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Letter

Chlorella supplementation decreases methylmercury concentrations of hair and blood in healthy volunteers

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ABSTRACT — An open-label clinical trial was performed to test the effects of unicellular green alga *Chlorella* supplementation on mercury concentrations of hair and blood in healthy subjects. Fifty-eight healthy participants (36 male and 22 female) were assigned to *Chlorella* and control groups. The *Chlorella* group of 35 subjects received *Chlorella* tablets (9 g/day) for an experimental period of 3 months while the control group of 23 subjects did not. Total mercury concentrations of hair and blood were analyzed at the beginning and end of the experimental period for estimation of methylmercury (MeHg) levels in the body. The hair mercury concentration of the *Chlorella* group (n = 33) was significantly decreased during the experimental period (p = 0.041) while the change in the control group (n = 23) was not significant (p = 0.362). Although the decrease in blood mercury concentration in the *Chlorella* group (n = 19) was not significant (p = 0.084), the change of values (values at end – values at beginning) in this group was significantly greater than that in the control group (n = 20, p = 0.038). The fish intake rates remained relatively constant during the experimental period in both the *Chlorella* and control groups. These results suggest that supplementation with *Chlorella* for 3 months in healthy subjects might reduce their body MeHg levels.

Key words: Methylmercury, *Chlorella*, Mercury, Detoxification, Hair, Blood

INTRODUCTION

Methylmercury (MeHg) is a neurotoxic metal compound that is formed by saprophyte microorganisms from inorganic mercury compounds in aquatic environments. It accumulates in fish and shellfish through the marine food web. Thus, the major route of human exposure to MeHg is the ordinary consumption of seafood (National Research Council, 2000; WHO, 1990). As the developmental effects of exposure on human fetuses are of great concern, pregnant women are being cautioned against consuming fish in many countries including United States and Europe (Food Safety Commission of Japan, 2005; FDA, 2011; European Commission, 2004).

Chlorella is a unicellular green alga that has been used as a food source and nutritional supplement for over half a century (Kay, 1991). *Chlorella* contains high amounts of protein and dietary fiber, as well as many kinds of vitamins, essential minerals, and essential fatty acids. It is also rich in plant pigments, including chlorophylls, lutein, zeaxanthin and β-carotene. Various health-promoting benefits, such as lowering of oxidative stress (Nakashima *et al*., 2009), lowering of serum lipids (Sano, 1982), and augmentation of immunity (Hasegawa *et al*., 1997) have been reported for *Chlorella*. Consumption of *Chlorella* has been also shown to remove various toxic substances

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from the body, such as cadmium (Shim *et al*., 2008), lead (Uchikawa *et al*., 2009), dioxins (Morita *et al*., 1999), heterocyclic amines (Lee *et al.,* 2013), and carbon tetrachloride (Li *et al.,* 2013).

Apart from its likely contamination by small amounts of MeHg, fish is generally an excellent source of many nutrients including omega-3 fatty acids, vitamin D, protein and selenium. Indeed, it has been shown that fish intake during pregnancy and infancy are beneficial for young children's cognitive development (Sakamoto *et al*., 2004; Daniels *et al*., 2004; EFSA, 2015). Thus, the discovery of a food material that could help detoxify the body from MeHg would be considered to beneficial to human health. We previously reported that *Chlorella* promoted the excretion of MeHg in both feces and urine, such that long-term administration of *Chlorella* enhances the elimination of tissue methylmercury in mice (Uchikawa *et al*., 2010, 2011). Here we studied the effect of 3-month *Chlorella* supplementation on mercury concentrations of blood and hair in healthy subjects.

MATERIAL AND METHODS

Subjects

Fifty-eight healthy participants (36 male and 22 female) were recruited from Chikugo region, Fukuoka, Japan. The exclusion criteria of this study were obvious disease such as heart, neurological or metabolic disorders, taking warfarin, ongoing *Chlorella* supplementation, and pregnancy or lactation. Participants who submitted signed consent forms were assigned to one of two groups based on their preferences: a *Chlorella* group of 35 subjects and a control group of 23 subjects. Of these 58 subjects, 37 subjects agreed to provide both blood and hair samples, 19 subjects agreed to provide a hair sample only and 2 subjects agreed to provide a blood sample only. Accordingly, hair samples were collected from 56 subjects (33 from the *Chlorella* group and 23 from the control group), and blood samples were collected from 39 subjects (19 from the *Chlorella* group and 20 from the control group). 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 Chlorella Industry (ethics no. K-01025). All participants gave their written informed consent prior to the study.

Protocol

The subjects of *Chlorella* group took *Chlorella* tablets (Chlorella Industry Co. Ltd., Tokyo, Japan) for an experimental period of 3 months. The dose was 9 g/day of *Chlorella* (45 tablets/day), in portions of 15 tablets after each main meal, a recommended intake by a manufacturing company. The average compliance with *Chlorella* consumption was 95% with a confidence interval at the 95% level of 93-97%. The subjects of the control group were prohibited from taking *Chlorella*. All subjects submitted responses to a questionnaire regarding their daily fish intake rates during 60 days: 30 days prior to the experiment and last 30 days during the experimental period.

Blood and hair were collected at the beginning and end of the experimental period. Blood samples were stored at -80°C until mercury analysis. Hair was cut close to the scalp in the occipital area and stored in clean polyethylene bags at room temperature until mercury analysis.

Chlorella tablets

The *Chlorella* tablets were composed of 100% *Chlorella* powder (*Parachlorella beijerinckii*) that was cultured and manufactured by Chlorella Industry Co. Ltd. According to our analysis, each tablet is composed of the following (/100g): protein, 62 g; lipid, 11g; dietary fiber, 11 g; vitamin B_1, 1.8 mg; vitamin B_2, 5 mg; vitamin B_{12}, 500 μg; vitamin C, 60 mg; folic acid, 2500 μg; biotin, 300 μg; α-tocopherol, 30 mg; vitamin K_1, 3000 μg; iron, 75 mg; potassium, 1000 mg; magnesium, 350 mg; chlorophylls, 3.2 g; lutein, 270 mg; β-carotene, 90 mg; zeaxanthin, 30 mg; α-carotene, 7 mg; linoleic acid, 2.0 g; and α-linolenic acid, 1.9 g.

Measurements

Blood samples were digested by the wet-ashing method in a mixture of nitric acid, sulfuric acid and perchloric acid as a pretreatment (Ministry of the Environment, Japan, 2004); total mercury levels were then determined by the reducing-vaporization method using a Mercury Analyzer RA-3320 (Nippon Instruments Corp. Tokyo, Japan). The analytical results were qualitatively confirmed by analyzing a reference material of fish meat, DORM-2 (National Research Council, Canada), with a certified value of 4.64 ± 0.26 μg/g for total mercury. Our total mercury level from a quintuple analysis was 4.58 ± 0.07 μg/g.

Hair samples were analyzed at the National Institute for Minamata Disease, Japan. The 1-cm proximal end of the scalp hair was washed in detergent using an ultrasonic washer and soaked in acetone twice to remove the water. The sample was dissolved in 2N NaOH by heating at 60°C for 30 min., and subjected to total mercury analysis according to an oxygen combustion-gold amalgamation method using an atomic absorption detector MD-1 (Nippon Instruments Corp., Tokyo, Japan). The analytical results were qualitatively confirmed by analyzing a ref-

erence material of human hair, NIES CRM No. 13, with a certificate value of 4.42 ± 0.2 μg/g for total Hg. Our total mercury level from a quintuple analysis was 4.55 ± 0.05 μg/g.

Statistical analysis

Mercury concentrations of hair and blood, and ages of subjects are expressed as the median and 25th and 75th percentiles. The Mann-Whitney U test was used to compare data between the *Chlorella* and control groups. For comparison of values prior to and at the end of the experimental period, Wilcoxon signed-ranks test was used. Fish intake rate (times/week) was expressed as an average and standard deviation. The student's t test was used to compare data between the *Chlorella* and control groups. Comparison of values for 30 days prior to 30 days during the experimental period were analyzed by period t-tests. Fisher's exact test was used for comparisons of the gender ratio between the *Chlorella* and control groups. Differences were considered significant at $p < 0.05$. Statistical calculations were performed using Excel Toukei 2015 software for Windows (SSRI, Tokyo, Japan).

RESULTS AND DISCUSSON

Mercury concentrations in human hair and blood are often used to estimate exposure levels to MeHg (National Research Council, 2000; WHO, 1990). In the present study we examined the possible effect of *Chlorella* in reducing the body burden of MeHg in healthy humans through analyzing hair and blood mercury concentrations. The number, gender and age of subjects who provided hair and blood samples in this study are summarized in Tables 1 and 2. The stools of subjects in the *Chlorella* group displayed green discoloration, but this was due to excretion of chlorophyll in the *Chlorella*. No other adverse reaction by *Chlorella* supplementation was observed.

Hair mercury concentrations at the beginning and end of the experimental period, and the changes of values (values at end – values at beginning) are shown in Fig. 1. The hair mercury concentration in the *Chlorella* group was significantly decreased during the experimental period ($p = 0.041$), while the change in the control group was not significant ($p = 0.362$). However, there was no significant difference in the changes of values (values at end – values at beginning) between the *Chlorella* group and control group (Fig. 1-B).

Blood mercury concentrations at the beginning and end of the experimental period, and the changes of values are shown in Fig. 2. Although the decrease in blood mercury concentration in the *Chlorella* group during the experimental period was not significant ($p = 0.084$, Fig. 2-A), the change of values in the *Chlorella* group was significantly greater than that in the control group ($p = 0.038$, Fig. 2-B). The blood mercury level in the control group was relatively unchanged during the experimental period ($p = 0.117$). No significant differences in alterations of hair and blood mercury levels could be found when groups were separated into males and females, probably due to the small sample sizes.

Hair and blood mercury levels at the beginning of the experiment in *Chlorella* group were somewhat higher than that in the control group. This was probably due to the higher fish intake rate in *Chlorella* group (6.4 ± 2.7 times/week) than in the control group (5.0 ± 3.0 times/week). Changes of the fish intake rates were quite small during the experimental period in both groups (data

Table 1. Number, gender and age of subjects who provided hair sample.

	Chlorella group	Control group	p value [a]
Subjects who provide hair (n)	33	23	
Gender (Male/Femal)	24/9	12/11	0.158
Age [b] (years)	51.0 (28.0, 56.0)	52.0 (48.5, 58.0)	0.115

[a] between two groups, [b] median (25th percentile, 75th percentile).

Table 2. Number, gender and age of subjects who provided blood sample.

	Chlorella group	Control group	p value [a]
Subjects who provide blood (n)	19	20	
Gender (Male/Femal)	16/3	11/9	0.082
Age [b] (years)	56.0 (51.5, 57.5)	51.0 (46.3, 55.5)	0.254

[a] between two groups, [b] median (25th percentile, 75th percentile).

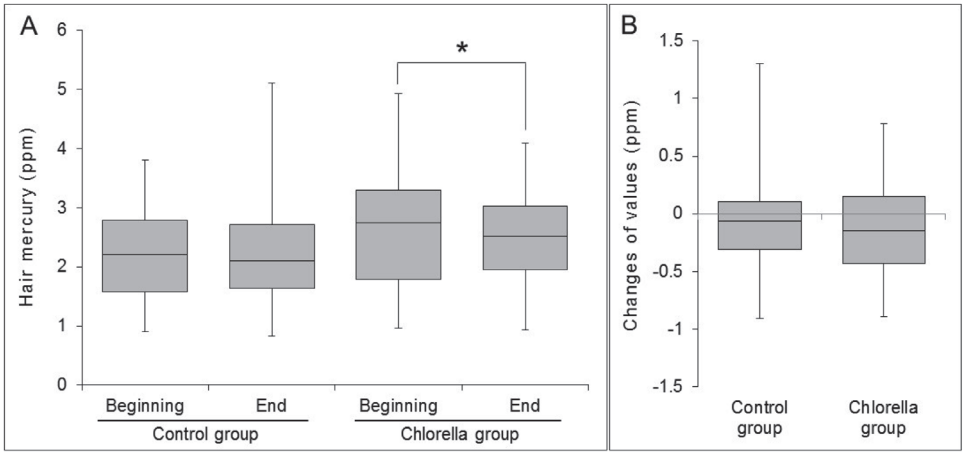

Fig. 1. Hair mercury concentrations at the beginning and end of the experimental period (A), and the changes of values (values at end – values at beginning) (B). The results are represented by box-and-whiskers graphs. The box plots show median and interquartile range. The whiskers indicate the lowest and highest values, respectively. *, $p < 0.05$.

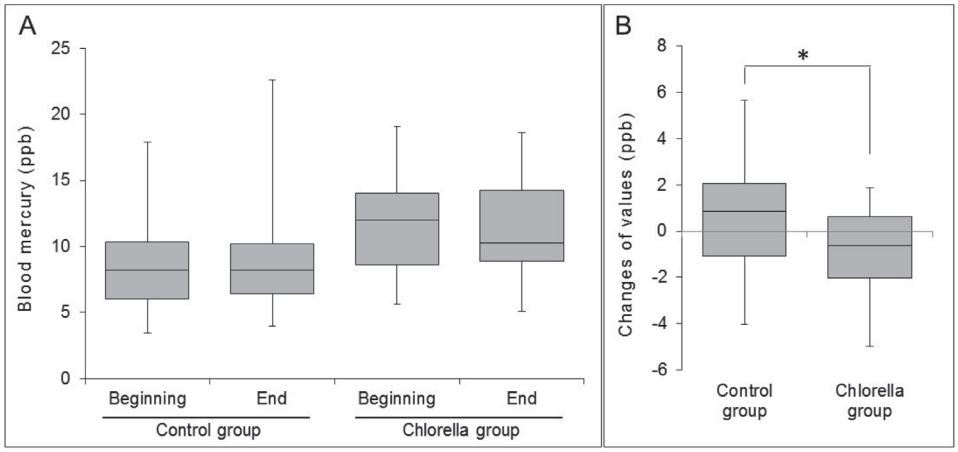

Fig. 2. Blood mercury concentrations at the beginning and end of the experimental period (A), and the changes of values (values at end – values at beginning) (B). The results are represented by box-and-whiskers graphs in the same way as Fig. 1. *, $p < 0.05$.

not shown). In addition, it may be related to the difference of the mercury concentrations at the beginning of the experiment and the fact that the male/female ratio of *Chlorella* group was higher than that of control group (Tables 1, 2). Hair mercury levels are reported to be higher in males than in females, though there is no difference in the amount of fish intake per body weight (Schaefer *et al*., 2014; Yasutake *et al*., 2004).

In the present study, a high correlation between hair and blood mercury concentrations was observed (Pearson's correlation coefficient r = 0.86), and the average hair-to-blood ratio was 250 with a 95% confidence interval of 238-261. The ratio obtained here is well matched to reported values (National Research Council, 2000; WHO, 1990).

Previously, we found that *Chlorella* increased mercury excretion and lowered tissue mercury levels in MeHg-treated mice (Uchikawa *et al*., 2010, 2011). Fecal excretion constitutes a major route for the elimination of MeHg, about 90% of the total elimination, in most animal species including humans (Norseth and Clarkson, 1970; Miettinen *et al.*, 1971; Komsta-Szumska *et al*., 1983; WHO, 1990). A significant portion of MeHg in the liver is secreted as glutathione complex via the bile duct; then

a small portion can be excreted in the feces (Ballatori and Clarkson, 1983; Hirayama *et al*., 1987). *Chlorella* contains high amounts of protein and dietary fiber, as well as all kinds of vitamins, essential fatty acids, and many kinds of essential minerals. Of these ingredients, the dietary fiber and the complex lipid fraction, which is rich in polyunsaturated fatty acids, have been shown to inhibit the re-absorption of bile acid in the intestinal tract, resulting in accelerated bile secretion in the enterohepatic circulation (Sano, 1982). In addition, the dietary fiber contained in whole *Chlorella* increases the amount of feces excreted by animals (Sano, 1982; Uchikawa *et al*., 2011) and humans (Fujiwara *et al.*, 1998), and dietary fiber has been shown to absorb some MeHg *in vitro* (Uchikawa *et al*., 2010). These above-mentioned characteristics of *Chlorella* are considered to be related to the increase in excretion of MeHg in the feces. Accordingly, the promotion of fecal MeHg excretion by the acceleration of bile secretion, the absorption of MeHg with dietary fiber in the intestinal tract and the increase of feces production might contribute to the lowering of the hair and blood mercury levels observed in the present study.

The present study, a non-placebo, open-label clinical design, was the first trial to assess the effects of supplementation with *Chlorella* on MeHg levels of the body in healthy subjects. The results obtained here suggest that supplementation with *Chlorella* for 3 months in healthy subjects reduces mercury levels in hair and blood. These results could provide evidence for its efficacy and be used to design future comprehensive studies.

Conflict of interest---- Chlorella Industry Co. Ltd. provided the test food used in the study. Hisao Kitsuki and Akira Yasutake have no competing interests.

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