

Discussion

For isolation of α-cryptoxanthin, fresh green carrot leaves from ecological production were used. Since these plants were cultivated without the application of pesticides, the leaf extract did not contain anthropogenic compounds with a possible health risk for participants. However, the amount of α-cryptoxanthin available was very limited (3·8 mg/kg leaves). As a consequence, only three participants were included in the pilot study, designed to acquire basic data about the intestinal absorption of α-cryptoxanthin administered as multiple oral doses. Although α-cryptoxanthin is usually not present as a major xanthophyll in the human diet and the resulting plasma levels are consequently extremely low, a 2-week depletion phase preceded the study to reduce the overall carotenoid concentrations usually found in plasma to a base level and to approximate the individual levels of the participants.

Xanthophylls are lipid soluble and follow the same absorption pathway as other dietary lipids. Absorption of xanthophylls occurs via incorporation into chylomicrons, which subsequently enter the bloodstream. As α-cryptoxanthin represents a comparatively polar carotenoid (one hydroxyl function), it is distributed approximately equally between the HDL and LDL fractions (Furr & Clark, 1997). After daily ingestion of α-cryptoxanthin supplements, plasma concentration of all participants increased continuously except for a slight decrease on day 9, indicating that all participants were responders. We have no explanation for this temporary decrease. Due to the time lag it cannot be explained by hepatic circulation and lipoprotein incorporation, as is known for the biphasic plasma response to β-carotene within 2 d (Rock, 1997). Furthermore, we do not think that the decrease is a consequence of ingestion of 'banned' carotenoid-rich food possibly leading to a competing effect, since it was observed in all plasma samples. The second increase found in the α-cryptoxanthin concentration cannot be explained by ingestion of food rich in α-cryptoxanthin because this xanthophyll usually forms a minor component and is present in the European diet in extremely low amounts. Moreover, we do not believe that it is an analytical artefact because all analytical steps were controlled accurately. A second peak, which is usually observed in concentration-time plots because xanthophylls are incorporated in VLDL and LDL, did not appear in the present study.

It is understood that the low number of subjects (*n* 3) is a limitation of the present study. However, the variance in plasma response between individual subjects (see Fig. 3) was quite low, a finding being in contrast to results of former studies reporting broad interindividual variations for other carotenoids. One explanation may be that all subjects were male and were within the same age range (27·3 (SD 4·7) years).

The results of the present pilot study for the first time suggest a high intestinal absorption of α-cryptoxanthin even from a minimally formulated cellulose-based supplement. Although potential health functions of α-cryptoxanthin have not yet been investigated, this xanthophyll may serve as a future dietary supplement with both vitamin A activity and further biological values, not yet known. After optimisation of the extraction step from carrot leaves – which usually are regarded as plant waste material – there is a virtual possibility to launch an optimised formulation on the market.

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