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ORIGINAL ARTICLE

Blood Kinetics of A Self-emulsifying Formulation Containing Astaxanthin, Lutein/Zeaxanthin, and Water-soluble Powders： A Randomized, Double-blind, Crossover Study

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Abstract

Objective：Carotenoids, including astaxanthin and lutein/zeaxanthin, are beneficial for eye health；however, they are fat-soluble and have poor bioavailability. In order to improve absorption, we developed a self-emulsifying formulation containing astaxanthin and lutein/zeaxanthin along with water soluble powders. Our study aimed to compare the blood kinetics of self-emulsifying and control formulations containing astaxanthin and lutein/zeaxanthin.

Methods：Twenty-two participants were enrolled. The participants ingested the formulations while in a fasting state. Blood samples were collected from the participants for a total of 12 times between the time of ingestion to 48 hours after ingestion.

Results：The primary endpoint was the area under a curve（AUC）of blood concentrations of astaxanthin and lutein/zeaxanthin. Following a dietary survey, data from 19 enrolled participants were included in the analysis. An increase in blood concentrations of astaxanthin and lutein/zeaxanthin were observed in participants ingesting the self-emulsifying formulation compared to those ingesting the control formulation（AUC；$p<0.05$）.

Conclusions：Our findings indicate that our self-emulsifying formulation can enhance

the absorption of astaxanthin and lutein/zeaxanthin as compared to conventional formulation, despite the presence of water-soluble powders.

Introduction

Carotenoid intake is reportedly associated with the risk of developing breast[1], lung[2], and prostate cancer[3]. Given that humans cannot synthesize carotenoids internally, their distribution in the body depends on the amount ingested through the daily diet ; however, daily carotenoid intake via diet is limited. Therefore, supplementation is necessary to maintain high blood concentration and achieve targeted pharmacological effects. Among carotenoids, astaxanthin and lutein/zeaxanthin—belonging to the xanthophyll family—have been reported to have strong antioxidant properties and may be useful in preventing cardiovascular diseases (CVD)[4][5] and eye diseases[6][7]. Furthermore, astaxanthin intake reduces skin aging[8], and lutein/zeaxanthin intake reportedly contributes to brain health[9]. Astaxanthin is found in crustaceans, including shrimp and crab, and trout, such as salmon trout. Lutein/zeaxanthin is found in particularly high concentrations in green and yellow vegetables, such as kale and spinach. Recently, research findings have suggested that formulations containing a combination of these xanthophyll carotenoids may improve eye function and symptoms of eye diseases[10]. Therefore, our present study developed a formulation combining astaxanthin and lutein/zeaxanthin. However, because these carotenoids are fat-soluble and thus have low solubility in water, their dissolution in the digestive tract and micelle solubilization are rate-limiting factors, resulting in low absorption via oral ingestion. Moreover, carotenoids are chemically unstable and can degrade upon exposure to heat, light, and oxygen[11]. When designing a formulation containing carotenoids, it is necessary to consider physical stability and solubility/dispersibility for better absorption. Although conventional emulsifying formulations can improve dispersibility, their physical stability is affected by environmental factors such as temperature, pH, ionic strength, and dilution. Recently, stable self-emulsifying formulations have attracted attention because they naturally emulsify in the body[12]. Therefore, we developed a soft capsule-type self-emulsifying formulation containing astaxanthin and lutein/zeaxanthin to improve the dispersibility and absorption of these carotenoids.

Self-emulsifying technology is considered a useful approach for improving solubility and bioavailability ; however, the blood kinetics of self-emulsifying formulations containing both astaxanthin and lutein/zeaxanthin remain unknown. Thus, we aimed to investigate the effects of a self-emulsifying formulation containing astaxanthin and lutein/zeaxanthin on blood kinetics in humans by comparing it with a control formulation consisting of astaxanthin and lutein/zeaxanthin that was formulated using medium-chain fatty-acid triglycerides and beeswax, both of which are commonly used in soft capsules. Furthermore, we added water soluble powders to our self-emulsifying formulation consisting of astaxanthin and lutein/zeaxanthin. The

addition of water-soluble powders usually requires increased viscosity to prevent powder settling, which in turn makes it difficult to improve dispersibility. Therefore, we also explored formulation designs capable of maintaining the self-emulsifying properties of these carotenoids when combined with water-soluble powders. Finally, we investigated the absorbability of this novel self-emulsifying formulation combining both astaxanthin and lutein/zeaxanthin and water-soluble powders.

Ⅰ Materials and methods

1. Study design

This randomized, double-blind, crossover clinical trial consisted of two study periods, with patients taking either self-emulsifying or the control formulation in the first period, and vice versa in the second period. Considering the half-life of lutein, the washout period was set at 4 weeks. Participants in this study were Japanese men and women aged 20-59 years at the time of obtaining consent, with a body mass index (BMI) of ≥18.5 and ＜25.0 kg/m^2, recruited through a paid volunteer bank. The study was conducted between July 2025 and November 2025 at Watanabe Hospital, a medical corporation, using the participant management and research implementation systems of KANAMORI GIKEN Co., Ltd. (Kanagawa, Japan). All work related to the study, including participant guidance, consent acquisition, interview surveys, and research system management, was conducted by research physicians and staff affiliated with these institutions. APO PLUS STATION Co., Ltd. (Tokyo, Japan) conducted monitoring activities such as registration status, consent of study participants, checking compliance, dealing with adverse events among study participants, examination of records, study procedures including ethics committee procedures, and confirming the continuity of the implementation system. Participants who passed the screening test were randomly assigned in a 1：1 ratio to be administered either the self-emulsifying or the control formulations. The allocation was performed by Statcom Co., Ltd. (Tokyo, Japan), a third-party organization not involved in the study. The randomization officer signed and stamped a bound allocation decision form together with the participant allocation list. These documents, along with a sealed study formulation allocation record (identification code table), were stored in a locked cabinet until allocation disclosure.

During each study period, allocated participants were admitted to the hospital the day before ingesting the test formulation and remained hospitalized until 48 h after ingesting the test formulation. On the day of the test, the participants were instructed to skip breakfast and instead orally ingest (swallow without chewing) the two soft gel capsules that contained the test formulation with a glass of water. Participants were told to resume eating 4 h after ingestion. Venous blood samples, consisting of approximately 9 mL (3 mL of serum) per sample, were collected from the study participants before ingestion of the test formulation, and at 2, 4, 6, 8, 10, 12, 24, 28, 32, 36, and 48 h after ingestion during the first and second periods of the study. In total, 12 samples were taken from each participant.

2. Ethical considerations

This study was reviewed and approved

by the Watanabe Hospital Institutional Review Board（IRB approval number：22000038, protocol code：BA1-2502）and an independent third-party organization（IRB approval number：BA12502_F05_001_000_20250711_1, approval date：July 11, 2025）. Based on the tenets of the Declaration of Helsinki (revised in October 2024), this study was conducted in accordance with the "Ethical Guidelines for Medical and Biological Research Involving Human"（Notification No. 1 of the Ministry of Education, Culture, Sports, Science and Technology；the Ministry of Health, Labour and Welfare；and the Ministry of Economy, Trade and Industry, March 23, 2021 [partially revised March 27, 2023]）and the "Guidance on Ethical Guidelines for Medical and Biological Research Involving Human Subjects"（the Ministry of Education, Culture, Sports, Science and Technology；the Ministry of Health, Labour and Welfare；and the Ministry of Economy, Trade and Industry, April 16, 2021 [partially revised April 1, 2024]）. Before the study, the principal investigator explained the purpose and protocol of the study to the participants. Written informed consent was obtained from all participants. Only those who provided written consent were included in the study. Prior to the study initiation, the study details were registered with the University Hospital Medical Information Network Clinical Trial Registry System (clinical trial registration number：UMIN 000058436).

3. Screening test

For the screening test, the participants completed a self-reported demographic survey, underwent physical examination, and underwent blood tests. Height was measured using a scale, while weight was measured using a body composition scale. BMI was calculated by dividing the weight（kg）by the square of the height（m）. The body temperature was measured using a thermometer. Systolic and diastolic blood pressure and pulse rate were measured in the sitting position following 5-min rest duration using an automated blood pressure measuring device. Blood tests used for screening included measurements of white blood cells, red blood cells, hemoglobin, hematocrit, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, total protein, aspartate aminotransferase, glutamic acid transaminase, γ-glutamyl transferase, lactate dehydrogenase, alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin, total protein, blood urea nitrogen, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, urea nitrogen, creatinine, uric acid, creatine kinase, blood sugar, and hemoglobin A1c. All blood tests were performed at the LSI Medience Corporation laboratory（Tokyo, Japan）using standardized methods.

4. Study population

After screening tests were administered for each of the 44 participants who provided written consent. The inclusion criteria for participants were Japanese men and women aged 20-59 years at the time of consent, with a BMI of ≥ 18.5 and < 25.0 kg/m^2. Exclusion criteria included a history of serious illness or gastrointestinal surgery；diseases affecting gastric excretion or digestion/absorption；regular symptoms of indigestion, constipation, or diarrhea；abnormal renal/hepatic function, blood pressure, or glucose/lipid metabolism；

regular use of pharmaceuticals (including herbal medicines), quasi-drugs, or supplements ; regular consumption of foods for specified health benefits, functional foods, or other foods with potential functional properties that may affect test results and could not be discontinued during the study period ; heavy alcohol consumption ; current smoking or smoking cessation within the past year ; plans for overseas travel ; irregular work schedules or day–night shifts ; inability to maintain a restricted diet during the study period ; food allergies or risk of allergy related to the test foods or prescribed diets ; pregnancy, breastfeeding, or intention to become pregnant during the study period ; participation in another clinical trial within 1 month before consent or planned participation during the study period ; and any other condition deemed inappropriate by the investigator.

5. Dietary management

During the study period, participants were instructed to avoid excessive intake of general foods containing lutein and astaxanthin (green and yellow vegetables, eggs, shrimp, crabs, salmon, salmon roe, and other seafood), and to especially limit their intake as much as possible for 3 days prior to their visit (i.e., the overnight stay at the hospital). They were also asked to keep a record of their diet. Participants' dietary compliance was confirmed using a daily diary from 3 days prior to ingesting the test formulation.

Participants' dinner on the day before ingesting the test formulation consisted of a low-fat meal that comprised only of rice and miso soup. On the day of ingesting the test formulation, participants were asked to skip breakfast, consume the test formulation while in a fasting state, and eat lunch 4 h later. Meals were provided at 4, 10, 24, 28, and 34 h after ingestion and at the end of blood collection. Participants consumed only carotenoid-free meals until the end of the blood collection time period, which was 48 h after ingestion.

6. Test formulations

The self-emulsifying and control formulations consisted of two soft capsules per serving, and the active ingredients were astaxanthin (6 mg), lutein (10 mg), and zeaxanthin (2 mg). Astaxanthin and lutein/zeaxanthin oil preparations were derived from *Haematococcus* algae and marigold, respectively. Other ingredients included vitamins B2 and B6, eriodictyol-6-C-glucoside, antioxidants (catechins), edible processed fats and oils, gelatin, glycerin, cocoa color, and phytic acid. The self-emulsifying formulation was comprised of edible processed fats and oils as the base, which also worked as a thickener, together with glycerin fatty-acid esters as the emulsifiers. The control formulation was comprised of medium-chain fatty-acid triglycerides as the base oil and beeswax as the dispersant, both of which are commonly used in soft-capsule manufacturing. Appearance, capsule shape, taste, and color were adjusted to be equivalent to those of the self-emulsifying formulation capsules in order to ensure comparability and double blinding.

The self-emulsifying and control formulations were standardized to a viscosity suitable for soft-capsule filling. The final dosage forms met the specified disintegration criteria as prescribed by the Japanese Pharmacopoeia, confirming their appropriateness as oral soft-capsule preparations. The test formulations

were provided by FANCL Co., Ltd.(Yokohama, Japan).

7. Serum carotenoid analysis

The collected blood was immediately centrifuged (4℃ ; 3000 rpm ; 10 min) in the dark, the obtained serum was dispensed into Eppendorf tubes, and the tubes were stored at or below −70℃ until further analysis. To measure serum astaxanthin concentrations, 100 μL of serum sample was pretreated with 200 μL of ethanol/tetrahydrofuran (1 : 9), vortexed, and subsequently centrifuged (4℃ ; 3000 rpm ; 20 min). The supernatant was collected and analyzed using high-performance liquid chromatography (HPLC). For serum lutein/zeaxanthin measurements, 200 μL of α-tocopherol acetate was added to 200 μL of serum sample and vortexed. Next, 800 μL of n-hexane was added and vortexed, followed by centrifugation (5000 rpm ; 3 min). The hexane layer was collected and evaporated to dryness in vacuo. The residue was redissolved in 20 μL of ethyl acetate and 105 μL of ethanol and analyzed by HPLC. The serum carotenoid analysis was performed at the Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd. (Shizuoka, Japan).

8. Sample size calculation

Sample size was calculated based on our preliminary considerations. We predicted that the standard deviation of the difference between the test formulations would be 1.5 times the mean difference. To achieve a statistical power of 80%, a minimum of 20 participants was required. With an estimated 10% dropout rate, we ultimately enrolled 22 participants.

9. Data management and statistical analysis

Before the key opening, we reviewed the data handling method and conducted case studies. Subsequently, the key opening was carried out after the data was locked, and approval was obtained from the principal investigator. Case reviews were considered based on dietary records from 3 days prior to ingesting the test formulation, and participants who had consumed food containing carotenoids were excluded from the study as they were in violation of compliance requirements. For serum astaxanthin concentrations, values below the detection limit of 3 ng/mL were assigned a value of 0.

For the physical examination items investigated during screening, we showed the 25%, median, and 75% values as quartile. The primary endpoint was the area under the curve (AUC) of the blood astaxanthin and lutein/zeaxanthin concentrations. The AUC of the blood concentration was calculated using the trapezoidal method as part of non-compartmental analysis. The least squares mean ± standard error, and p-value for the primary endpoint were calculated using linear mixed models with the participant ID as a random effect and the test period and sequence and baseline (0 h) values as a fixed effect. This analysis allowed us to adjust for test period, sequence and baseline values, and confirmed carryover effects. For sensitivity analysis of the primary endpoint, the same analysis was performed on the full analysis set, which included all the participants. As a precaution, we also checked the statistical analysis by logarithmically transforming the AUC values. The secondary endpoints were blood carotenoid levels at each time point and the maximum blood concentration (C max). For the secondary outcome measures, Wilcoxon signed-rank test was performed on the measured values using a non-parametric

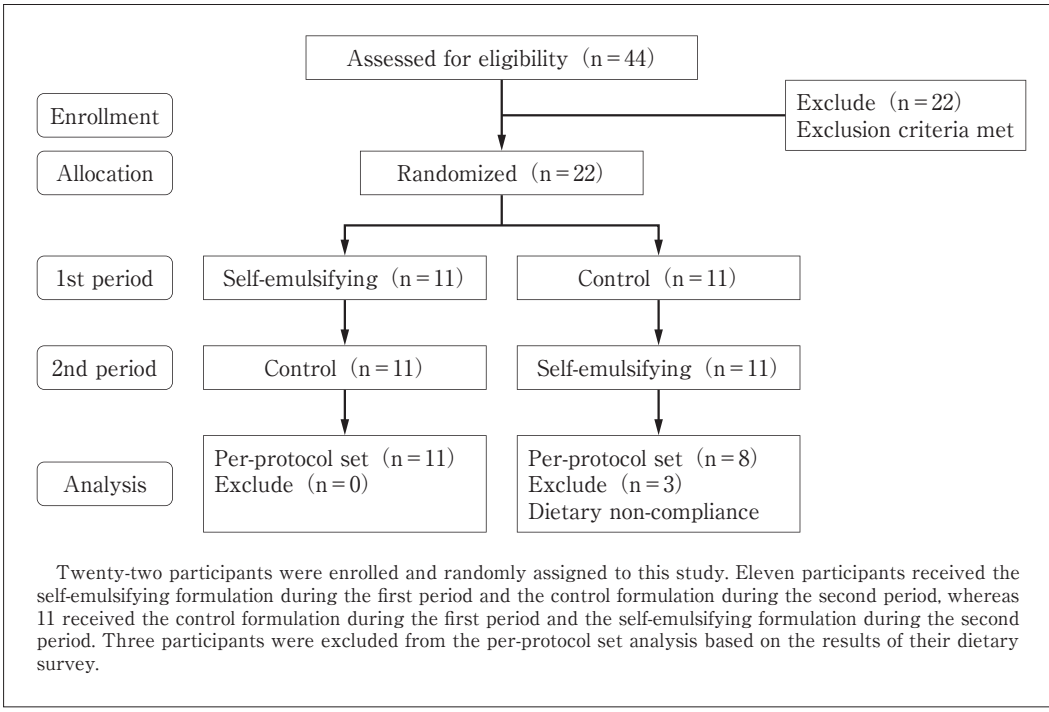

Figure 1　Flow diagram of participants

method. The measured values for each time point are also shown as the mean±standard deviation. Statistical significance was set at $p<0.05$ in a two-tailed test. All statistical analyses were performed using JMP® Ver. 18 and 19 software（SAS Institute, Inc., Cary, NC, USA）. Data management before key opening was performed by APO PLUS STATION Co., Ltd., and statistical analysis was performed by FANCL Co., Ltd.

Ⅱ Results

A flow diagram of the participants is shown in **Figure 1**. Twenty-two participants were enrolled and randomly assigned to the study. Eleven participants received the self-emulsifying formulation during the first period and the control formulation during the second period, whereas 11 participants received control formulation during the first period and the self-emulsifying formulation during the second period. All participants completed the study；however, 3 men were excluded from the per-protocol set analysis population based on the results of their dietary survey conducted 3 days prior to ingestion of the test formulation. No adverse events were observed. The clinical characteristics of the study participants are summarized in **Table 1**. The sex distribution of the study participants in the per-protocol set was：11 women and 8 men. Additionally, the blood test results were normal, and all participants were considered healthy.

Tables 2 and **3** show the mean and standard deviation of the time points and C max for blood concentrations of astaxanthin and lutein/

Table 1 Clinical characteristics

		Women（N＝11）			Men（N＝8）		
Variables	unit	25%	Median	75%	25%	Median	75%
Age	years	33.0	35.0	47.0	36.3	44.5	49.3
BMI	kg/m^2	20.2	21.3	23.1	21.8	22.7	24.2
SBP	mmHg	100.0	104.0	110.0	116.3	125.0	129.3
DBP	mmHg	62.0	66.0	70.0	68.8	75.0	79.8
Pulse rate	bpm	74.0	78.0	84.0	65.0	69.0	90.8

Eleven women and 8 men were included in the analysis. The median and 25%-75% of the physical examination results of the study participants are provided in this table.

Table 2 The time profile and C max of blood astaxanthin concentrations

	Self-emulsifying	Control	
Hours	Mean ± S.D.	Mean ± S.D.	p value
0	2.6 ± 3.22	1.7 ± 2.42	0.178
2	2.5 ± 2.87	1.5 ± 2.12	0.161
4	3.4 ± 3.06	1.8 ± 2.64	0.051
6	8.2 ± 4.50	1.7 ± 2.08	＜0.01
8	12.6 ± 6.69	1.8 ± 2.25	＜0.01
10	14.2 ± 7.30	2.5 ± 2.65	＜0.01
12	20.4 ± 10.36	3.2 ± 3.28	＜0.01
24	13.7 ± 7.29	2.1 ± 2.45	＜0.01
28	13.5 ± 6.96	1.9 ± 2.46	＜0.01
32	11.9 ± 6.31	1.8 ± 2.23	＜0.01
36	10.3 ± 5.25	1.1 ± 1.94	＜0.01
48	6.5 ± 4.06	0.7 ± 1.80	＜0.01
C max	20.4 ± 10.28	3.9 ± 3.20	＜0.01

Blood astaxanthin concentrations（ng/mL）
P values were calculated using Wilcoxon signed-rank test.

Nineteen participants were included in the analysis. The time points and C max for blood concentrations of astaxanthin show the mean and standard deviation.

zeaxanthin, respectively. Blood astaxanthin concentrations were significantly higher after ingestion of the self-emulsifying formulation as compared to after ingestion of the control formulation from 6 h to 48 h after ingestion of the respective formulation, as was the C max. On the other hand, there were no significant differences in blood lutein/

Table 3 The time profile and C max of blood lutein/zeaxanthin concentrations

	Self-emulsifying	Control	
Hours	Mean ± S.D.	Mean ± S.D.	p value
0	23.6 ± 7.75	23.6 ± 7.22	0.883
2	23.9 ± 8.38	23.8 ± 7.52	0.709
4	23.2 ± 7.81	24.0 ± 7.98	0.746
6	22.8 ± 7.31	23.2 ± 7.36	0.993
8	23.0 ± 7.34	22.8 ± 7.17	0.687
10	23.5 ± 7.39	22.7 ± 6.91	0.462
12	23.3 ± 7.08	21.9 ± 6.59	0.358
24	25.2 ± 8.02	22.8 ± 7.33	0.145
28	24.5 ± 7.67	22.1 ± 7.18	0.104
32	23.5 ± 7.14	21.6 ± 7.09	0.178
36	22.8 ± 6.92	21.1 ± 6.94	0.199
48	22.8 ± 7.20	21.3 ± 6.89	0.471
C max	26.3 ± 7.83	25.1 ± 7.61	0.496

Blood lutein/zeaxanthin concentrations (μg/dL)
P values were calculated using Wilcoxon signed-rank test.

Nineteen participants were included in the analysis. The time points and C max for blood concentrations of lutein/zeaxanthin show the mean and standard deviation.

zeaxanthin concentrations and C max at each measurement point. **Figure 2** shows the time profiles and AUC of the measured blood astaxanthin concentrations. The least squares mean and the corresponding standard error of the AUC for blood astaxanthin concentrations analyzed with a linear mixed model were 534.1 ± 40.65 (ng/mL・h) for the self-emulsifying formulation and 109.0 ± 40.51 (ng/mL・h) for the control formulation. The AUC of blood astaxanthin concentrations was significantly higher after ingestion of the self-emulsifying formulation as compared to after ingestion of the control formulation ($p<0.05$). **Figure 3** shows the time profiles and AUC of the measured blood lutein/zeaxanthin concentrations. The least squares mean and the corresponding standard error of the AUC for blood lutein/zeaxanthin concentrations analyzed with a linear mixed model were 1131.5 ± 18.02 (μg/dL・h) for the self-emulsifying formulation and 1064.7 ± 17.98 (μg/dL・h) for the control formulation. The AUC of blood lutein/zeaxanthin concentrations was significantly higher after ingestion of the self-emulsifying formulation as compared to after ingestion of the control formulation ($p<0.05$).

For the sensitivity analysis of the primary outcome measure, the same analysis was

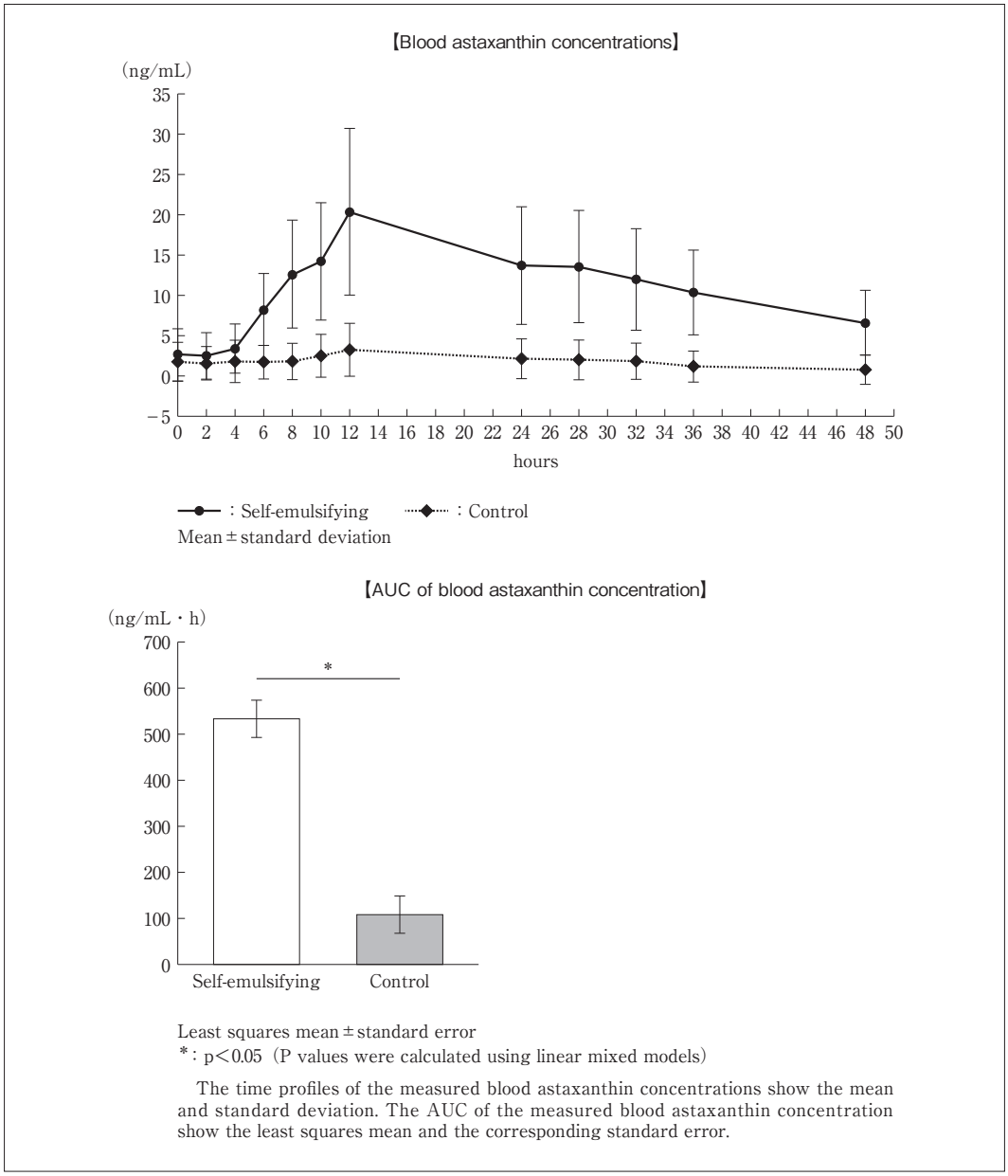

Figure 2　The time profile and AUC of blood astaxanthin concentrations

performed on the full analysis set including all participants and on logarithmically transformed values, and the results were confirmed to be similar. These AUCs were also significantly higher after ingestion of the self-emulsifying formulation as compared to after ingestion of the control formulation ($p<0.05$). Furthermore, no significant differences in the sequence (carry over) effect were observed in the AUC of blood astaxanthin and lutein/zeaxanthin concentrations. Incidentally, since no increase

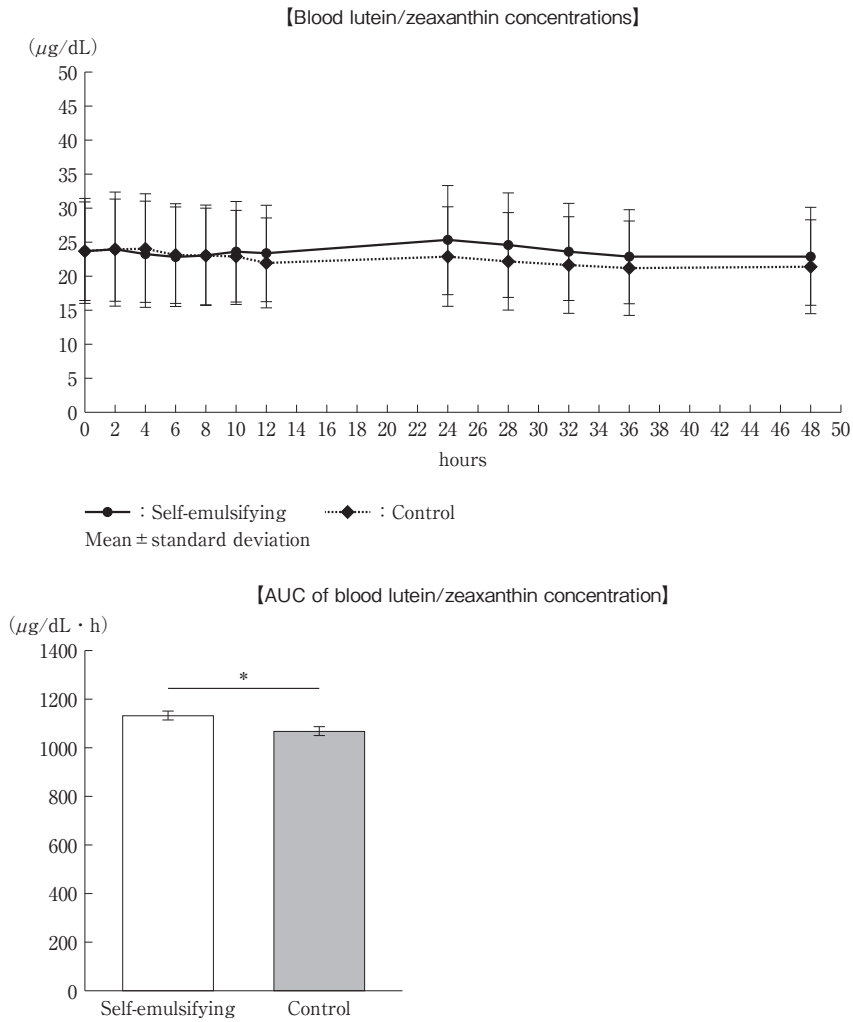

Figure 3 The time profile and AUC of blood lutein/zeaxanthin concentrations

in blood astaxanthin and lutein/zeaxanthin concentrations was observed in some cases up to 48 h after ingestion of the test formulation, T max was not calculated.

Ⅲ Discussion

This randomized, double-blind, crossover trial examined the effects of a self-emulsifying formulation containing astaxanthin and lutein/zeaxanthin combined with water-

soluble powders on the blood kinetics of astaxanthin and lutein/zeaxanthin in healthy participants. The results of this study confirmed an increase in blood astaxanthin and lutein/zeaxanthin levels after ingestion of the self-emulsifying formulation compared with the control formulation. Notably, both astaxanthin and lutein/zeaxanthin are fat-soluble carotenoids belonging to the xanthophyll family, and these carotenoids have low bioavailability. However, self-emulsifying formulations facilitate fine emulsion formation after ingestion, thereby improving the dissolution and potential bioavailability of astaxanthin and lutein/zeaxanthin in the gastrointestinal tract. Both self-emulsifying and control formulations met the Japanese Pharmacopoeia disintegration requirements, making it unlikely that differences in disintegration were the primary contributing factors. Therefore, the improved absorption observed in this study was possibly due to improved emulsification and dispersibility in the digestive tract. Our findings suggest the usefulness of self-emulsifying formulations using a combination of edible processed fats, oils, and glycerin fatty-acid esters to improve the absorption of fat-soluble carotenoids.

The test formulation used in this study also contained water-soluble components, including eriodictyol-6-C-glucoside, to further improve eye health[13]. These results suggest that the self-emulsifying formulation used in this study was effective in improving the absorption of astaxanthin and lutein/zeaxanthin, despite the presence of water-soluble powders. It is generally physically difficult to combine fat-soluble and water-soluble powders in the same formulation ; however, the self-emulsification formulation used in this study suggests that the absorption of carotenoids is improved, even when combined with water-soluble powders. Most conventional self-emulsifying formulations consist of fat-soluble ingredients alone, and there are limited reports on self-emulsifying formulations that incorporate water-soluble powders. In test formulations containing water-soluble powders in the liquid fill, it is necessary to increase the viscosity to prevent powder sedimentation, which may reduce dispersibility. In addition, interactions with the soft-capsule shell limit the choice of usable oils and emulsifiers ; therefore, a formulation that does not compromise shell plasticity is required. Consequently, designing self-emulsifying soft capsules containing water-soluble components is generally challenging. In our study, we optimized the self-emulsifying formulation by using edible processed fats and oils as the base, which also worked as a thickener, together with glycerin fatty-acid esters as the emulsifier. This combination helped prevent the water-soluble powders from settling in the formulation and allowed the system to self-emulsify when in contact with gastrointestinal fluid. The significance of this finding is that it confirmed that the self-emulsifying formulations used in this study, which simultaneously combine fat-soluble carotenoids and water-soluble powders, improve absorption of carotenoids. The improved bioavailability observed in this study may enhance the potential use of other self-emulsifying formulations containing both fat-soluble and water-soluble powders.

In this study, blood astaxanthin concentrations increased noticeably after

ingestion of the self-emulsifying formulation compared with the control formulation. Carotenoids are fat-soluble compounds absorbed in a manner similar to lipids and transported to the liver via the lymphatic system. Carotenoids mix with bile acids in the small intestine and form micelles after emulsification[14]. Carotenoid-containing micelles are incorporated into chylomicrons by intestinal mucosal cells. They subsequently enter the bloodstream via the lymphatic vessels. Astaxanthin and lutein/zeaxanthin are both polar carotenoids with similar molecular weights ; however, their structural differences suggest that they may have different absorption profiles. A study comparing xanthophyll carotenoids using a Caco-2 *in vitro* model has reported that astaxanthin was absorbed more efficiently compared to other carotenoids[15]. Additionally, while blood lutein/zeaxanthin levels increased, they increased at a slower rate than that of blood astaxanthin levels, and there were differences in their blood profiles. Previous studies have reported similar results, suggesting that lutein and zeaxanthin are continuously absorbed over a long period of time by binding to the gastrointestinal mucosa and awaiting transport via chylomicron formation[16].

As the absorption of carotenoids is reportedly affected by dietary lipids and bile acid secretion, the test formulation was administered while participants were in a fasting state to examine the effects of spontaneous emulsifying and dispersing properties of the self-emulsifying formulation on the absorption process under conditions that minimize the influence of exogenous factors. The participants continued fasting for 4 h after ingestion, which likely accounts for the minimal increases in blood astaxanthin and lutein/zeaxanthin concentrations observed after ingestion of the control formulation. Notably, blood astaxanthin levels showed a very slight increase at 4 h after ingestion but increased at 6 h after ingestion, likely due to bile acid secretion stimulated by the first meal 4 h after ingestion. Self-emulsifying formulations are useful for assisting and accelerating emulsification in the digestive tract. However, the subsequent micellization process may be influenced by the action of bile acids in the body. Conversely, it has been reported that micellar formulations of astaxanthin increase blood astaxanthin levels within a shorter period[17].

Moreover, the standard deviation of blood lutein/zeaxanthin concentrations was large after ingesting the test formulation, likely due to influence of carotenoid absorption in the diet before the ingestion of the test formulations. The participants were completely prohibited from consuming any food containing carotenoids in the evening meal on the day prior to the test formulation until the completion of all blood sampling. Since complete dietary restriction was not possible until the day before ingesting the test formulation, participants were asked to restrict their diet throughout the study and particularly from 3 days prior to the test formulation intake. However, since the prescribed diet restriction was not supervised, we believe that the restrictions were insufficient. Because the estimated half-life of lutein/zeaxanthin is approximately 5 days[18], strict dietary restrictions during the blood sampling period may have reduced the concentration of lutein absorbed into the

body from daily meals before the ingestion of the test formulations. A more rigorous dietary restriction observation period is required to more accurately assess the profile of blood lutein/zeaxanthin levels.

Efficiently increasing the blood levels of xanthophyll carotenoids, such as astaxanthin and lutein/zeaxanthin, is of great clinical importance. As blood lutein/zeaxanthin levels are associated with the cardiovascular-kidney-metabolic status in the general adult population and mortality risk for CVD in adults with hypertension[19)20)], improving their levels may be beneficial for cardiometabolic health[21)]. Moreover, blood xanthophyll carotenoid concentrations are associated with macular pigment optical density (MPOD)[22)23)], and studies indicate that lutein/zeaxanthin intake enhances MPOD and improves visual function in patients with age-related macular degeneration in a dose-dependent manner[24)25)]. Furthermore, studies suggest that astaxanthin is essential for treating eye diseases in a dose-dependent manner[26)], and it has been reported that taking astaxanthin may improve eye fatigue[27)]. Thus, our findings, in line with previous findings, suggest that increasing blood levels of xanthophyll carotenoids, such as astaxanthin and lutein/zeaxanthin, using self-emulsifying formulations may effectively prevent the progression of eye disease and promote eye health.

This study has some limitations. First, the blood sampling period and time points were insufficient to completely confirm the blood lutein/zeaxanthin concentration profiles. Although the half-life of blood lutein/zeaxanthin is approximately 5 days, blood sampling was limited to 48 h after ingestion in this study. Furthermore, owing to ethical considerations, the number of blood samples taken in this study was kept to a minimum, while still being sufficient to confirm the blood astaxanthin concentration profile. Therefore, it is possible that blood lutein/zeaxanthin profiles could not be as accurately determined as they could have been if the blood collection window was extended. Second, the test formulation was administered in a fasting state to eliminate the effects of food. Given that supplements are often consumed in various states, clinical trials targeting their administration before or after meals are also necessary. Third, the supplement used in this study was a combination of astaxanthin and lutein/zeaxanthin. Co-administration of multiple carotenoids may result in synergistic effects[28)], and the results may differ from those reported in this study if individual components were administered as self-emulsifying formulations. Finally, the clinical significance of long-term administration of the self-emulsifying formulation used in this study is unknown. Consuming various carotenoids, primarily through food, can contribute to health promotion[29)] ; however, further research is needed to determine the effects of long-term intake of specific carotenoids as supplements.

Conclusions

The results of our study suggest that a self-emulsifying formulation using a combination of edible processed fats and oils and glycerin fatty-acid ester could improve the absorption of astaxanthin and lutein/zeaxanthin, even when they contain water-soluble powders. Future clinical evaluations

at various intake time points and long-term trials will be necessary to validate the effectiveness of self-emulsifying formulations.

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Conflicts of interest

M.T., A.H., T.A., S.T., Y.N., and Y.I. are employees of FANCL. FANCL has applied for a patent for a self-emulsifying formulation, wherein A.H. is named as the inventor. T.W. declares that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The authors declare that this study received funding from FANCL Co. Ltd. The funders played a role in the decision to submit this study for publication.

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原著

アスタキサンチン，ルテイン・ゼアキサンチンおよび水溶性粉末を含む自己乳化製剤の血中動態
――無作為化二重盲検クロスオーバー試験――

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要　旨

目的：アスタキサンチンやルテイン・ゼアキサンチンなどのカロテノイドは目の健康に有益であるが，脂溶性であるため生体内利用能が低い。そこで，吸収性を向上させるためにアスタキサンチンとルテイン・ゼアキサンチンを含む自己乳化製剤を開発した。なお，試験製剤には水溶性粉末も含まれていた。本研究はアスタキサンチン，ルテイン・ゼアキサンチンおよび水溶性粉末を含む自己乳化製剤と対照製剤の血中動態を比較することを目的とした。

方法：本試験には合計22名が参加し，試験製剤を空腹時に摂取した。各来院時に試験製剤摂取前から摂取後48時間までに計12回，血液サンプルを採取した。

結果：主要評価項目はアスタキサンチンおよびルテイン・ゼアキサンチンの血中濃度の曲線下面積とした。食事調査の結果から19名の参加者が解析対象となった。自己乳化製剤摂取後，対照製剤と比較してアスタキサンチンおよびルテイン・ゼアキサンチンの血中濃度の曲線下面積の上昇が認められた（$p<0.05$）。

結論：本研究の結果は，水溶性粉末も含まれる自己乳化製剤摂取において，アスタキサンチンおよびルテイン・ゼアキサンチンの吸収を促進する可能性を示唆している。

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