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Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations

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Abstract

Astaxanthin is a carotenoid with antioxidant properties, synthesised by plants and algae, and distributed in marine seafood. Astaxanthin is also available as a food supplement, but, like other carotenoids, is a very lipophilic compound and has low oral bioavailability. However, bioavailability can be enhanced in the presence of fat. There is not much information in the literature about the pharmacokinetics of oral astaxanthin in humans. In this open parallel study, healthy male volunteers received a single dose of 40 mg astaxanthin, as lipid based formulations or as a commercially available food supplement, followed by blood sampling for further analysis of plasma concentrations. Pharmacokinetic parameters were calculated to evaluate the extent and rate of absorption from each formulation. The elimination half-life was 15.9±5.3 h (n=32), and showed a mono-phasic curve. Three lipid based formulations: long-chain triglyceride (palm oil) and polysorbate 80 (formulation A), glycerol mono- and dioleate and polysorbate 80 (formulation B), and glycerol mono- and dioleate, polysorbate 80 and sorbitan monooleate (formulation C), all showed enhanced bioavailability, ranging from 1.7 to 3.7 times that of the reference formulation. The highest bioavailability was observed with formulation B, containing a high content of the hydrophilic synthetic surfactant polysorbate 80.

Keywords: Astaxanthin; Pharmacokinetics; Lipid based formulations; Polysorbate 80; Bioavailability

1. Introduction

Astaxanthin is a naturally occurring carotenoid with strong antioxidant properties, does not have vitamin A activity and belongs to the xanthophylls group. The lipid soluble pigment is synthesised by plants and algae, and distributed mainly in marine seafood (Kurashige et al., 1990; Clark et al., 2000). Astaxanthin is commercially available as an antioxidant food supplement, approved by the Swedish Health Food Council Advisory Board, produced from the microalgae *Haematococcus pluvialis*; the recommended daily intake is 4 mg according to the manufacturer's product information.

Carotenoids are thought to be associated with a number of health benefits, and epidemiologic studies have shown an inverse relationship between the presence of various cancers and cardiovascular diseases, and a high intake of carotenoid containing food, such as fruit and vegetables. The biological mechanisms for this protection are currently unclear, but, apart from their provitamin A activity, carotenoids exhibit antioxidant properties, can affect cell growth regulation, and modulate gene expression and immune response, all of which are possible mechanisms of relevance (Rock, 1997; Paiva and Russell, 1999). A recent publication has shown astaxanthin to inhibit LDL (low density lipoprotein) oxidation in humans (Iwamoto et al., 2000).

To better understand the potential benefits of high intakes of carotenoids in food, or as a food supplement, recent studies have focused on the absorption, distribution and metabolism of diverse carotenoids in humans (Parker, 1996; Zaripheh, 2002). However there is not much information regarding the pharmacokinetics of astaxanthin in humans.

The bioavailability of carotenoids, which are very lipophilic compounds, is low. It varies widely, from less than 10% from raw uncooked vegetables to more than 50%

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in oily solutions or in synthetic commercial formulations (Olson, 1999). Apart from incomplete release of carotenoids from the matrices of foods, one explanation for the low bioavailability is probably dissolution limitations in the gastrointestinal fluids. Another factor suggested to limit absorption, is a saturated capacity of incorporation into bile micelles (micelles are formed during lipolysis, and facilitate absorption of lipophilic compounds) seen at high doses (Parker, 1996). The enhanced bioavailability of carotenoids after co-administration of fat is thought to be mainly due to the presence of conjugated bile salt, and its ability to form bile salt micelles (Olson, 1994; Rock, 1997). The explanation for the enhanced oral bioavailability of a poorly water soluble compound in the presence of fat, is, however complex and due to numerous mechanisms (Charman et al., 1997; Porter and Charman, 2001a).

Lipid based formulations can range from pure triglyceride oils, through mixed glycerides, lipophilic surfactants, and hydrophilic surfactants to water-soluble co-solvents (Pouton, 2000). Surfactants are, like bile salt, amphiphilic, and are commonly used for emulsification of a lipid vehicle. The emulsification process increases the surface area for release of drug from the vehicle. The surfactants can also mimic formation of bile salt micelles, and thereby increase the solubility characteristics of a poorly water-soluble drug. If dissolution and solubilisation characteristics of a hydrophobic drug change, then the rate and extent of absorption can be affected (Humberstone and Charman, 1997; Gershanik and Benita, 2000).

The primary objective of this study was to see if the bioavailability of astaxanthin could be increased in humans by incorporating into lipid formulations of different compositions. In a commercially available food supplement, astaxanthin is present as a triglyceride rich algal meal, and therefore work on our formulations has mainly been focused on the effects of the addition of different surfactants. The secondary objective of this study was to gain more knowledge about the pharmacokinetics of astaxanthin in humans.

2. Material and methods

2.1. Preparation of lipid based formulations

The reference formulation was a commercial formulation consisting of algal meal and dextrin in hard gelatin capsules (Napro Pharma, Brattvaag, Norway). For formulation A, the surfactant, polysorbate 80 (Grünau Illertissen, Germany), and palm oil (Karlshamn, Sweden) were mixed with an Ultra turrax TP 18/10 (Janke and Kunkel, Staufen, Germany). Formulations B and C were kindly provided by Paul Goggin, R.P. Scherer, UK (Table 1). Formulations A–C were packed by hand in hard gelatin capsules identical to those used for the reference formulation. The source of astaxanthin in all formulations was homogenised and spray dried cells of the unicellular green alga *H. pluvialis* (AstaCarotene, Gustavsberg, Sweden).

Table 1
Lipid formulations: ratio of components in each of the different formulations

Formulation	Algal meal[b] (fat)	Palm oil	MG and DG[c]	Polysorb 80 (H)[d]	Polysorb 80 (H)+SPAN 80 (L)[e]
Reference[a]	0.30 (0.12)				
A	0.32 (0.13)	0.63		0.05	
B	0.24 (0.10)		0.20	0.56	
C	0.24 (0.10)		0.20		0.56

[a] For the reference, the remaining content is dextrin (not shown).
[b] 40% of the algal meal is algal fat.
[c] Glycerolmono- and dioleate.
[d] Polysorbate 80 (H, hydrophilic).
[e] Approximately 1/3 polysorbate 80 and 2/3 sorbitan monooleate (SPAN 80) (L, lipophilic).

2.2. Subjects

A total of 32 healthy male subjects, age 20–46 (mean 26.5) years, weight 51–110 (mean 81.5) kg, and height 170–200 (mean 184) cm, were entered into the study. Prior to entry, the subjects gave written informed consent after receiving both verbal and written information. All subjects were considered healthy according to medical history, physical examination and laboratory investigation. None of the subjects smoked nor did they use any medications suspected of interfering with the outcome of the study. The University Ethics Committee approved the study.

2.3. Design

The study was of open parallel design where the subjects received one of four different treatments: reference formulation, formulation A, formulation B or formulation C. Formulations A–C contained different lipid formulations. All treatments nominally contained 40 mg astaxanthin, which was given as a single dose. A parallel design was chosen due to the long half-life of astaxanthin. In a previous study, doses of up to 22 mg/day for 14 days were given to healthy volunteers (Iwamoto et al., 2000) without any adverse events.

On the study day, the subjects arrived fasting (intake of water was allowed) since the previous evening (22:00 h) to the clinic. An intravenous indwelling catheter was inserted in an arm vein for blood sampling. The subjects were then served breakfast, containing ~15 g fat. The formulation drug was then administered 15 min after the end of the meal, together with a standard amount of tap water. Blood samples were collected according to the schedule, given in Section 2.5. Lunch was served 4 h after intake of the drug formulation. No food or drink restrictions were applied 10 h after intake of the drug formulation.

2.4. Adverse event

Adverse events were recorded after open questioning and with spontaneous reports during the study days.

2.5. Blood sampling

Samples were obtained pre-dose and 2, 4, 8, 12 and 24 h and 2, 4 (±1), 7 (±1), 14 (±2) and 28 (±3) days after dose. All samples were collected in 5-ml EDTA tubes. The samples were protected from light, and the plasma was separated after centrifugation and then stored at −70 °C until analysis.

2.6. Analytical method

Analysis of *trans*-astaxanthin was performed using liquid–liquid extraction for sample preparation and high performance liquid chromatography with visible spectrometric detection. Plasma samples and control plasma were thawed at room temperature under conditions of reduced light. To extract the carotenoids 4 ml of acetone was added to 0.5 ml of plasma in 10-ml glass tubes. After mixing vigorously for 10 s, the tubes were left on a reciprocating shaker for 1 h whilst protected from light. Thereafter 4.0 ml hexane was added and the samples mixed vigorously 10 s before two-phase separation for 1 h. The upper phase was then removed and dried down under a stream of nitrogen. The dried extract was dissolved in 75–150 μl chloroform:methanol (1:1, v/v). A Rheodyne injection system (model 7010) was used to inject 25 μl of sample into a reverse-phase column (ReproSil-Pur 120 C18-AQ). The carotenoids were eluted using a linear gradient with methanol:water:ethyl acetate, 82:8:10 at the start and 29:1:7 after 20 min, using a Merck-Hitachi L6200A Intelligent pump. Astaxanthin was detected at 474 nm by a Merck-Hitachi L4200 detector. Integration was performed using CSW version 1.5 software.

Quality control plasma samples and calibration standards were prepared from a stock solution of *trans*-astaxanthin (>98% pure, Sigma-Aldrich) in acetone, 30 μg/ml. Lower limit of quantification (LLOQ), based on peak area, was 20 ng/ml with linearity demonstrable to 1000 ng/ml. Intra-assay precision values, based upon coefficients of variation (CV) of quality control samples were less than or equal to 14.0%.

2.7. Pharmacokinetic calculations

The following kinetic parameters were estimated for astaxanthin: C_{max} (maximum observed plasma concentration), t_{max} (the time to reach maximum concentration), t_{lag} (the lag time observed before absorption), $AUC_{(0-\infty)}$ (area under the curve calculated to infinity) and $t_{1/2}$ (elimination half-life). C_{max}, t_{max} and t_{lag} were read directly from the observed concentration versus time data. $AUC_{(0-\infty)}$ and $t_{1/2}$, based on plasma concentrations of astaxanthin, were calculated by means of a non-compartmental analysis, using model 200 of WinNonlin version 1.5 (Scientific Consulting, Apex, NC, USA). A linear-log trapezoidal method was used when calculating $AUC_{(0-\infty)}$. We used the Wagner-Nelson method (Gibaldi and Perrier, 1982) to estimate the amount of astaxanthin absorbed over time.

For each treatment, one dose was saved for post-trial analysis. Not all treatments contained exactly 40 mg. All raw data (concentration) have therefore, apart from the reference (40 mg/10 capsules), been dose adjusted: formulation A (40.3±3.0 mg/9 capsules), formulation B (40.9±1.4 mg/12 capsules), and formulation C (41.8±2.0 mg/12 capsules).

2.8. Statistical analysis

We assumed C_{max} and $AUC_{(0-\infty)}$ for astaxanthin to be normally distributed when log transformed, and $t_{1/2}$ to be normally distributed. For the primary objective of the study, to find formulations which were superior to the reference, a comparison of means was made between groups using one-way ANOVA followed by Student's *t*-test, 95% CI. Non-parametric tests (Mann–Whitney) were used to compare the non-continuous variables t_{max} and t_{lag}. Statistical analyses were carried out using the programme SPSS version 11.0.

3. Results

3.1. Safety

All the subjects (n=32) completed the study in accordance with the protocol. Three mild adverse events were reported within the first 48 h after dose; the most common was headache. During the rest of the blood sampling period of <28 days, minor adverse events were reported, and unlikely to be caused by the treatment.

3.2. Concentration versus time and descriptive statistics

Concentrations versus time curves for the different formulations are given in Fig. 1. A one-compartment model with linear elimination could describe the disposition of astaxanthin in the body. The estimated half-life ($t_{1/2}$) was 15.9±5.3 h. The values for C_{max}, t_{max}, t_{lag}, $t_{1/2}$ and $AUC_{(0-\infty)}$ are given in Table 2. There was no astaxanthin detectable in the pre-dose samples.

3.3. Bioavailability

The bioavailability of astaxanthin was enhanced with all the lipid formulations tested (Fig. 2). The *AUC*s (Table 2)

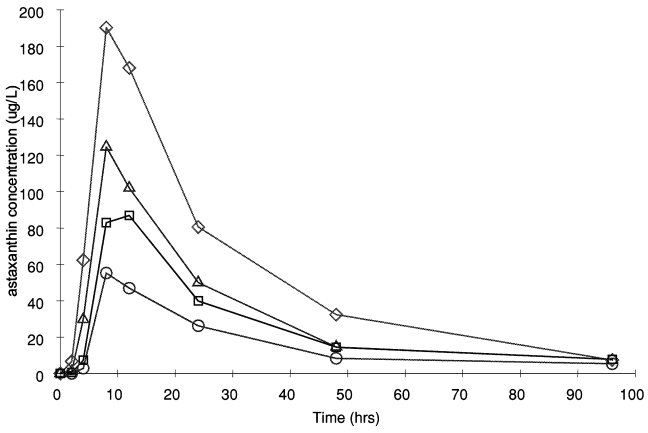

Fig. 1. Plasma astaxanthin concentration–time profiles (mean values, $n=8$) as a function of formulations, following oral administration of 40 mg astaxanthin. Four different formulations were used: reference formulation (○), formulation A (□), formulation B (◇) and formulation C (△).

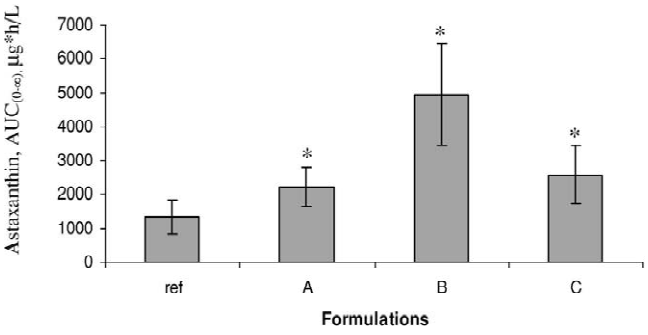

Fig. 2. Astaxanthin, observed area under the curve to infinity ($AUC_{(0-\infty)}$). The bars represent mean values of *AUC* and standard deviation (S.D.) for all formulations: reference, and formulations A, B and C. *$P<0.05$, statistically significant compared to the reference.

of the test formulations compared to the reference exhibited a 3.7-fold (2.7–5.1; 95% CI, $P<0.001$) increase for formulation B, while formulation C had nearly a doubling of the bioavailability at 1.9 (1.4–2.6; 95% CI, $P<0.001$), while formulation A was 1.7 (1.2–2.3; 95% CI, $P=0.002$) times higher.

The time for absorption to reach maximum concentration for these three formulations is also similar, looking at the parameter t_{max} (Table 2), and there are no statistically significant differences for this variable between the favorable formulations compared to the reference. The delay between drug administration and beginning of absorption, t_{lag}, is reduced for formulations B and C ($P<0.05$). However, while t_{lag} and t_{max} are dependent on the schedule of blood samples, statistically significant differences should be considered with caution.

When using the Wagner-Nelson method to estimate the amount of astaxanthin absorbed over time, we found that the median amount absorbed after 4 h was 4% (0–20, min–max) for the reference, 6% (0–28) for formulation A, 15% (10–40) for formulation C and 34% (23–61) for formulation B. This further verifies the increased absorption observed for formulations B and C.

4. Discussion

In this clinical trial, 32 healthy males received a single dose of astaxanthin, either as a commercial food supplement or in different lipid based formulations, in order to observe the effect on bioavailability. All three lipid based formulations enhanced the bioavailability of astaxanthin, with formulation B being 3.7-fold better.

Carotenoids are lipid-soluble molecules, which follow the absorption of dietary fat (Erdman and Deming, 1999). Many xanthophylls, including astaxanthin, are mainly present in esterified form (mono- and diesters) and must be hydrolysed before absorption (Zaripheh, 2002). The absorption process from the intestinal tract into the enterocytes is thought of as mainly a passive process not involving special epithelial transporters (Olson, 1999). Dissolution from matrix and incorporation into mixed micelles are two important steps preceding absorption over the membrane. In the enterocytes the xanthophylls are incorporated in chylomicrons, and then released into the lymphatic system before entering the systemic circulation (Zaripheh, 2002). Xanthophylls not incorporated in chylomicrons are thought to be returned back to the lumen with the turnover of mucosal cells. There is apparently no evidence for significant portal absorption of carotenoids in humans or other mammals (Parker, 1996; Zaripheh, 2002).

Table 2
Descriptive statistics of astaxanthin kinetic parameters for each of the different formulations

Formulation	Subjects (n)	$AUC_{(0-\infty)}$±S.D. (µg*h/l)	C_{max}±S.D. (µg/l)	$t_{1/2}$±S.D. (h)	t_{max} (h) (min–max)	t_{lag} (h) (min–max)
Reference	8	1347±501	55.2±15.0	16.7±7.2	8 (8–8)	3 (2–4)
A	8	2216±574*	90.1±29.3*	15.7±5.0	10 (8–12)	2 (0–4)
B	8	4960±1504*	191.5±59.3*	19.1±2.4	8 (8–12)	1 (0–2)*
C	8	2580±850*	125.7±38.2*	12.1±3.3	8 (8–12)	2 (0–2)*

For the parameters relative area under the curve to infinity ($AUC_{(0-\infty)}$), maximum concentration (C_{max}) and terminal half life ($t_{1/2}$), mean values±standard deviation (S.D.) are given. For the parameters time to maximum concentration (t_{max}) and time before absorption are observed (t_{lag}), median together with minimum and maximum are given.

*$P<0.05$, statistically significant difference compared to the reference.

Astaxanthin, in our study, is a food supplement from the alga *H. pluvialis* and is rich in triglycerides. The supplement was taken after a standardised breakfast. Thus, conditions in the gastrointestinal tract, regarding bile salt secretion and lipase activity due to food and fat abundance, should theoretically have been optimised. However, the bioavailability of astaxanthin could be further enhanced when adding oil and/or surfactants with different characteristics.

The absorption increased most from formulation B, where two surfactants were used, glycerol mono- and dioleate (lipophilic) and polysorbate 80 (hydrophilic).

The difference between formulations B and C (Table 1) is the presence of another surfactant, sorbitan monooleate (lipophilic), and a parallel reduction in the amount of hydrophilic polysorbate 80. However, the overall ratio of polysorbate 80+SPAN 80 and the glycerol mono- and dioleate remains the same (Table 1). The reduced bioavailability in formulation C compared with B might therefore be due to a reduction in the hydrophilic polysorbate 80 or the addition of more lipophilic sorbitan monooleate.

For both formulations B and C, the pharmacokinetic parameter t_{lag} was shorter compared to the reference. It cannot be excluded that a more frequent blood sampling schedule would have revealed a shorter t_{max} and higher C_{max}. These pharmacokinetic changes might mainly be due to the dissolution and solubility properties of astaxanthin in the formulations B and C. Thus, high concentrations of hydrophilic surfactants are thought to produce very fine dispersions under conditions of gentle agitation in an aqueous environment, which provide for a good self-emulsifying performance, and can result in more rapid absorption (Pouton, 2000). The calculated percent of dose absorbed over time, using the Wagner-Nelson method, also indicates that astaxanthin underwent increased absorption from formulations B and C.

The parameter t_{lag} for formulation A was not as clearly reduced as for formulations B and C. However, the bioavailability of formulation A was increased similar to formulation C. One explanation for the enhanced bioavailability observed may be the increased formation of chylomicrons and increased lymphatic transport of astaxanthin, due to the presence of palm oil, a long chain triglyceride (LCT) in formulation A.

In a study in humans the incorporation of beta-carotene into chylomicrons was lower after administration of an emulsion containing medium-chain triglycerides, MCT, than long-chain triglycerides, LCT (Borel et al., 1998). Medium-chain glycerides are mainly transported by portal blood and not favoring lymphatic transport. Co-administration of long-chain triglycerides enhanced lymphatic transport of a lipophilic compound (Caliph et al., 2000; Porter and Charman, 2001b). However, in the study of MCT and LCT, the emulsions contained 40 g of fat, compared to ~4–6 g used in our formulations, which might be too small to stimulate a further chylomicron response during the time of absorption.

Our results show that the choice of surfactant is critical, favoring hydrophilicity, but that the difference in ratio of oil:surfactant, and the surfactant concentration can also be important. However, large quantities of non-ionic surfactants are reported to irritate the gastrointestinal (GI) tract, and this can be a problem when long-term dosing is necessary (Gershanik and Benita, 2000), and an optimal formulation should therefore contain only low amounts of surfactants. In formulation A, a very low amount of polysorbate 80 was added, but its contribution, if any, to the observed effect on bioavailability cannot be estimated from our data. Further studies, with different non-GI-irritating concentrations of polysorbate 80 in oil might form the basis for further development of a pharmaceutical formulation of astaxanthin. The formulations also need to be tested in a fasted state, since the postprandial effect might contribute to the effects on bioavailability observed in our study.

In our study the pharmacokinetics of astaxanthin could be adequately described using a one-compartment model. The elimination was linear, with a half-life of 15.9±5.3 h.

In the only previously published study regarding the pharmacokinetics of astaxanthin in humans, three men were given 100 mg of synthetic astaxanthin as a single dose. The plasma astaxanthin concentration–time curves were monophasic and measured over 72 h, with a half-life of 21±11 h (Osterlie et al., 2000).

In conclusion, the relative bioavailability of the food supplement astaxanthin given as a high single dose could be enhanced by its incorporation into lipid formulations of various compositions. Formulation B, containing a high amount of polysorbate 80, increased the bioavailability nearly 4-fold, compared with the commercial reference formulation. The kinetics of astaxanthin is described as a one-compartment model, with a half-life of 15.9±5.3 h. The high dose, 40 mg, given to healthy volunteers on one occasion was well tolerated.

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