Ingestion of *Chlorella* Reduced the Oxidation of Erythrocyte Membrane Lipids in Senior Japanese Subjects

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Abstract: Accumulation of phospholipid hydroperoxide (PLOOH) in erythrocyte membranes is an abnormality found in patients with senile dementia, including those with Alzheimer's disease. In our previous studies, dietary xanthophylls (polar carotenoids such as lutein) were hypothesized to inhibit lipid peroxidation. In the present study, we conducted a randomized, double-blind, placebo-controlled human trial to assess the impact for a total of 2 months *Chlorella* supplementation (8 g *Chlorella*/day/person; equivalent to 22.9 mg lutein/day/person) on PLOOH and carotenoid concentrations in erythrocytes as well as plasma of 12 normal senior subjects. After 1 or 2 months of treatment, erythrocytes and plasma lutein concentrations increased in the *Chlorella* group but not in the placebo group. In the *Chlorella*-supplemented group, erythrocyte PLOOH concentrations after a total of 2 months of treatment were lower than the concentrations before supplementation. These results suggest that *Chlorella* ingestion improved erythrocyte antioxidant status and lowered PLOOH concentrations. These reductions might contribute to maintaining the normal function of erythrocytes and prevent the development of senile dementia.

Key words: *Chlorella*, antioxidant, carotenoids, lipid oxidation, erythrocytes, senile dementia

1 INTRODUCTION

Senile dementia, including Alzheimer's disease (AD), is a serious neurodegenerative disorder in humans¹⁾. Erythrocytes of AD patients are known to be in an excessively oxidized state^{2,3)}. In 1992, Miyazawa *et al.*⁴⁾ investigated phospholipid hydroperoxide (PLOOH) concentrations as a specific marker for membrane lipid oxidative injury and discovered that erythrocyte in AD patients were significantly higher than in normal control subjects. Erythrocytes having high levels of PLOOH have been recognized as aged cells. It has been suggested that such cells might have a decreased ability to transport oxygen to the brain and could also impair blood rheology, thus facilitating senile dementia⁵⁻⁷⁾. We considered that oxygen dissociation from oxidized hemoglobin may be inhibited by the presence of PLOOH in the phospholipid bilayers in the PLOOH-rich erythrocytes⁵⁻⁷⁾.

For human erythrocytes, α -tocopherol and the xanthophylls (polar carotenoids such as lutein) are important lipo-

philic antioxidants⁸⁾. In our recent studies⁹⁾, erythrocyte lutein concentrations in AD patients were found to be significantly lower than in control subjects, and an inverse relationship was confirmed between erythrocyte lutein and PLOOH concentrations in the AD patients. In our murine study¹⁰⁾, oral administration of a lutein concentrate showed its incorporation into erythrocytes and distribution in the phospholipid bilayers. This treatment resulted in decreased erythrocyte PLOOH levels. We also confirmed in a human study that oral intake of lutein capsules increased lutein concentrations and prevented PLOOH accumulation in erythrocytes¹¹⁾. On the basis of our data obtained in human and animal studies, we suggested that dietary xanthophyll like lutein has the potential to act as an important antioxidant in erythrocytes. We further hypothesized that it could contribute to the suppression of PLOOH accumulation in erythrocyte membranes and might therefore reduce AD. Thus, in daily life, continuous dietary intake of foods rich in lutein might help prevent the development of AD.

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However, to the best of our knowledge, no extensive study of food resources that can efficiently prevent erythrocyte lipid oxidation has been undertaken.

Chlorella pyrenoidosa is a unicellular green alga¹²⁾ rich in protein, minerals and lutein^{13–15)}. In the present study, we hypothesized that continuous dietary intake of *Chlorella* might prevent PLOOH accumulation in the erythrocytes of senior Japanese subjects. To evaluate the hypothesis, we performed a randomized, double-blind, placebo-controlled human trial to investigate the efficacy of 1- and 2-month *Chlorella* supplementation (8 g *Chlorella*/day/person, equivalent to 22.9 mg lutein/day/person).

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Zeaxanthin, β -cryptoxanthin, lycopene, and lutein were purchased from Extrasynthese (Lyon, France). α -carotene, β -carotene, echinenone, pyrogallol, methanol, ammonium acetate, and methyl *tert*-butyl ether (MTBE) were obtained from Wako (Osaka, Japan). *Chlorella* tablets (product name: Sun Chlorella A[®]) were provided by Sun Chlorella Co., Ltd. (Kyoto, Japan). *Chlorella* tablets, consisting of Sun Chlorella A[®], were provided by Sun Chlorella Co., Ltd. (Kyoto, Japan). The daily intake of 8 g of tablets contained 22.9 mg lutein and 5 mg β -carotene for carotenoids. This dosage was determined according to our previous studies¹¹⁾. All other reagents were of analytical grade.

2.2 Methods

2.2.1 Supplementation study

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of the New Drug Development Research Center, Inc. (number PMS-2012-001). Exclusion criteria included pregnancy, lactation, and severe medical illness. Twelve healthy subjects (aged 50–68 years; 7 men and 5 women) participated in this study. Written informed consent was obtained from all subjects according to the criteria of the ethics committee.

For the *Chlorella*-supplemented group (3 men and 3 women), subjects took 40 *Chlorella* tablets (8 g, containing 22.9 mg lutein and 5 mg β -carotene) per day for a total of 2 months. For the placebo group (4 men and 2 women), placebo tablets (85% lactose) were ingested for a total of 2 months. At 1 or 2 months after ingestion of the tablets, blood was collected into a tube with heparin as an anticoagulant. The blood samples were subjected to centrifugation at 1000 g for 10 min at 4°C. After the plasma and buffy coat were removed, erythrocytes were washed 3 times with phosphate buffered saline (pH 7.4) to prepare packed cells. The packed cells were immediately subjected to determi-

nation of carotenoids and PLOOH (combined values obtained for phosphatidylcholine hydroperoxide (PCOOH) and phosphatidylethanolamine hydroperoxide (PEOOH)). Plasma samples were stored at -80°C for later analysis.

2.2.2 Extraction and determination of PLOOH

For the determination of erythrocyte PLOOH concentrations, total lipids were extracted from packed cells with a mixture of 2-propanol and chloroform (1:1, v/v, containing 5 nmol/L EDTA-2Na). Using total erythrocyte lipids, PLOOH (PCOOH and PEOOH) was determined by HPLC coupled with chemiluminescence (CL) detection^{4, 16)}. The column was a 4.6×250 mm, 5 μm Finepak SIL NH2-5 column (Japan Spectroscopic Co., Tokyo, Japan). The eluent was 2-propanol:methanol:water (135:45:20, v/v/v), and the flow rate was 1 mL/min. Post-column CL detection was carried out using a CLD-100 detector (Tohoku Electronic Industries Co., Sendai, Japan). A mixture of luminol and cytochrome C in 50 mmol/L borate buffer (pH 10) was used as a hydroperoxide-specific post-column CL reagent and the flow rate was 1.8 mL/min¹⁶⁾. Calibration was carried out using PCOOH and PEOOH standards that were prepared by the methoxypropene method¹⁷⁾. Plasma PCOOH was measured by HPLC-CL¹⁸⁾.

2.2.3 Extraction and determination of carotenoids

Two mL of packed cells were diluted with 2 mL of water and were mixed with 4 mL of 80 mmol/L ethanolic pyrogallol, 0.8 mL of 1.8 mol/L aqueous KOH, and 125 μL of one $\mu\text{mol/L}$ ethanolic echinenone (an internal standard). After addition of 1 mL of 0.1 mol/L aqueous sodium dodecyl sulfate, the sample was mixed with 12 mL of hexane/dichloromethane (5:1, v/v, containing 2.4 mmol/L butylated hydroxytoluene) for extraction of erythrocyte carotenoids. The extract was purified with a Sep-Pak silica cartridge (Waters, Milford, MA), and then subjected to HPLC coupled with UV detection^{8, 19, 20)}. These extraction procedures were conducted under subdued (yellow) light to minimize photo degradation of the carotenoids.

A C30 carotenoid column (4.6×250 mm, 5 μm ; YMC, Kyoto, Japan) was used; the column was eluted using a binary gradient consisting of the following HPLC solvents: A, methanol/MTBE/water (83:15:2 v/v/v, containing 3.9 mmol/L ammonium acetate), and B, methanol/MTBE/water (8:90:2 v/v/v, containing 2.6 mmol/L ammonium acetate)^{19, 20)}. The gradient profile was as follows: 0–12 min, 10–55% B linear; 12–20 min, 55–100% B linear; 20–25 min, 100% B; 25–27 min, 100–10%. The flow rate was adjusted to one mL/min, and the column temperature was maintained at 30°C . The column eluate was sent to a UV detector (UV-2075 PLUS, Japan Spectroscopic Co., Tokyo, Japan) for monitoring lutein and other carotenoids at 463 nm. Concentrations of erythrocyte carotenoids were calculated using an equation corresponding to the standard curve of each carotenoid and were adjusted by the percentage recovery of the added echinenone (the internal

standard). Plasma lutein and other carotenoids were determined by HPLC coupled with ultraviolet (UV) detection²¹⁾. Plasma and erythrocyte tocopherols were measured by HPLC with fluorescence detection^{8, 22)}. Blood characteristics (parameters as given in **Table 1** and **Table 2**) were analyzed using standardized methods.

2.2.4 Statistical analyses

Data were expressed as mean values and standard deviations. All data were analyzed by a normality test and a Friedman test followed by Scheffé's method with non-normally distributed data. Comparisons of data collected before and after supplementation were analyzed by Student's t-test with normally distributed data, and Mann-Whitney U test for non-normally distributed data. These statistical analyses were conducted using Excel Toukei 2010 (Social Survey Research Information, Tokyo, Japan).

3 RESULTS

Table 1 shows physical and haematological parameters such as blood pressure, platelet, leucocyte, neutrophil, monocyte, erythrocyte, and lymphocyte counts before and after a total of 2 months oral intake of *Chlorella* or placebo tablets. No change was observed after the intake of the *Chlorella* or placebo tablets. **Table 2** shows blood biochemical parameters such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), guanosine 5'-triphosphate (γ -GTP) after the intake of *Chlorella* or placebo tablets. No differences were observed among them, and the values were found to be within normal ranges.

As shown in **Table 3**, erythrocyte PLOOH concentrations (5.0 ± 1.7 pmol/mL packed cells) were significantly lower after a 2 months oral intake of 8 g *Chlorella*/day than before the supplementation (11.9 ± 5.5 pmol/mL packed cells). No difference was observed in erythrocyte PLOOH concentrations after 1 month of *Chlorella* intake (7.9 ± 3.7 pmol/mL packed cells). In the erythrocytes of the placebo group, no change in PLOOH concentrations was observed after treatment. For erythrocyte carotenoids, 6 carotenoids (lutein, zeaxanthin, β -cryptoxanthin, α -carotene, β -carotene, and lycopene) were detected (**Table 3**). Among these carotenoids, lutein was the predominant xanthophyll found, and it increased 4.6-fold (from 49.7 to 230.7 pmol/mL packed cells) after a total of 2 months of *Chlorella* ingestion. Erythrocyte tocopherol levels were not changed after *Chlorella* ingestion. These results indicate that daily *Chlorella* intake would be effective for improving and maintaining erythrocyte antioxidant status and lutein concentrations in humans.

Table 4 shows plasma PCOOH, carotenoids and tocopherol concentrations before and after a total of 2 months of

Chlorella ingestion. PCOOH is a representative lipid hydroperoxide present in plasma; concentrations were not changed after *Chlorella* ingestion or placebo treatment. For plasma carotenoids, concentrations of lutein, α -carotene, and β -carotene significantly increased (4.4-fold for lutein, 2.1-fold for α -carotene, 1.8-fold for β -carotene) after *Chlorella* supplementation (**Table 4**). In the placebo group, no changes were observed for plasma carotenoids or tocopherols after the treatment (**Table 4**).

4 DISCUSSION

In the present study, we conducted a randomized, double-blind, placebo-controlled human trial to investigate the impact of 1 and 2 months of *Chlorella* supplementation on carotenoid compositions and PLOOH concentrations in erythrocytes as well as the plasma of 12 senior healthy subjects. Subjects ingested 8 g *Chlorella*/day/person, equivalent to 22.9 mg lutein/day/person. This research was carried out to assess the possibility that *Chlorella* ingestion could prevent the increase of erythrocyte PLOOH in senior Japanese volunteers.

Previously, we discovered that erythrocyte PLOOHs in AD patients were significantly higher than in normal subjects^{4, 9)}. In normal volunteers, erythrocyte PLOOH of senior subjects (56-92 of age) was higher than the young subjects (21-27 of age)¹⁶⁾. The magnitude of PLOOH change is not proportional to subject body weight. PCOOH and PEOOH are the major PLOOHs in human erythrocytes, and PCOOH is the principal PLOOH in human plasma^{4, 9)}. The data suggested that biochemically older erythrocytes circulated in the blood of patients with senile dementia than in normal subjects. Lutein is a polar carotenoid and important antioxidant present in erythrocyte lipid bilayers⁸⁾. We analyzed the effect of *Chlorella* supplementation as a lutein-rich nutrient (contains an average of 0.29% by dry weight) on the prevention of erythrocyte membrane lipid oxidation.

In the present study, *Chlorella* intake resulted in no meaningful changes of blood biochemistry or parameters (**Table 1** and **Table 2**). These data might indicate the safety of *Chlorella* supplementation. As shown in **Table 3**, *Chlorella* supplementation was effective at elevating the lutein concentration in the erythrocyte lipid bilayers. The increased erythrocyte β -cryptoxanthin concentrations found after ingestion might have been due to food intake (such as mandarin oranges, rich in β -cryptoxanthin) by two volunteers during the ingestion period (from October 15th to December 17th). Decreased lycopene concentrations found in erythrocytes and plasma after the 2-month *Chlorella* treatment were recognized due to the lack of lycopene in the ingested *Chlorella* tablets and meals during the study (**Table 3** and **Table 4**). Erythrocyte and plasma

Table 1 Physical and haematological parameters before and after a total of 2 months oral intake of 0 (placebo) or 8 g/day *Chlorella* (Mean values and standard deviations).

Daily chlorella supplementation		0 g (placebo group)		8 g		<i>p</i> ‡
Parameters		Mean	SD	Mean	SD	
Age (years)		57	7	58	7	0.840
Total number of subjects		6		6		
Men		4		3		
Women		2		3		
Height (cm)		161.9	10.0	161.8	6.8	0.984
Weight (kg)	0 month	59.3	10.4	61.4	11.5	0.747
	1	60.0	12.1	61.8	11.2	0.791
	2	60.1	12.6	61.7	11.2	0.819
BMI (kg/m ²)	0 month	22.5	2.5	23.4	3.4	0.728
	1	22.7	2.7	23.5	3.2	0.654
	2	22.7	2.7	23.5	3.2	0.681
Systolic blood pressure (mmHg)	0 month	112.2	8.9	130.2	18.6	0.069
	1	116.5	12.3	127.8	24.1	0.338
	2	110.5	13.9	128.5	31.3	0.239
Diastolic blood pressure (mmHg)	0 month	74.2	9.0	83.3	14.5	0.224
	1	75.7	9.8	81.5	12.0	0.380
	2	72.5	11.2	82.2	18.4	0.304
Platelets (x10 ⁴ /μL)	0 month	22.9	5.4	23.6	6.8	0.851
	1	24.1	5.2	24.9	5.9	0.801
	2	24.8	5.3	24.4	6.7	0.900
Leucocytes (/μL)	0 month	4716.7	762.7	4483.3	643.2	0.580
	1	4500.0	784.9	5483.3	980.6	0.086
	2	4400.0	469.0	4650.0	653.5	0.466
Neutrophil (%)	0 month	64.0	9.8	52.2	10.0	0.065
	1	60.7	7.6	60.1	6.9	0.883
	2	62.6	7.4	52.9	8.1	0.056
Eosinophil granulocyte (%)	0 month	2.7	2.1	2.5	0.9	0.863
	1	2.5	1.5	2.8	1.1	0.676
	2	2.5	1.4	3.9	2.5	0.275
Basophil granulocyte (%)	0 month	0.5	0.4	0.6	0.4	0.567
	1	0.5	0.3	0.6	0.3	0.783
	2	0.6	0.3	0.8	0.4	0.472
Monocyte (%)	0 month	6.0	1.7	5.9	1.0	0.843
	1	5.7	1.4	4.7	1.1	0.208
	2	5.6	1.4	5.7	1.0	0.910
Erythrocytes (x10 ⁴ /μL)	0 month	466.0	27.0	450.8	35.8	0.428
	1	466.8	21.1	455.8	32.6	0.506
	2	459.0	30.3	451.8	46.7	0.760
lymphocyte	0 month	26.8	9.6	38.8	9.6	0.057
	1	30.6	7.1	31.9	6.9	0.760
	2	28.8	6.4	36.8	7.4	0.073
Hb (g/dL)	0 month	14.0	1.3	13.8	1.0	0.756
	1	14.2	1.2	14.1	1.1	0.941
	2	13.9	1.5	13.8	1.5	0.882
Haematocrit (%)	0 month	42.7	2.9	42.0	2.8	0.680
	1	42.9	2.2	42.4	3.0	0.743
	2	42.3	3.6	42.3	4.2	1.000
Total bilirubin (mg/dL)	0 month	0.7	0.1	0.7	0.3	0.900
	1	0.7	0.2	0.7	0.2	1.000
	2	0.6	0.1	0.8	0.3	0.245
MCV (fL)	0 month	91.7	4.6	93.4	4.4	0.535
	1	92.1	4.4	93.1	4.6	0.690
	2	92.2	5.1	93.7	3.7	0.566
MCH (pg)	0 month	30.0	2.2	30.6	1.4	0.599
	1	30.4	2.3	31.0	1.8	0.451
	2	32.0	2.5	30.6	1.5	0.274
MCHC (%)	0 month	32.7	1.0	32.7	1.0	0.955
	1	33.0	1.2	33.3	0.9	0.588
	2	32.9	1.1	32.6	0.9	0.630

‡ Student's t-test among groups.

BMI, body mass index; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 2 Blood biochemical paramaters before and after a total of 2 months oral intake in the 0 (placebo) or 8 g/day *Chlorella* (Mean values and standard deviations).

Daily chlorella supplementation		0 g (placebo group)		8 g		$p^{\ddagger}$
Parameters		Mean	SD	Mean	SD	
Total protein (g/dL)	0 month	7.3	0.3	7.4	0.3	0.474
	1	7.4	0.3	7.5	0.2	0.645
	2	7.3	0.5	7.3	0.3	0.950
Albumin (g/dL)	0 month	4.4	0.2	4.4	0.3	0.769
	1	4.5	0.3	4.4	0.3	0.510
	2	4.4	0.4	4.3	0.4	0.785
Creatinine (mg/dL)	0 month	0.7	0.1	0.7	0.2	0.973
	1	0.8	0.1	0.7	0.2	0.785
	2	0.8	0.1	0.8	0.2	0.973
Homocysteine (nmol/mL)	0 month	13.8	1.1	15.0	8.0	0.728
	1	13.3	1.0	15.0	9.7	0.683
	2	13.0	1.4	14.8	7.5	0.579
GOT (U/L)	0 month	24.5	10.5	20.8	3.5	0.447
	1	22.5	5.5	21.7	3.7	0.765
	2	23.7	3.5	20.5	1.5	0.083
GPT (U/L)	0 month	19.8	9.7	22.0	8.9	0.696
	1	20.7	7.1	22.0	7.6	0.761
	2	22.7	4.8	21.7	6.2	0.761
LDH (U/L)	0 month	198.2	35.9	191.8	22.6	0.723
	1	187.8	23.5	188.3	29.8	0.975
	2	189.7	21.5	185.3	22.0	0.737
ALP (U/L)	0 month	181.3	21.0	235.5	34.4	0.011
	1	183.0	31.3	254.3	47.2	0.014
	2	184.5	39.9	245.7	50.5	0.044
γ -GTP (U/L)	0 month	26.2	12.7	32.7	14.7	0.433
	1	27.0	9.1	34.2	18.1	0.414
	2	27.2	11.0	32.3	19.7	0.591
CPK (U/L)	0 month	208.2	234.1	108.2	42.6	0.348
	1	139.0	56.7	104.0	63.5	0.338
	2	148.3	75.7	90.0	27.6	0.124
Uric acid (mg/dL)	0 month	5.1	1.0	5.2	2.2	0.922
	1	5.4	0.7	5.1	1.9	0.743
	2	5.7	1.2	5.1	1.5	0.460
Urea (mg/dL)	0 month	13.6	2.7	14.1	4.1	0.804
	1	13.6	1.7	12.4	1.6	0.209
	2	14.4	2.6	13.6	1.6	0.510
Glucose (mg/dL)	0 month	82.8	7.7	89.3	9.8	0.231
	1	83.5	6.1	89.3	9.4	0.236
	2	85.3	9.6	89.2	10.9	0.534
Total cholesterol (mg/dL)	0 month	226.0	7.7	219.0	31.3	0.615
	1	228.8	11.7	219.3	29.8	0.493
	2	225.5	9.9	216.3	30.5	0.510
HDL cholesterol (mg/dL)	0 month	73.0	19.4	58.5	14.5	0.176
	1	75.3	17.0	56.0	13.1	0.053
	2	73.2	16.7	57.7	14.7	0.119
LDL cholesterol (mg/dL)	0 month	134.7	20.6	135.7	27.9	0.945
	1	136.7	24.1	136.7	28.0	1.000
	2	136.0	23.1	130.8	25.0	0.718
TG (mg/dL)	0 month	77.2	35.2	124.7	78.8	0.220
	1	66.5	13.0	156.0	75.8	0.034
	2	62.2	18.7	145.7	94.6	0.084

$\ddagger$ Student's t-test among groups.

CPK, creatine phosphokinase; TG, triglyceride.

Table 3 Erythrocyte phospholipid hydroperoxides (PLOOH), carotenoids and tocopherol levels in the subjects before and after a total of 2 months oral intake of the 0 (placebo) or 8 g/day *Chlorella* (Mean values and standard deviations).

Daily chlorella supplementation		0 g (placebo group)		8 g		
Parameters		Mean	SD	Mean	SD	<i>p</i> †
PLOOH (pmol/mL packed cells)						
PCOOH	0 month	8.2	4.1	8.4	4.2	0.936
	1	9.7	8.8	6.6	3.2	0.749
	2	4.1	2.2	3.7	1.3	0.936
PEOOH	0 month	1.1	0.4	3.5	3.8	0.261
	1	1.4	0.8	1.2	0.7	0.630
	2	1.0	0.9	1.3	1.0	0.336
PLOOH (PCOOH+PEOOH)	0 month	9.3	4.3	11.9	5.5	0.423
	1	11.1	9.0	7.9	3.7	0.747
	2	5.1	2.9	5.0*	1.7	0.522
Carotenoids (pmol/mL packed cells)						
Lutein	0 month	47.4	13.2	49.7	29.9	0.873
	1	54.3	19.8	192.9*	122.8	0.109
	2	55.5	26.3	230.7	129.4	0.025
Zeaxanthin	0 month	9.4	5.2	9.2	3.5	1.000
	1	8.6	3.0	11.1	7.7	0.631
	2	11.2	6.5	7.1	3.1	0.200
β-Cryptoxanthin	0 month	3.9	2.1	2.0	0.8	0.078
	1	4.2	2.9	3.5	1.8	0.522
	2	6.5	4.5	7.3*	3.9	0.749
α-Carotene	0 month	0.7	0.7	1.8	2.2	0.262
	1	0.8	0.4	1.6	1.4	0.337
	2	1.0	0.9	3.2	2.5	0.150
β-Carotene	0 month	4.2	3.1	4.7	3.0	1.000
	1	4.4	2.4	9.8	13.1	0.631
	2	5.9	5.6	6.9	4.2	0.522
Lycopene	0 month	4.6	0.5	4.7	0.7	0.522
	1	4.8	1.2	4.9	1.3	0.749
	2	4.8	0.7	3.7*	0.3	0.004
Tocopherols (nmol/mL packed cells)						
α-Tocopherol	0 month	4.2	1.7	5.4	1.2	0.262
	1	4.5	1.7	7.6	1.2	0.010
	2	5.8	1.7	6.9	1.9	0.337
γ-Tocopherol	0 month	0.9	0.4	1.2	0.5	0.423
	1	0.9	0.7	1.7	0.7	0.037
	2	1.6	0.8	1.6 [§]	0.6	1.000

Mean values were significantly different in the Friedman test with Scheffe's method between before administration and 1 or 2 month after administration. * $p < 0.05$

Mean values were significantly different in the Friedman test with Scheffe's method between 1 month administration and 2 month after administration. § $p < 0.05$

† Mann-Whitney U test among groups.

tocopherol concentrations were recognized to reflect the contents of daily meals during the experimental period (Table 3 and Table 4). This may reflect the scarcity of any tocopherols in the *Chlorella* tablets employed in the present study.

After the 2-month treatment, erythrocyte PLOOH con-

centrations in the *Chlorella* group were significantly reduced (5.0 ± 1.7 pmol/mL packed cells vs. 11.9 ± 5.5 pmol/mL packed cells). The *Chlorella* group initially showed rather high values compared to the placebo group (9.3 ± 4.3 pmol/mL packed cells). Comparing the placebo and *Chlorella* groups after 2 months of treatment, there

Table 4 Plasma phospholipid hydroperoxides (PLOOH), carotenoids and tocopherol levels in the subjects before and after a total of 2 months oral intake of 0 (placebo) or 8 g/day *Chlorella* (Mean values and standard deviations).

Daily chlorella supplementation		0 g (placebo group)		8 g		
Parameters		Mean	SD	Mean	SD	<i>p</i> †
PLOOH (pmol/mL plasma)						
PCOOH						
	0 month	171.1	59.8	115.1	23.6	0.150
	1	138.1	12.0	115.6	14.5	0.025
	2	133.3	33.6	143.1	55.5	0.631
Carotenoids (pmol/mL plasma)						
Lutein	0 month	351.3	73.8	370.1	199.6	0.749
	1	408.2	137.5	1523.9*	648.8	0.055
	2	430.5	213.3	1620.3*	926.3	0.037
Zeaxanthin	0 month	84.7	61.6	127.1	29.6	0.200
	1	69.3	28.6	78.8	56.3	1.000
	2	88.4	47.2	81.4	52.7	0.749
β-Cryptoxanthin	0 month	111.1	67.6	106.7	68.2	0.631
	1	172.4	127.7	151.4	54.0	0.873
	2	209.4	109.2	298.2	197.6	0.522
α-Carotene	0 month	130.5	56.1	180.3	173.3	1.000
	1	109.1	46.8	364.3*	184.7	0.025
	2	99.5	39.0	382.5*	231.3	0.037
β-Carotene	0 month	380.1	118.3	371.7	220.1	0.749
	1	411.2	229.9	644.2*	326.6	0.262
	2	414.9	270.2	659.6*	358.9	0.150
Lycopene	0 month	250.0	136.3	226.7	112.3	0.873
	1	259.5	89.7	154.2	78.6	0.078
	2	199.2	77.7	106.3*	52.3	0.037
Tocopherols (nmol/mL plasma)						
α-Tocopherol	0 month	24.6	5.4	29.0	7.0	0.423
	1	22.1	4.6	29.2	8.3	0.078
	2	26.1	3.6	28.7	7.1	1.000
γ-Tocopherol	0 month	6.0	2.2	7.4	4.3	0.749
	1	4.4	2.6	5.0	1.9	0.631
	2	6.3	2.3	5.7	1.3	0.423

Mean values were significantly different in the Friedman test with Scheffe's method between before administration and 1 or 2 month after administration. **p* < 0.05

† Mann-Whitney U test among groups.

was no difference in PLOOH concentrations. However, we confirmed a significant decrease of PLOOH after the 2 months of *Chlorella* ingestion (Table 3). There was a wide dispersion of PLOOH data found in the placebo group (Table 3).

Our results indicate for the first time that *Chlorella* ingestion improves erythrocyte antioxidant status and lowers the PLOOH concentration. Thus, *Chlorella* supplementation could maintain the normal function of erythrocytes.

Generally, aged erythrocytes contain more oxidized lipids than young erythrocytes¹⁶⁾. *Chlorella* intake may prevent the development of senile dementia, including AD, due to its suppression of the accelerated aging of erythrocytes in senior subjects.

5 CONCLUSIONS

In summary, we performed a randomized, double-blind, placebo-controlled human trial to investigate the impact of a total of 2 months *Chlorella* supplementation on erythrocytes antioxidant status. The present study provides the first evidence that *Chlorella* is an effective dietary source of antioxidant carotenoids that prevents PLOOH accumulation in the erythrocytes of senior Japanese subjects. We previously found that there was a higher accumulation of PLOOH, an oxidative stress marker, in erythrocytes of dementia patients^{4,9}. Erythrocytes high in lipid hydroperoxides have been suggested to have a decreased ability to transport oxygen to the brain and may impair blood rheology, thus facilitating dementia. The present findings confirm the efficacy of *Chlorella* supplementation for the prevention of erythrocyte lipid oxidation in senile dementia, including AD.

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