Short Communication

JYP

Kinetics of plasma and erythrocyte-astaxanthin in healthy subjects following a single and maintenance oral dose

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ABSTRACT

Aim and Background: Astaxanthin is a unique carotenoid of predominantly marine origin providing the pink-red color to certain microalgae and accumulating in various animals higher in the food chain. It is an antioxidant without pro-oxidant properties or known side-effects following oral intake. **Materials and Methods**: We investigated astaxanthin kinetics in plasma and erythrocytes (red blood cells [RBC]) of four healthy adults after a single oral 40 mg dose. Plasma- and RBC-astaxanthin were measured during 72 h. Subsequently, an 8 mg/day dose was given during 17 days. Plasma- and RBC-astaxanthin were measured each morning. **Results:** Plasma-astaxanthin reached a peak (from 79 to 315 nmol/L) after 8 h and then declined (half-life, 18 h). Within 72 h, plasma-astaxanthin had returned to baseline. RBC-astaxanthin reached a peak (from 63 to 137 nmol/L packed cells) at 12 h and subsequently disappeared (half-life, 28 h). During the daily dose, plasma-astaxanthin increased until day 10 (187 nmol/L) and then decreased to a steady concentration similar to that reached after 2 days. RBC-astaxanthin appeared to be highly variable (group median concentration, 86 nmol/L packed cells). **Conclusion:** We found high intra- and inter-individual variations, especially in RBC, possibly due to non-standardized time difference between astaxanthin intake and sampling, fluctuating background intake from the diet, variable bioavailability, large distribution volume, degradation or others. Oral astaxanthin is rapidly absorbed and incorporated into RBC. The subsequent rapid decline suggests that, for a higher-than-baseline status, astaxanthin should be taken daily, at least in an early phase when total body equilibrium, if any, has not been reached yet.

Key words: Absorption, antioxidant, carotenoid, half-life, humans, status

INTRODUCTION

A diet rich in natural antioxidants supports health[1,2] strengthens the antioxidant network and is thereby associated with lower oxidative stress and inflammation, leading to decreased risk of cardiovascular disease, neurodegenerative diseases, certain cancers, and other diseases.[3] Astaxanthin has recently received attention for its potent antioxidant activity[4] without pro-oxidant properties.[5] It is a unique carotenoid belonging to the xanthophyll family, synthesized by plants and algae providing them with a pink-red color[6] and accumulating in certain animals higher in the food chain, such as flamingoes, salmon, shrimps, and crayfish.[7] Natural astaxanthin is optically distinct from synthetic astaxanthin. It is commercially available as a food supplement from the algae *Hematococcus pluvialis*.[8]

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Orally administered astaxanthin incorporates into both plasma and erythrocytes (red blood cell [RBC]) of healthy subjects, improves RBC antioxidant status and decreases membrane phospholipid peroxidation after a 12-week daily supplementation.[9] Following its ingestion, astaxanthin has been shown to reach a peak in plasma at about 7 h and to decline with a median half-life of about 21 h.[10] The kinetics of astaxanthin in RBC are currently unknown. We investigated the kinetics of astaxanthin in both plasma and RBC after a single oral 40 mg dose and its distribution in both compartments during a 17-day 8 mg/day maintenance dose.

MATERIALS AND METHODS

Study group

Four apparently healthy volunteers (1 male, 3 females) aged 29-41 years (weight 59-70 kg, height 1.73-1.80 m, individual body-mass indices, 19.7, 20.7, 22.1, and 22.9 kg/m^2), participated in this pilot intervention study. Throughout the study period, subjects were instructed to maintain their usual lifestyle. Age, weight, and length were self-reported. All participants received verbal and written explanation of the objectives and procedure of the study and subsequently provided us with written informed consent.

Astaxanthin supplementation

The pilot study consisted of two well-defined parts. In the first part, a single dose of 40 mg astaxanthin (10 soft gel gelatin capsules containing an astaxanthin extract from the algae *H. pluvialis*; Cyanotech, Hawaii) was given to the 4 subjects together with a fat-containing breakfast, as astaxanthin absorption is improved in the presence of lipid based formulations.[11] Each soft gel capsule contained 4 mg astaxanthin, 10 IU vitamin E (as d-alpha tocopherol), 64 μg β-carotene, 40 μg lutein and 72 μg canthaxanthin. The capsules contained glycerol and safflower oil as wetting and filling agents, respectively. Five days after, the 40 mg astaxanthin intake, the second part of the pilot was initiated, where the four participants were instructed to take a daily dose of 8 mg astaxanthin during 17 days. The astaxanthin capsules were taken in the evening together with, or just after, a fat-containing meal. Compliance was verbally checked by one of us on the following day.

Sample collection and analyses

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood (4 mL) was collected in the morning by venipuncture at baseline and at 2, 4, 6, 8, 10, 12, 24, 32, 48, and 72 h after the single oral 40 mg astaxanthin dose and every morning for 13 days (days 1-5, 8-12, and 15-17, respectively) following the daily 8 mg maintenance dose.

Ethylenediaminetetraacetic acid-blood was centrifuged for 10 min at 1,000 g in a cooled centrifuge (4°C) for the separation of plasma and RBC. The EDTA-plasma (200 μL) was transferred to a teflon-sealable sovirel tube containing 2.75 mL of an antioxidant solution containing EDTA, ascorbic acid, pyrogallol and butylated hydroxytoluene in methanol/water for the preservation of astaxanthin. Following the removal of plasma and buffy coat, RBC were washed 3 times with 0.9% saline. Phosphate buffered saline (pH = 7.4) was subsequently added to prepare an about 50% hematocrit suspension, from which 500 μL were transferred to a teflon-sealable sovirel tube containing 2.75 mL of the aforementioned antioxidant solution. The remainder was used for a total cell count including a hematocrit measurement (Sysmex, Etten-Leur, The Netherlands).

All tubes were frozen at −20°C until analyses. Plasma- and RBC-astaxanthin were determined with high-performance liquid chromatography (HPLC)/VIS using previously described procedures.[12,13] Briefly, this method includes hexane extraction, evaporation to dryness under nitrogen, and re-dissolution in methyl tert-butyl ether (MTBE) and ethanol. From this mixture, 50 μL are injected into the HPLC. The analytical system was composed of a carotenoid 250 × 2.1 mm ID column (YMC, Japan) with HPLC/VIS detection operated at a flow rate of 0.3 ml/min, using a gradient of solvent A (methanol: MTBE: H_2O = 81:15:4 v/v/v) and solvent B (methanol: MTBE = 7:93 v/v) at a detection wavelength of 450 nm.

The RBC-astaxanthin content (in nmol/L packed cells) was calculated by dividing the measured RBC-astaxanthin concentration by the hematocrit of the washed RBC suspension.

RESULTS

The between-series plasma-astaxanthin cyclic voltammograms (CVs)(n = 6) at mean levels of 42 and 111 nmol/L amounted to 5.3 and 3.8%, respectively, implying that the analytical reference change values (2.8 times the SDs of 2.2 and 4.2 nmol/L, respectively)[14] are about 6 and 12 nmol/L, at the low and higher levels, respectively.

Plasma- and RBC-astaxanthin kinetics after a single 40 mg oral dose

The individual and median courses of plasma- and RBC-astaxanthin up to 72 h after the single 40 mg dose

are presented in Figure 1. Large intra- and inter-individual variations in the courses were observed for plasma- and notably for RBC-astaxanthin, (Panels A and B). Due to the variations in time to reach the peak values and also peak heights, and in view of the small sample size, we refrained from statistical analyses. Nevertheless, all study subjects responded well beyond the analytical variation as their differences between baseline and peak levels were well beyond the reference change value for analytical variation.

In all four subjects, plasma-astaxanthin (Panel A) reached a peak (from a baseline median of 79-315 nmol/L) after 8 h and subsequently declined with an estimated half-life of 18 h. Within 72 h, plasma-astaxanthin had returned to baseline (median, 64 nmol/L). This is in agreement with Østerlie *et al*., who reported a plasma-astaxanthin peak at about 7 h following a 100 mg astaxanthin oral dose and a half-life of about 21 h.[10] RBC-astaxanthin (Panel B) reached a peak (from 63 at baseline to 137 nmol/L packed cells) at 12 h and subsequently disappeared with a half-life of about 28 h. Individual summits were reached after 6, 8, 8, and 12 h. Furthermore for RBC, the baseline was reached within 72 h (median, 27 nmol/L packed cells). The median percentage astaxanthin found in RBC throughout the 72 h observation period (with the plasma concentration set at 100%) amounted to 44% (range of individual medians: 26-56%). These RBC-plasma ratios, subject to a large variation, are comparable to the median of 43% (range: 35-48%) that we estimated from the data from Nakagawa *et al*.[9] who supplemented healthy adults with 6 and 12 mg oral astaxanthin/day for 12 weeks.

Plasma- and RBC-astaxanthin during a 17 days 8 mg/day oral maintenance dose

The individual and median courses of plasma- and RBC-astaxanthin during the 17 days maintenance dose are presented in Figure 2. Supplementation started 5 days after the single 40 mg oral dose. Baseline levels were taken from astaxanthin concentrations at 72 h after the single 40 mg astaxanthin oral dose. Plasma-astaxanthin (Panel A) increased slowly until day 10, reaching a maximum of 187 nmol/L. After this peak, plasma astaxanthin decreased to reach a steady concentration similar to that reached after 2 days. RBC-astaxanthin (Panel B) appeared to be highly variable, giving rise to a group median

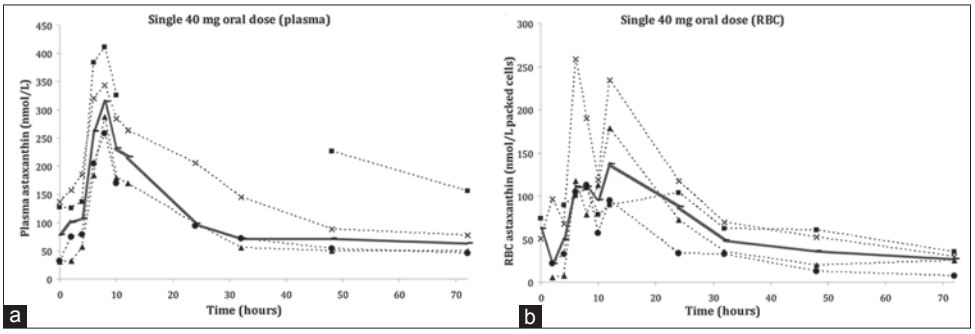

Figure 1: Courses of astaxanthin in plasma (a) and erythrocytes (b) after a single 40 mg astaxanthin oral dose. (Healthy subjects (*n* = 4) took a single 40 mg astaxanthin oral dose (from *Haematococcus pluvialis*; Cyanotech, Hawaii). Each soft gel capsule contained 4 mg astaxanthin, 10 IU vitamin E (as d-alpha tocopherol), 64 µg β-carotene, 40 µg lutein and 72 µg canthaxanthin. Dotted lines represent data of the 4 individuals and the bold lines represents their medians. RBC: Red blood cell)

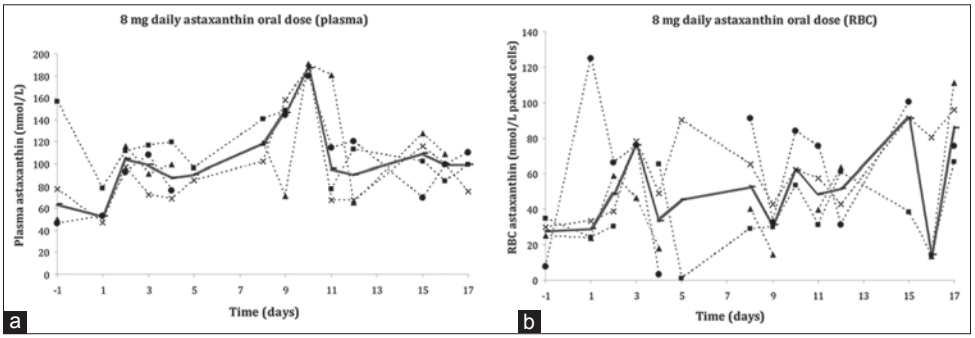

Figure 2: Courses of astaxanthin in plasma (a) and erythrocytes (b) during a 17 days maintenance dose of 8 mg astaxanthin/day (Healthy subjects (*n* = 4) took a daily dose of 8 mg astaxanthin (from *Haematococcus pluvialis*; Cyanotech, Hawaii) during 17 days. Supplementation started 5 days after the single 40 mg oral dose. Baseline levels (day 1) were taken from the astaxanthin concentrations at 72 h after the single 40 mg astaxanthin oral dose [Figure 1]. Each soft gel capsule contained 4 mg astaxanthin, 10 IU vitamin E (as d-alpha tocopherol), 64 µg β-carotene, 40 µg lutein and 72 µg canthaxanthin. Dotted lines represent data of the 4 individuals and the bold lines represents their medians. RBC: Red blood cell)

concentration of 86 nmol/L packed cells throughout the 17 days supplementation period. The median percentage astaxanthin found in RBC throughout the 17 days maintenance dose (with the plasma concentration set at 100%) was 49% (range of individual medians: 28-71%). This value is in agreement with the median of 44% (range of individual medians: 26-56%) of the study group throughout the 72 h observation period following the single 40 mg dose (see above), and comparable to the median of 43% (range: 35-48%) that we estimated from the data from Nakagawa *et al*.[9] Large intra- and inter-individual variations were observed for plasma- and RBC-astaxanthin, notably for the latter, during the 17 days supplementation period. This may be partly due to the low dose compared with the background intakes (from food). For instance, the median inter-individual plasma-astaxanthin CV during the 17 days maintenance dose amounted to 20% (range: 3-46%), and for RBC-astaxanthin this CV was 40% (range: 22-212%).

DISCUSSION AND CONCLUSIONS

The short plasma- and RBC-astaxanthin half-lives of 18 and 28 h, respectively, suggest the necessity to take astaxanthin on a daily basis to maintain a higher-than-baseline steady state, at least in the initial phase of supplementation. This early phase might in part be influenced by the tendency of astaxanthin to incorporate into all bodily cell membranes. Astaxanthin is likely to be subject to a large distribution volume, in which probably not all compartments are reached at equal rates. Both the single and maintenance oral doses gave rise to large intra- and inter-individual biological variations, especially in the RBC compartment. The variability in responses may, e.g., derive from non-standardized time difference between astaxanthin intake and blood sampling, a fluctuating background intake from the diet, variable bioavailability, large distribution volume, (induced) degradation and others. High intra- and inter-individual bioavailability of carotenoids has been previously reported[15-17] and the present data on astaxanthin appear as no exception to this rule.

Orally administered astaxanthin is rapidly absorbed and becomes rapidly incorporated into RBC. The subsequent rapid decline suggests that, for a higher-than-baseline status, astaxanthin should be taken daily, at least in an early phase when total body equilibrium, if any, has not been reached yet.

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