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Bioavailability of Astaxanthin in *Haematococcus* Algal Extract: The Effects of Timing of Diet and Smoking Habits

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Astaxanthin is a carotenoid that possesses strong antioxidant activity. Recently, many studies on biological activity have been reported. In general, the absorption of carotenoids is affected greatly by diet and by smoking. In this report, we investigated astaxanthin pharmacokinetics after administration of *Haematococcus* algal extract, a source of astaxanthin, to smokers and nonsmokers before and after a meal; astaxanthin was given before the meal to nonsmokers (n = 7), after the meal to nonsmokers (n = 6), and after the meal to smokers (n = 7), then serum samples were analyzed. The timing of administration greatly affected astaxanthin bioavailability including the area under the curve (AUC_{0–168}, 2,968 ± 959 µg h/l in the before-meal group vs. 7,219 ± 3,118 µg h/l in the after-meal group), indicating high availability in the after-meal group. Smoking also affected the pharmacokinetic parameters and reduced the half-life (t_{1/2}) of astaxanthin elimination significantly.

Key words: absorption; carotenoid; pharmacokinetics

Astaxanthin, a natural lipophilic compound with a deep red color, is a carotenoid-like β-carotene and lycopene. It is widely distributed, especially in marine organisms, including salmon, salmon roe, shrimp, crab, microalgae.¹ It possesses oxygen functional groups, and thus belongs to the xanthophylls. Plants, algae, and microorganisms can biologically synthesize carotenoids but animals lack the ability to synthesize these compounds. Therefore, in animals carotenoids must be acquired and accumulated from the diet.

Haematococcus is a green alga. It is known to start accumulating astaxanthin due to environmental stimuli such as high light intensity or oligotrophic conditions. *Haematococcus* alga is believed to be the most efficient natural source of astaxanthin.¹ Most of the astaxanthin accumulated in *Haematococcus* algae is in the ester form, and its hydroxyl functional groups are acylated with fatty acids. The hydroxyl functional group of *Haematococcus* astaxanthin molecule has the (3S,3′S)-form of chirality.²

In general, carotenoids possess high antioxidative ability. Astaxanthin is also reported to show a strong quenching effect against singlet oxygen, with a potency more than 100-fold higher than that of α-tocopherol. It also shows strong activity against lipid peroxidation.³ Other

carotenoids, such as β-carotene, lycopene, and zeaxanthin, also show high antioxidative ability, but it has been reported that they can also show pro-oxidative properties under certain conditions. On the other hand, it has been reported that astaxanthin shows pure antioxidative properties.⁴

Due to their high antioxidant properties, many nutraceuticals containing astaxanthin have potent effects against many symptoms and are coming onto the market. Recently, further functionalities of astaxanthin in addition to its antioxidative ability have also been reported, including anti-cancer,^{5,6} prevention of diabetic nephropathy,⁷ prevention of *H. pylori* infection,⁸ alleviation of eye fatigue,⁹ and immunomodulating ability.^{10,11}

Absorption of xanthophylls is believed to occur as follows: After ingestion, xanthophylls mix with bile acid to make micelles in the intestinum tenue. Next, the micelles containing xanthophylls are passively absorbed by intestinal mucosal cells. Xanthophylls are then incorporated into chylomicra by intestinal mucosal cells. Chylomicra with xanthophylls are released into the lymph and digested by lipoprotein lipase within the systemic circulation, and chylomicron remnants are rapidly removed by the liver and other tissues. Xanthophylls are then incorporated into very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) and transported to the tissues.¹²

In relation to the pharmacokinetics of astaxanthin, Østerlie reported a shift in plasma astaxanthin concentration after administration of a water-dispersible free racemic form of astaxanthin contained in beadlets with olive oil.¹³ Coral-Hinostroza reported the effects of the administration of a purified chiral isomeric mixture of diester astaxanthin as a dressing onto a pasta salad.¹⁴ With regard to the pharmacokinetics of *Haematococcus* astaxanthin, Odeberg reported the effects of soft capsule formulations on its bioavailability.¹⁵ Considering the above xanthophyll absorption mechanisms and the fact that dietary fat greatly stimulates the absorption of carotenoid,¹⁶ the bioavailability of astaxanthin must be affected greatly by the timing of its ingestion, before a meal or after. To our knowledge, however, there are no reports concerning the effect of ingestion time on astaxanthin absorption. In this study, therefore, we

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Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; AUC, area under the curve; C_{max}, maximum blood concentration; t_{max}, time of maximum blood concentration; t_{1/2}, elimination half-life

investigated the effects of various ingestion times and smoking habits on the pharmacokinetics of *Haematococcus* astaxanthin.

Materials and Methods

Subjects and study design. A total of 15 healthy males, aged 18–60 years, and five healthy females, aged 26–32 years, were enrolled. The procedures adopted were in accordance with the Helsinki Declaration. Prior to entry, written consent was obtained from each subject. This study was approved by the Yamaha Motor Ethics Committee.

The dose was set to ensure detection of serum astaxanthin based on the report of Odeberg,¹⁴ where the dose was set at 40 mg. All the subjects refrained from eating from previous evening to the morning of the study day. A single dose of astaxanthin (a total of 48 mg in the free form in 12 soft capsules, each containing 4 mg of astaxanthin) was administered with water before and after a meal in the morning. The capsule contained olive oil (78 mg), and vitamin E (20 mg), in addition to *Haematococcus* algal extract (52 mg). Astaxanthin in the *Haematococcus* algal extract (PURESTA, Yamaha Motor Co., Ltd., Japan) used in this study comprised 70% monoester form, 28% diester form, and less than 2% free form. The meal was sandwiches with egg and ham, a cup of yogurt, a glass of milk, and a few pieces of cut apple (about 420 kcal in total, protein 20 g, fat 21 g, carbohydrate 37 g). In the before-meal group, seven subjects including five males and two females took astaxanthin 2 h before the meal. In the after-meal group, 13 subjects including 10 males and three females took astaxanthin 10 min after the meal. Seven subjects of the above 13, all male, were smokers, and were classed as the smoking after-meal group; the remaining six subjects (non smoking) were classed as the after-meal group.

Blood sampling and sample preparation. A doctor's interview was conducted prior to blood sampling before (0) and 4, 6, 8, 24, 72, and 168 h after astaxanthin administration. All serum samples were prepared by centrifugation (4 °C, 2,500 rpm, 5 min) from the blood quickly after sampling, and were stored in a freezer (−20 °C) until analysis. Hematological, blood-biochemical, and astaxanthin analyses were also conducted on the 0 and 168-h blood samples. In hematological analysis, the numbers of platelets, and white and red blood cells, and the hemoglobin content, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentrations were analyzed. Aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, total cholesterol, HDL, LDL, triglyceride, uric acid, urea nitrogen, creatinine, and fasting blood glucose were analyzed in the blood-biochemical analysis.

Astaxanthin analysis. The astaxanthin concentration of serum samples prepared as described above was analyzed by the method described by Wada *et al.*¹⁷ Briefly, the procedure was as follows: Carotenoids were extracted from serum samples with ethanol in the presence of 2,6-dihydroxy toluene and an internal standard. β -apo-8'-carotenal was used as the internal standard. Extracts were concentrated, dissolved in chloroform/ethanol, and analyzed by high performance liquid chromatography. The samples were separated on Inertsil ODS-P 5 μ m (4.6 i.d. \times 250 mm) at 25 °C and eluted in gradient condition (methanol: tetrahydrofuran: H₂O=90.25:4.75:5 to 94.05:4.5:1). Carotenoid peaks were identified by comparison with authentic standards. The astaxanthin concentration was calculated from the peak area of the sample compared with the standard.

Data analysis. Pharmacokinetic parameters were estimated from the serum astaxanthin concentration with the Moment xls ver. 1.0 program (<http://www.pharm.kyoto-u.ac.jp/byoyaku/Kinetics/download.html#moment>).¹⁸ The area under the serum astaxanthin concentration-time curve (AUC₀₋₁₆₈) was calculated by the linear trapezoidal rule method to the maximum blood concentration (C_{max}), and by the log trapezoidal rule method after C_{max}. For analysis, the value calculated after subtracting the 0-h astaxanthin concentration for each subject from the concentration at each time point was used. The AUC from the last measured time point (168 h) to infinity (AUC_{0-∞}) was estimated on the basis of AUC_{C_{max}-168}.

Statistical analysis. A comparison of the means of each group, after confirmation of normality by Kolmogorow Smirnov D test, was conducted by the parametric method. Data without normality were analyzed non-parametric tests (Mann-Whitney). Statistical analyses were carried out using program SPSS version 15.0.

Results

Astaxanthin analysis

Typical HPLC chromatograms of serum before (a) and after (b) astaxanthin administration are shown in Fig. 1. The peak of astaxanthin was clearly separated from other carotenoids. In all of the serum samples analyzed, only the free form of astaxanthin was detected.

Adverse events

All of the subjects completed the study without any adverse events except for red coloration of feces within the test period. Hematological and bloodbiochemical data for the 0- and 168-h serum samples did not show any abnormalities or significant differences.

Serum astaxanthin concentration

The initial astaxanthin concentrations in the serum were different among the subjects (from 0.0 to 1.0 ng/ml). Average concentration-*vs.*-time curves for the before-meal and nonsmoking after-meal groups are shown in Fig. 2. The serum astaxanthin concentration of the before-meal group increased until the 8-h time point. It then gradually decreased until it reached the level before astaxanthin administration. In the after-meal group, the serum astaxanthin concentration gradually increased until the 24 h time point. After a slow decrease, as compared to the before-meal group, the serum astaxanthin concentration then also returned to the starting level after the 168 h time point. The average astaxanthin concentration of the after-meal group was 115 \pm 44.6 ng/ml at 24 h, and that of the before-meal group was 79.6 \pm 27.1 ng/ml at 8 h.

The average concentration-*vs.*-time curves for the smoking after-meal group are shown in Fig. 3. The serum astaxanthin concentration of the smoking after-meal group increased until the 6-h time point, and thereafter gradually decreased until it reached the starting level after 168-h. In the smoking after-meal group, the serum concentration of astaxanthin varied greatly from individual to individual. The average astaxanthin concentration was 135 \pm 123.5 ng/ml at 6 h.

Pharmacokinetic parameters

The serum astaxanthin pharmacokinetic parameters of the three groups are listed in Table 1. The time of maximum blood concentration (t_{max}) for the after-meal group (21.33 \pm 6.53 h) was about 3-fold that of the before-meal group (7.43 \pm 0.98 h). The value the smoking-after meal group (13.71 \pm 9.62 h) was midway between the other two groups. The smoking after-meal group (18.5 \pm 11.0 h) had the shortest t_{1/2}, at about 0.61 times that of the after-meal group ($p < 0.05$). The AUC₀₋₁₆₈ of the after-meal group (7,219 \pm 3,118 μ gh/l) was the highest, at 2.4 times the AUC₀₋₁₆₈ of the before-meal group (2,968 \pm 959 μ gh/l, $p < 0.05$). The AUC₀₋₁₆₈ of the smoking after-meal group varied largely from individual to individual. The mean value was 6,462 \pm 4,065 μ gh/l.

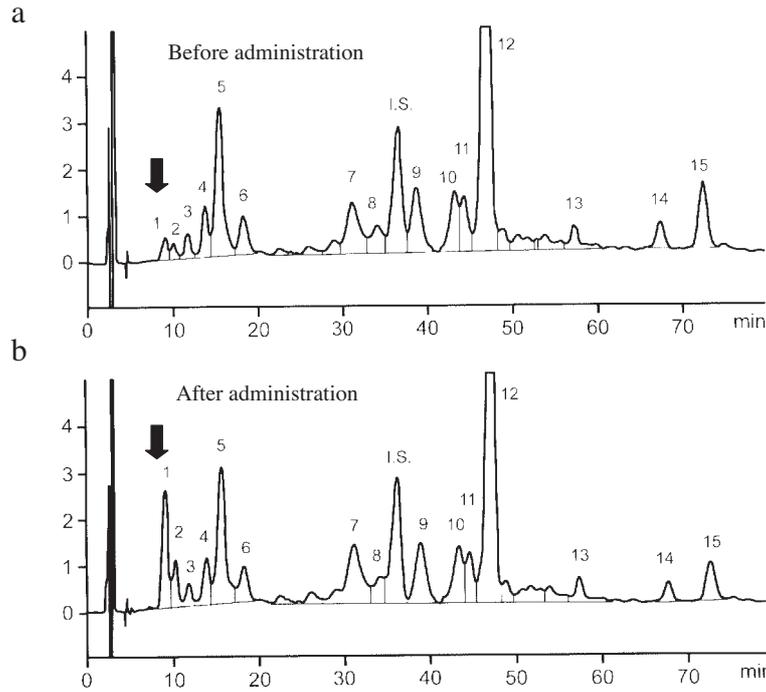


Fig. 1. Typical Chromatograms of Serum Carotenoids before (a) and after (b) Oral Administration of Astaxanthin. Arrows indicate peaks of astaxanthin. Peak identification: 1, astaxanthin; 2, ϵ,ϵ -carotene-3,3'-dione; 3, 3-hydroxy- ϵ,ϵ -carotene-3'-one; 4, 3-hydroxy- β,ϵ -carotene-3'-one; 5, lutein; 6, zeaxanthin; 7, anhydrolutein I; 8, anhydrolutein II; I. S.: 8'-apo- β -carotenal; 9, unidentified; 10, unidentified; 11, α -cryptoxanthin; 12, β -cryptoxanthin; 13, unidentified; 14, α -carotene; 15, β -carotene.

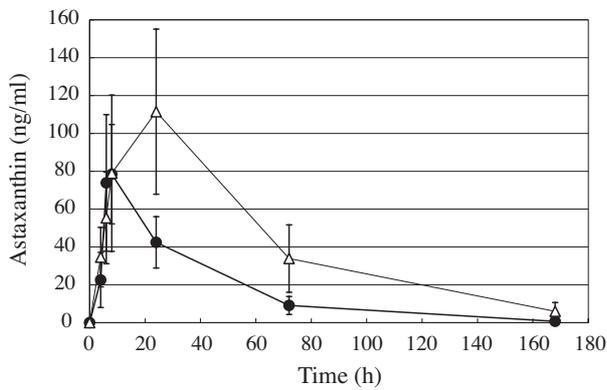


Fig. 2. Serum Concentration of Total Astaxanthin (ng/ml) after Oral Administration of 48 mg of Astaxanthin to Nonsmokers. Astaxanthin was given before (closed circle) and after (open triangle) the meal. Bars indicate standard deviation.

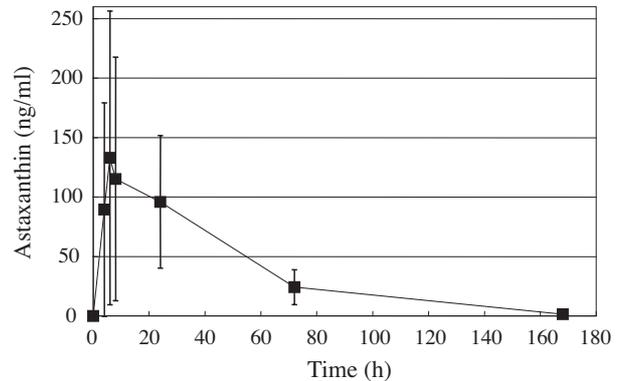


Fig. 3. Serum Concentration of Total Astaxanthin (ng/ml) after Oral Administration of 48 mg of Astaxanthin to Smokers after the Meal. Bars indicate standard deviation.

Table 1. Pharmacokinetic Parameters of Astaxanthin after Administration of a Single Oral Dose of 48 mg Astaxanthin before and after a Meal to Smokers and Non-Smokers (mean \pm standard deviation)

		Before meal administration (non-smokers)	After meal administration (non-smokers)	Statistical significance vs. before meal	After meal administration (smoker)	Statistical significance vs. after meal non-smoker
C_{max}	(ng/ml)	83.4 \pm 32.5	114.4 \pm 39.0	$p = 0.155$	149.7 \pm 111.7	$p = 0.886$
t_{max}	(h)	7.43 \pm 0.98	21.33 \pm 6.53	$p < 0.05$	13.71 \pm 9.62	$p = 0.082$
$t_{1/2}$	(h)	23.7 \pm 13.6	30.5 \pm 12.9	$p = 0.375$	18.5 \pm 11.0	$p < 0.05$
Elimination rate constant	(1/h)	0.05 \pm 0.03	0.03 \pm 0.03	$p = 0.307$	0.06 \pm 0.04	$p < 0.05$
$AUC_{(0-168)}$	($\mu\text{g h/l}$)	2,968 \pm 959	7,219 \pm 3,118	$p < 0.05$	6,462 \pm 4,065	$p = 0.718$
$AUC_{(0-\infty)}$	($\mu\text{g h/l}$)	2,996 \pm 969	7,526 \pm 3,300	$p < 0.05$	6,518 \pm 4,125	$p = 0.640$
Oral clearance	(l/h)	0.30 \pm 0.13	0.13 \pm 0.07	$p < 0.05$	0.15 \pm 0.06	$p = 0.553$
Volume of distribution	(l/kg)	9.01 \pm 3.20	5.88 \pm 2.04	$p = 0.064$	5.66 \pm 2.53	$p = 0.866$
$MRT_{(0-\infty)}$	(h)	31.6 \pm 7.8	48.5 \pm 10.2	$p < 0.01$	36.2 \pm 4.1	$p < 0.05$

MRT, mean residence time

Discussion

Olson has reported that xanthophyll esters must be hydrolyzed prior to absorption.¹⁹⁾ In this study, we used *Haematococcus* algal extract as the astaxanthin source. The majority of the astaxanthin of *Haematococcus* algal extract comprised ester forms. By contrast, the astaxanthin detected in serum samples was solely of the free form. Previous studies of Odeberg,¹⁵⁾ using astaxanthin from *Haematococcus*, and, Coral-Hinostriza,¹⁴⁾ using synthetic astaxanthin diesters, also found that only the free form was present in human blood. These results indicate that like other known xanthophylls, the ester forms of astaxanthin must be also hydrolyzed before absorption.

Smoking is known to affect the bioavailability of carotenoids.²⁰⁾ In our study, the $t_{1/2}$ of the smoking after-meal group was short as compared with the other two groups; it was about 60% of the nonsmoking after-meal group, despite showing a high C_{max} value. This indicates that smoking promotes astaxanthin elimination or metabolism. Galan *et al.* reported that people who smoke have lower blood levels of carotenoids than nonsmokers.²⁰⁾ The reason for these low levels might have been the shorter $t_{1/2}$ of carotenoids attributed to smoking. There have been studies on the relationship between oxidative stress and depletion of antioxidants. The abundance of free radicals in cigarette smoke might induce oxidative stress on both the respiratory and the circulatory system,^{21,22)} and the lower levels of antioxidants found in smokers might partly be a consequence of greater antioxidant depletion due to sustained smoke-related oxidant load.²³⁾ Thus blood astaxanthin might decrease comparatively quickly as a result of oxidative stress in smokers. A previous pharmacokinetics study by Coral-Hinostriza *et al.* showed that C_{max} was 0.28 mg/l and AUC_{∞} was 11 mg h/l when astaxanthin diesters were given as a dressing in a pasta salad.¹⁴⁾ The C_{max} of the after-meal group in our study was 114 μ g/l and AUC_{∞} was 7,500 μ g h/l. Thus the C_{max} and AUC_{∞} of Coral-Hinostriza were respectively about 2.5 times and 1.5 times the values in our study. Taking the amount of astaxanthin administered in their study into consideration (100 mg, about twice that in our study), these results appear reasonable. In this range of ingestion, astaxanthin show a slight dose-dependent absorption. Both the C_{max} (55–192 μ g/l) and AUC_{∞} (1,350–4,960 μ g h/l) of Odeberg *et al.*, in which homogenized *Haematococcus* dried cells were used as the astaxanthin source (40 mg in the free form), and the serum astaxanthin level of the after-meal group in our study were almost the same. On the other hand, Østerlie *et al.* reported a C_{max} of 1.3 mg/l and an AUC_{∞} of 42 mg h/l after free astaxanthin (100 mg) was administered as water-dispersible beadlets in olive oil.¹³⁾ The C_{max} and AUC_{∞} of that study were too high, about 11 and 5.6 times the respective values in our study. The reason for this difference is not clear, but, there are several possibilities, such as the difference in astaxanthin form (free form *vs.* ester), and the difference in formulation (water-dispersible beadlets *vs.* soft capsules containing oil). Odeberg *et al.* reported that ingestion of astaxanthin with emulsifier greatly affected both C_{max} and AUC_{∞} .¹⁵⁾ They found that the formulation caused a maximum

4-fold difference in AUC_{∞} . Based on these facts, the main reason for the different pharmacokinetics parameters in our study and Østerlie *et al.* might be difference in the preparation of astaxanthin.¹³⁾

In the case of β -carotene, absorption is markedly reduced when the intake of fat is low. The addition of a small quantity of fat to the diet greatly improves β -carotene absorption.¹⁶⁾ Judging by previous studies, diet must have a large effect on the absorption of carotenoids, but, there are no reports on the effects of dietary timing on the bioavailability of astaxanthin. In the before-meal group, the concentration of astaxanthin in the serum increased until the 8-h time point after administration and decreased relatively faster (Fig. 2) than in the after-meal group, where the concentration increased until 24-h and then gradually decreased. The bioavailability of astaxanthin in the after-meal group was significantly higher than in the before-meal group, and the AUC_{0-168} was about 2.4 times that of the before-meal group. The reason for the increment in absorption of carotenoids with dietary fat might be as follows: The presence of fat stimulates the excretion of bile. Sufficient amounts of bile help the dispersion of carotenoids in the digestive canal, which then leads to effective absorption of carotenoids.¹²⁾

Recently, much research on the health benefits of astaxanthin has been reported. The number of astaxanthin nutraceuticals on the market with potent properties against many symptoms has also been increasing, and most of the products are in the form of soft capsules. When *Haematococcus* astaxanthin is ingested as soft capsules, the timing of ingestion greatly affects astaxanthin bioavailability.

In conclusion, this study indicates that both the timing of diet and the smoking habit greatly affect the pharmacokinetic parameters of astaxanthin. The bioavailability (AUC_{0-168}) of the after-meal group was significantly higher than that of the before-meal group. The smoking habit also affected the pharmacokinetics greatly and reduced the $t_{1/2}$ of the serum astaxanthin concentration after astaxanthin administration.

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