

FJ46703

D 40613

Food Sci. Technol. Res., 15 (4), 449–452, 2009

Note

Bioavailability of Lutein in *Chlorella* Powder: A Single Ingestion of *Chlorella* Powder Raises Serum Lutein Concentrations in Healthy Human Volunteers

Shinya SHIBATA* and Kazuhito HAYAKAWA

Yakult Central Institute for Microbiological Research., 1796 Yaho, Kunitachi, Tokyo 186-8650, Japan

Received December 15, 2008; Accepted March 13, 2009

Chlorella powder (CP), a dietary supplement made from a green alga, contains abundant lutein (2.8 mg/g), but the bioavailability of lutein in CP has not been evaluated. In the present study, we assessed the effect of a single ingestion of CP (3 or 6 g) in tablet form on serum lutein concentrations in humans ($n = 21$). At 1 d, serum lutein concentrations increased from baseline values by 34% (25.6 nM/mg-lutein intake) after 3 g CP and by 66% (21.4 nM/mg-lutein intake) after 6 g CP. These results suggest that lutein in CP is highly bioavailable.

Keywords: bioavailability, *Chlorella*, green alga, lutein

Introduction

Lutein is the most abundant carotenoid in nature, and green leafy vegetables are the most popular source of dietary lutein. Lutein and zeaxanthin exist in the lens of the eye and the macular region of the retina (Krinsky *et al.*, 2003). Many studies have indicated that the consumption of lutein- and zeaxanthin-rich food is associated with a reduced risk of age-related macular degeneration (AMD) and cataracts (Moeller *et al.*, 2000; Seddon *et al.*, 1994). Seddon *et al.* (1994) have reported that daily intake of 6 mg of lutein was associated with a reduced risk of AMD. Richer *et al.* (2004) reported that a daily intake of 10 mg of lutein improved the vision of AMD patients.

During the absorption of carotenoids from food, the carotenoids are initially transferred from the intact food matrix into emulsified lipid droplets. Subsequently, they are mixed into micelles, as are other dietary lipids, and then absorbed by the small intestine. These processes are influenced by various characteristics of the food; for example, fat facilitates the absorption of carotenoids, whereas dietary fiber inhibits it (van het Hof *et al.*, 2000). Thus, to consume adequate amounts of vegetables and fruits in order to ensure sufficient intake of carotenoids, we must determine the bioavailability of carotenoids in food, not just the carotenoid content of the

food.

Chlorella species are unicellular green algae with high photosynthetic efficiency, and are rich in protein, essential nutrients, and plant pigments. Some *Chlorella* species are widely used as a dietary supplement in many countries, especially in East Asia (e.g., Japan, Taiwan, Korea). *Chlorella regularis* has been reported to potentially prevent lifestyle-related diseases (Shibata *et al.*, 2003; Shibata and Sansawa, 2006; Shibata *et al.*, 2007). *Chlorella* powder (CP) made from *C. regularis* contains large amounts of carotenoids such as lutein, α -carotene, and β -carotene (approx. 2.5 mg/g, 250 μ g/g, and 500 μ g/g, respectively). Thus, the ingestion of CP could improve low dietary intake of lutein due to low consumption of vegetables and fruits, because only a few grams of CP can supply 6 to 10 mg of lutein. However, to our knowledge, there is no available information about carotenoid bioavailability of CP.

The aim of the present study was to determine the bioavailability of lutein from CP in humans. We assessed the effect of a single ingestion of CP (3 or 6 g) in tablet form on serum lutein concentrations in humans.

Materials and Methods

Foods and reagents We purchased CP from Nihon Chlorella Co. (present name, Yakult Pharmaceutical Industry Co., Ltd., Kunitachi, Tokyo, Japan). The CP was a spray-dried powder of *C. regularis* cells cultivated heterotrophically

*To whom correspondence should be addressed.

E-mail: shinya-shibata@yakult.co.jp

cally in sterilized fermenters. CP in tablet form (200 mg) was manufactured using a tableting machine, vacuum-packed, and stored at -20°C until ingestion. Standards used for the carotenoids (lutein, α -carotene, and β -carotene) in the HPLC analysis were purchased from DHI (Hoersholm, Denmark). The internal standard (β -apo-8'-carotenal) for HPLC analysis was purchased from Sigma-Aldrich (St. Louis, MO).

Study design The total experiment period was 11 days (from -7 d to 3 d). During the experiment, subjects maintained their usual lifestyle (eating habits, exercise, sleep, and daily work); however, they were instructed not to take vitamin supplement tablets, drink nutritional supplements, or drink vegetable juice during the study, because these products potentially contain large amounts of lutein. Before ingestion of the CP tablets, subjects ($n = 21$) were divided into three groups with the same initial serum lutein levels (-7 d), BMI, and age (Table 1). A fasting blood sample was collected in the morning (08:00 to 09:00) at -7 , 0, 1, 2, and 3 d. On the morning of 0 d, after the blood sampling, subjects each took a single CP dose (0, 3, or 6 g) in tablet form with bread (40 g), olive oil (10 g), and water (280 mL). During the experiment, the subjects kept food diaries regarding their consumption of vegetables, fruits, and eggs to let us estimate their dietary intake of lutein. The carotenoid content of the foods was estimated from data in release 20 of the USDA National Nutrient Database for Standard Reference (i). The carotenoid content of the 3 and 6 g tablets was (respectively) 8.6 and 17.3 mg of lutein, 1.3 and 2.6 mg of α -carotene, and 2.1 and 4.2 mg of β -carotene. The study protocol was approved by the Human Research Ethics Committee of the Yakult Central Institute and complied with the Helsinki Declaration. Written informed consent was obtained from all subjects.

Analysis of serum carotenoid concentrations Blood samples (5 to 10 mL) were drawn from a forearm vein, and serum was separated from the blood cells by centrifugation. Serum was stored at -80°C until analysis. Sample prepara-

tion and serum carotenoid analysis using HPLC was carried out by the method of Nakamura *et al.* (2006). The coefficients of variation (CV) for serum lutein concentrations for the same sample run ($n = 6$), which were all conducted on the same day, were 4.0%.

Statistical analysis Measurements were expressed as mean \pm SEM. Data were analyzed using ANOVA, but the amount of lutein intake from green leafy vegetables, fruits, and egg yolk during the study was assessed by nonparametric test (Man Whitney *U* test). Tukey's multiple-comparison test was used to compare serum lutein concentrations and changes in serum lutein concentrations from the baseline values (0 d). Differences were considered to be significant at $P < 0.05$. Statistical analyses were performed using version 8.2 of the SAS software (SAS Institute Inc., Cary, NC).

Results

Throughout the study, there were no significant differences in daily lutein intake from green leafy vegetables, fruits, and egg yolks among the groups (data not shown). Serum lutein concentrations were significantly different among the groups at 1 d (Table 2). At 1 d, serum lutein concentrations in the 6-g CP group were significantly higher than those in the control group (Table 2). The significant changes in serum lutein concentrations at 1 d from the baseline levels at 0 d were $0.22 \pm 0.06 \mu\text{M}$ (34% increase) in the 3-g CP group and $0.37 \pm 0.04 \mu\text{M}$ (66% increase) in the 6-g CP group (Table 2). Significant increases in serum lutein concentrations from the baseline values were also observed at 2 d (3-g CP and 6-g CP groups) and at 3 d (6-g CP group). Increases in serum lutein concentrations at 1 d per mg of ingested lutein in CP were 25.6 nM/mg-lutein intake in the 3-g CP group and 21.4 nM/mg-lutein intake in the 6-g CP group.

Discussion

Our results clearly show that a single ingestion of 3 or 6 g of CP in tablet form raised serum lutein concentrations in

Table 1. Initial characteristics of the subjects.

	Dose of <i>Chlorella</i> (g)			ANOVA (<i>P</i> value)
	0 ($n = 6$)	3 ($n = 7$)	6 ($n = 8$)	
Age (year)	38.0 ± 4.5	40.4 ± 4.9	35.9 ± 4.3	0.770
Body weight (kg)	71.0 ± 5.3	66.6 ± 1.2	67.3 ± 2.5	0.612
Height (cm)	169.7 ± 2.2	172.0 ± 2.9	170.5 ± 2.5	0.831
BMI (kg/m^2)	24.6 ± 1.7	22.6 ± 0.6	23.2 ± 0.7	0.420
Serum lutein concentration at -7 d (μM)	0.60 ± 0.09	0.65 ± 0.05	0.55 ± 0.06	0.623

Values are means \pm SEM.

Table 2. Serum lutein concentrations and changes in serum lutein concentrations from the baseline values (0 d) before and after ingestion of each dose of Chlorella (0, 3, 6 g) in healthy adult men.

Time (d)		Dose of Chlorella (g)			ANOVA P value
		0	3	6	
-7	lutein (μM)	0.60 \pm 0.09	0.65 \pm 0.05	0.55 \pm 0.06	0.623
	change (μM) ^d	0.02 \pm 0.03	-0.01 \pm 0.02	-0.01 \pm 0.05	0.881
	change (%) ^e	(103)	(100)	(98)	
0	lutein (μM)	0.58 \pm 0.08	0.65 \pm 0.03	0.56 \pm 0.10	0.700
	change (μM)	—	—	—	—
	change (%)	(100)	(100)	(100)	
1	lutein (μM)	0.60 \pm 0.08a	0.87 \pm 0.05ab	0.93 \pm 0.11b	0.048
	change (μM)	0.02 \pm 0.01a	0.22 \pm 0.06b	0.37 \pm 0.04c	<0.001
	change (%)	(103)	(134)	(166)	
2	lutein (μM)	0.63 \pm 0.08	0.85 \pm 0.04	0.85 \pm 0.11	0.166
	change (μM)	0.05 \pm 0.02a	0.20 \pm 0.04b	0.28 \pm 0.04b	<0.001
	change (%)	(109)	(131)	(152)	
3	lutein (μM)	0.66 \pm 0.08	0.81 \pm 0.05	0.85 \pm 0.10	0.281
	change (μM)	0.08 \pm 0.03a	0.16 \pm 0.04ab	0.29 \pm 0.04b	<0.001
	change (%)	(114)	(125)	(152)	

Values are means \pm SEM.

a,b,c; Means in rows followed by different letters were significantly different by Tukey's multiple comparison test ($p < 0.05$).

d; Changes in serum lutein concentrations from the baseline values (0 d).

e; Values in parenthesis are the relative mean serum lutein concentrations (%) to the mean values at 0 d.

healthy humans. The resultant serum lutein concentrations in the present study were directly proportional to the amount of ingested CP (34% in the 3 g CP group and 66% in the 6 g CP group). Food diaries recorded by the subjects suggested that daily lutein intake from vegetables, fruits, and egg yolks was smaller than 1.0 mg (data not shown) by all subjects on days 0 and 1, thus the increases in serum lutein concentrations in the two CP groups were caused almost entirely by the lutein provided in the CP.

Reported values of serum or plasma responses to consumption of lutein from vegetables and supplements differed among studies (Riso *et al.*, 2003; Chung *et al.*, 2004; Granado *et al.*, 2006). For example, lutein in supplements was more highly bioavailable than the lutein in cooked mixed vegetables in one study (van het Hof *et al.*, 1999). In contrast, Chung *et al.* (2004) reported that the bioavailability of lutein from supplements and spinach were similar. This inconsistency may result from differences in study design, such as different washout periods for carotenoids before ingestion, different carotenoid levels in the blood of the subjects, and different dietary compositions during the study. A single ingestion of 200 g broccoli, including 8 mg lutein, affected serum lutein concentrations slightly (Riso *et al.*, 2003) and ingestion of 200 g broccoli, including 2.4-3.1 mg lutein, for 7 days increased serum lutein concentrations

by only 0.1 μM (Granado *et al.*, 2006). Thus, lutein bioavailability from broccoli seems to be low. Other researchers have measured increases in serum or plasma lutein concentrations at 1 d after ingestion of a single quantity of spinach (7.3-16.3 nM/mg-lutein intake), broccoli (5.0 nM/mg-lutein intake), or a supplement (5.3-33.3 nM/mg-lutein intake) (Riso *et al.*, 2003; Chung *et al.*, 2004). The bioavailability of lutein from CP (21.4 to 25.6 nM/mg-lutein intake) in the present study was higher than that of spinach and broccoli and comparable to that of the supplement. However, the research protocols in these other studies differed from that in our study in terms of the washout period and the diet of subjects during the examination.

Other research has suggested that a daily intake of 6 to 10 mg lutein contributes to maintaining eye health (Seddon *et al.*, 1994; Richer *et al.*, 2004). However, daily intake of lutein is thought to be smaller than 6 mg in most industrialized countries; for example, Rock *et al.* (2002) estimated a value of 1.3 \pm 0.9 mg ($n = 2786$) in the United States. Foods that contribute to lutein intake include leafy green vegetables, spinach, and broccoli (Granado *et al.*, 2003). To obtain 6 to 10 mg of lutein from these vegetables would require consumption of 49 to 82 g of raw spinach, 347 to 578 g of raw lettuce, and 428 to 713 g of broccoli, based on the USDA reference (i). However, these amounts of vegetables are un-

realistically high for daily consumption. In contrast, CP can supply 6 to 10 mg of lutein at a small dose (2.1 to 3.5 g).

In conclusion, we found that the lutein in CP was highly bioavailable to humans and that CP is a good dietary source of lutein, as it remarkably enhanced serum lutein concentrations at a small dose (3 to 6 g). CP could thus be a potent food supplement to compensate for low intake of green vegetables.

Acknowledgements We thank the volunteers who participated in the study. We thank Dr. Yumi Arai of the Ohkubo Medical Clinic (Kunitachi, Tokyo, Japan) for blood sampling and Keiko Kasaha for technical assistance.

References

- Chung, H.Y., Rasmussen, H.M., and Johnson, E.J. (2004). Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. *J. Nutr.*, **134**, 1887-1893.
- Granado, F., Olmedilla, B., and Blanco, I. (2003). Nutritional and clinical relevance of lutein in human health. *Brit. J. Nutr.*, **90**, 487-502.
- Granado, F., Olmedilla, B., Herrero, C., Pérez-Sacristán, B., Blanco, I., and Blázquez, S. (2006). Bioavailability of carotenoids and tocopherols from broccoli: in vivo and in vitro assessment. *Exp. Biol. Med.*, **231**, 1733-1738.
- Krinsky, N.I., Landrum, J.T., and Bone, R.A. (2003). Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu. Rev. Nutr.*, **23**, 171-201.
- Moeller, S.M., Jacques, P.F., and Blumberg, J.B. (2000). The potential role of dietary xanthophylls in cataract and age-related macular degeneration. *J. Am. Coll. Nutr.*, **19**, 522S-527S.
- Nakamura, M., Sugiura, M., and Aoki, N. (2006). High beta-carotene and beta-cryptoxanthin are associated with low pulse wave velocity. *Atherosclerosis*, **184**, 363-369.
- Richer, S., Stiles, W., Statkute, L., Pulido, J., Frankowski, J., Rudy, D., Pei, K., Tshipursky, M., and Nyland, J. (2004). Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry*, **75**, 216-230.
- Riso, P., Brusamolino, A., Ciappellano, S., and Porrini, M. (2003). Comparison of lutein bioavailability from vegetables and supplement. *Int. J. Vitam. Nutr. Res.*, **73**, 201-205.
- Rock, C.L., Thornquist, M.D., Neuhouser, M.L., Kristal, A.R., Neumark-Sztainer, D., Cooper, D.A., Patterson, R.E. and Cheskin, L.J. (2002). Diet and lifestyle correlates of lutein in the blood and diet. *J. Nutr.*, **132**, 525S-530S.
- Seddon, J.M., Ajani, U.A., Sperduto, R.D., Hiller, R., Blair, N., Burton, T.C., Farber, M.D., Gragoudas, E.S., Haller, J., Miller, D.T., *et al.* (1994). Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*, **272**, 1413-1420.
- Shibata, S., Natori, Y., Nishihara, T., Tomisaka, K., Matsumoto, K., Sansawa, H. and Nguyen, V.C. (2003). Antioxidant and anti-cataract effects of Chlorella on rats with streptozotocin-induced diabetes. *J. Nutr. Sci. Vitaminol.*, **49**, 334-339.
- Shibata, S. and Sansawa, H. (2006). Preventive effects of heterotrophically cultured Chlorella regularis on lifestyle-associated diseases. *Annu. Rep. Yakult Central Inst. Microbiol. Res.*, **26**, 63-72.
- Shibata, S., Hayakawa, K., Egashira, Y. and Sanada, H. (2007). Hypocholesterolemic mechanism of Chlorella: Chlorella and its indigestible fraction enhance hepatic cholesterol catabolism through up-regulation of cholesterol 7 α -hydroxylase in rats. *Biosci. Biotechnol. Biochem.*, **71**, 916-925.
- van het Hof, K.H., Brouwer, I.A., West, C.E., Haddeman, E., Steegers-Theunissen, R.P., van Dusseldorp, M., Weststrate, J.A., Eskes, T.K. and Hautvast, J.G. (1999). Bioavailability of lutein from vegetables is 5 times higher than that of beta-carotene. *Am. J. Clin. Nutr.*, **70**, 261-268.
- van het Hof, K.H., West, C.E., Weststrate, J.A. and Hautvast, J.G. (2000). Dietary factors that affect the bioavailability of carotenoids. *J. Nutr.*, **130**, 503-506.

URL Cited

- i) <http://www.nal.usda.gov/fnic/foodcomp/Data/SR20/nutrlist/sr20w338.pdf> (Dec 08, 2008)