Simultaneous Effects of Green Tea Extracts and Fish Oil on Mercury Accumulation and Antioxidant Defenses in Methylmercury-exposed Adult Mice

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Received May 23, 2013; Accepted June 25, 2013

Male mice (4 months old) were fed green tea extract- (GTE; 0.25% w/w) and/or fish oil- (FO; 5% w/w) containing diets with 8 ppm methylmercury (MeHg) chloride for 4 months to investigate the effects of simultaneous intakes on brain functions, antioxidant defenses against MeHg, and MeHg accumulation in tissues. In mouse maze tests, intake of GTE or FO significantly improved the learning ability of MeHg-exposed mice. GTE and FO also significantly decreased liver and brain catalase activities in MeHg-exposed mice. Total Hg concentrations in muscle were significantly lowered by dietary GTE and FO in the MeHg-exposed group, though no remarkable differences were observed in the brain. These data indicate that simultaneous intake of GTE and FO effectively prevents MeHg-mediated oxidative stress and reduces the effects of Hg exposure with fish consumption.

Keywords: fish oil, green tea, methylmercury, ascorbic acid, thiobarbituric acid reactive substances

Introduction

Fish contains several nutrients that are beneficial to human health. However, fish has been known to contain hazardous components such as methylmercury (MeHg), which has been linked to decreased neuropsychological function (Farina *et al.*, 2011) and increased risk of cardiovascular diseases (Choi *et al.*, 2009; Virtanen *et al.*, 2007). In contrast, n-3 polyunsaturated fatty acids (PUFA) contained in fish improve brain function (Guesnet *et al.*, 2011; Mourek and Mourek, 2011) and reduce the risk of cardiovascular diseases (Cottin *et al.*, 2011). Some reports indicate that dietary fat can influence oxidative DNA damage, nephrotoxicity, and steroidogenic enzyme activities after MeHg exposure (Grotto *et al.*, 2011; Jin *et al.*, 2008, 2009; McVey *et al.*, 2008). However, the human diet varies widely, and some reports have shown that foodstuffs other than seafood may also prevent MeHg toxicity and accumulation (Abdalla *et al.*, 2010;

Farina *et al.*, 2005; Lee *et al.*, 1999; Rowland *et al.*, 1986; Sumathi *et al.*, 2012). Therefore, daily fish consumption may alleviate relatively few health risks.

In general, the Japanese drink a few cups of green tea every day, and often accompany sushi meals with green tea. Therefore, a close relationship exists between green tea and fish oil in the Japanese diet. Canuel *et al.* (2006) concluded that tea might accelerate the enterohepatic MeHg cycle and contribute to temporary MeHg bioamplification in the bloodstream. Green tea contains large quantities of polyphenols that have antioxidant effects and accelerate cadmium (Cd) excretion by forming chelates (Abib *et al.*, 2011; Yu *et al.*, 2007; Wang *et al.*, 2012). Some studies indicate that green tea consumption reduces the risk of coronary heart disease (Bøhn *et al.*, 2012; Chacko *et al.*, 2010) and prevents age-related neurodegeneration (Andrade and Assunção, 2012). Thus, these green tea polyphenol properties may be effective against MeHg toxicity.

The aim of this study was to determine the interactive influence of fish oil and green tea on the effects of MeHg ex-

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posure, antioxidant enzyme activities in the liver and brain of adult mice, MeHg tissue accumulation, and animal behavior.

Materials and Methods

Diet The experimental diet contained 5% fat (lard or fish oil), 48.8% corn starch, 20.0% casein, 15.0% granulated sugar, 5.0% cellulose powder, 4.0% salt mixture, 2.0% vitamin mixture, and 0.2% L-methionine. Vitamin and mineral mixtures were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). The mineral mixture contained 14.56 g $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 25.72 g KH_2PO_4 , 9.35 g NaH_2PO_4 , 4.66 g NaCl , 35.09 g Ca-lactate, 3.18 g Fe-citrate, 7.17 g MgSO_4 , 0.11 g ZnCO_3 , 0.12 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.03 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 0.01 g KI per 100 g. The vitamin mixture contained 0.1 g vitamin A-acetate, 0.25 mg vitamin D_3 , 0.5 g vitamin E-acetate, 0.52 g vitamin K_3 , 0.12 g vitamin $\text{B}_1\text{-HCl}$, 0.4 g vitamin B_2 , 0.08 g vitamin $\text{B}_6\text{-HCl}$, 0.05 mg vitamin B_{12} , 3.0 g vitamin C, 2.0 mg D-biotin, 0.02 g folic acid, 0.5 g calcium pantothenate, 0.5 g p-aminobenzoic acid, 0.6 g niacin, 0.6 g inositol, 20 g choline chloride, and 73.1 g cellulose powder per 100 g. MeHg chloride (75% purity) was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Lard (16:0, 27.4%; 18:0, 18.2%; 18:1n-9, 35.8%; 18:2n-6, 9.9%; 18:3n-3, 0.8%) was purchased from Oriental Yeast Co., Ltd. Fish oil (FO) (16:0, 20.9%; 18:0, 5.6%; 18:1n-9, 16.7%; 18:2n-6, 1.2%; 20:5n-3, 6.1%; 22:6n-3, 23.7%) was supplied by Nippon Suisan Kaisha Ltd (Tokyo, Japan). Green tea extracts (GTE) (Polyphenon GTM; total catechin 27.5%, caffeine 7.0%) was supplied by Mitsui Norin Co., Ltd (Tokyo, Japan) (Hara, 2001). MeHg chloride was added to mouse diets at 8 ppm. This dose was selected to prevent mortality during the feeding period due to MeHg toxicity (Shirasu *et al.*, 1983; Eto *et al.*, 1997; Heath *et al.*, 2010). In GTE free diets, GTE (at 0.25% w/w) was replaced with cornstarch. Experimental diets were prepared once a month and stored below -30°C to prevent oxidation.

Animals Male Crlj:CD-1 (ICR) mice (4 weeks old) were obtained from Charles River Japan, Inc. (Atsugi, Kanagawa, Japan). All mice were switched from laboratory chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) to experimental diets at 4 months of age. Mice were randomly divided into 5 groups of 10–11 animals each and fed the following experimental diets: control, MeHg, MeHg + GTE, MeHg + FO, MeHg + FO + GTE. Mice were housed in polycarbonate cages containing shredded paper. The animal room was maintained at $24 \pm 0.5^\circ\text{C}$, with a relative humidity of $65 \pm 5\%$ and a 12-h light/dark cycle. Diets and water were provided *ad libitum*. Food consumption did not differ between experimental groups. Body weights were measured once a month. Animals were handled according to the guidelines

Fig. 1. Experimental maze.

for laboratory animal studies of the Ministry of Agriculture, Forestry and Fisheries. All animal procedures were reviewed and approved by the Animal Care and Use Committee of the National Food Research Institute, National Agriculture and Food Research Organization (NARO), Japan.

Assessment of Maze-behavior An analytical video tracking and motion system (Library Co., Ltd., Tokyo, Japan) was used to assess maze behavior in mice. Direct records of X and Y co-ordinates were derived for each mouse movement and stored on the computer. Maze behavior was assessed 4 months after the start of the feeding trial. Prior to the maze-behavior test, all mice were conditioned in a simple maze of three partition walls. This procedure involved training thirsty mice to search for water that was placed outside the maze exit. A maze arrangement of 8 blind alleys was used in the experimental trial (Fig. 1). The trial was conducted after overnight water deprivation so that thirsty mice sought water that was placed at the maze exit in the same way as in the training exercise. The time required and distance traveled to reach the maze exit, and the number of times that a mouse strayed into blind alleys in the maze, were recorded. Two trials (trial 1 and 2) were performed under the same conditions every day, and improvements in times and distances traveled to the exit were calculated.

Preparation of plasma and brain samples Blood was collected from the inferior vena cava using a heparinized syringe under anesthesia with isoflurane. Whole brains of mice were subsequently removed and immediately flash frozen in liquid nitrogen. Plasma was separated by centrifugation at 900 g for 20 min at 4°C . Plasma and brain samples were stored at -80°C until analysis. Brains were homogenized in 50 mmol/L ice-cold phosphate buffer (pH 7.5) and 0.5% Triton X-100 (4.5 mL/g of tissue) using a Teflon-glass homogenizer. Antioxidant enzyme activities, thiobarbituric acid reactive substances (TBARS), and potential antioxidant (PAO) activity were measured in sample supernatants, which were centrifuged at 10,000 g for 10 min at 4°C . Total protein in the supernatant was determined using the Lowry method (1951). Plasma chemical parameters, such as urea nitrogen (UN), uric acid (UA), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT), were de-

terminated using Urea N B-test Wako, Uric acid C-test Wako, and Transaminase CII-test Wako test kits, respectively (Wako Pure Chemical Industries, Osaka, Japan). Ascorbic acid and TBARS concentrations in plasma and brain were determined using Vitamin C (Shima Laboratories Co., Ltd, Tokyo, Japan) and TBARS assay kits (Cayman Chemical Company, MI, USA). PAO was measured using an antioxidant assay kit (Japan Institute for the Control of Aging [JICA], Nikken Seil Co., Ltd., Shizuoka, Japan). PAO value was represented as the antioxidant ability of urea. Total glutathione (GSH) content in whole brains was determined using a GSH Assay Kit (Bioxytech GSH-420; OXIS Health products, Inc., OR, USA). Catalase, glucose 6-phosphate dehydrogenase (G6PDH), and glutathione reductase (GR) activities in brain supernatants were measured according to the methods described by Aebi (1984), Kelley and Kletzien (1984), and Ellerby and Bredesen (2000), respectively.

Total Hg and selenium (Se) analysis Muscle and brain homogenates were decomposed in an acid mixture containing sulfuric acid, nitric acid, and perchloric acid at a ratio of 2:1:1 at 220°C overnight. Hg concentrations were measured in the acidic decomposition solution using a Cold Vapor Atomic Absorption Spectrometry HG-310 analyzer (Hiranuma Sangyo Co., Ltd., Ibaraki, Japan). Se concentrations

were measured using a fluorometric assay with 2,3-diaminonaphthalene (Watkinson, 1966).

Statistical analyses Data are presented as means $\pm$ SE. Statistical analyses were performed using Microsoft Excel add-in software (Social Survey Research Information Co., Ltd., Tokyo, Japan). Differences between control and MeHg-exposed groups were determined using a t-test. The effects of FO and/or GTE with Hg were analyzed using one-way ANOVA with Dunnett's test and considered significant at $p < 0.05$.

Results

The time required and distance traveled to reach the maze exit, and the number of times that mice strayed into blind alleys, are presented in Fig. 2. The control group made significantly fewer mistakes and reached the maze exit in significantly shorter time than those in the first trial. In the second trial, the GTE-treated group achieved significant reductions in all three parameters compared with that in the first trial. The FO-treated group also reached the exit in significantly shorter time in the second trial.

Average food intake did not vary between experimental diet groups (3.6 – 3.7 g/day/mouse). Body composition is shown in Table 1. No significant differences in body composition parameters were observed between control and MeHg-

Fig. 2. The effects of GTE and FO on MeHg-induced alterations in the maze performance of mice. Parameters assessed were the time required and distance traveled to reach the maze exit, and the numbers of times that a mouse strayed into blind alleys. *: $p < 0.05$ vs. Trial 1.

Table 1. Body composition of mice fed experimental diets.

	Control	MeHg	MeHg + GTE	MeHg + FO	MeHg + GTE + FO
Body weight (g)	51.9 $\pm$ 1.2	53.6 $\pm$ 1.0	48.2 $\pm$ 2.5	48.5 $\pm$ 1.3	46.0 $\pm$ 1.8 *
Liver (g)	2.1 $\pm$ 0.1	2.3 $\pm$ 0.1	2.1 $\pm$ 0.1	1.9 $\pm$ 0.1	2.0 $\pm$ 0.1
(%)	4.0 $\pm$ 0.1	4.2 $\pm$ 0.1	4.4 $\pm$ 0.2	4.0 $\pm$ 0.1	4.3 $\pm$ 0.2
Fat tissue (g)	2.0 $\pm$ 0.2	2.2 $\pm$ 0.2	1.6 $\pm$ 0.3	1.5 $\pm$ 0.1	1.2 $\pm$ 0.2 **
(%)	3.8 $\pm$ 0.3	4.1 $\pm$ 0.3	3.1 $\pm$ 0.6	3.1 $\pm$ 0.2	2.5 $\pm$ 0.3 *
Brain (g)	0.51 $\pm$ 0.01	0.52 $\pm$ 0.01	0.52 $\pm$ 0.01	0.52 $\pm$ 0.01	0.52 $\pm$ 0.01
(%)	0.99 $\pm$ 0.03	0.98 $\pm$ 0.02	1.11 $\pm$ 0.07	1.09 $\pm$ 0.03	1.15 $\pm$ 0.05 *

#: $p < 0.05$, ##: $p < 0.01$ vs. Control *: $p < 0.05$, **: $p < 0.01$ vs. MeHg (n = 10 – 11).

exposed groups. However, the GTE + FO-treated group showed significantly reduced body and fat tissue weights and higher brain mass compared with the MeHg-exposed group. In contrast, GTE- and FO-treated groups showed a tendency towards lower percent fat tissue mass compared with the MeHg-exposed group.

Total Hg and Se concentrations in tissues are presented in Fig. 3. Hg was detected at trace levels or was not detected in the muscle and brain tissues from the control group. Total Hg concentrations in muscle were significantly lower in GTE-, FO-, and GTE + FO-treated groups than in the MeHg-exposed group. However, total Hg concentrations in brain tissues were not significantly influenced by the experimental diets. Total Se concentrations were significantly reduced in the muscle tissues of MeHg supplemented mice; in comparison, levels were significantly increased in the muscle tissues of FO- and FO + GTE-treated groups. Total Se concentrations in brains were not significantly influenced by experimental diets.

Plasma chemical parameters of mice fed the experimental diets are shown in Table 2. The MeHg-exposed group showed significant lowering of urea nitrogen and uric acid concentrations in comparison with the control group. GTE- and GTE + FO-treated groups had significantly lower ratios of GOT to GPT than the MeHg-exposed group. The GTE + FO-treated group had a significantly higher plasma urea

nitrogen concentration compared with the MeHg-exposed group.

Antioxidant parameters in plasma, brain, and liver samples are presented in Table 3. The MeHg-exposed group had significantly lower brain TBARS and plasma PAO concentrations and higher brain PAO concentrations than the control group. The GTE-treated group had significantly reduced liver TBARS and brain PAO concentrations compared with the MeHg-exposed group. The FO-treated group showed significantly higher brain and liver TBARS, and significantly lower brain PAO and ascorbic acid concentrations than the MeHg-exposed group. In contrast, the GTE + FO-treated group had significantly increased plasma and liver PAO concentrations compared with the MeHg-exposed group. This group also had a significant increase in brain TBARS and reduced brain PAO and ascorbic acid concentrations compared with the MeHg-exposed group.

Antioxidant enzyme activities in the liver and brain are presented in Fig. 4. The MeHg-exposed group had significantly higher GR and G6PDH activities in the liver than the control group. Although catalase and GR activities in the GTE-treated group were significantly lower than those in the MeHg-exposed group, G6PDH activity was significantly higher. Catalase, GR, and G6PDH activities in FO- and FO + GTE-treated groups were significantly lower than those in the MeHg group. Significantly increased brain catalase and

Fig. 3. The total mercury and selenium concentrations in the muscle and brain of mice fed experimental diets.

#: $p < 0.05$, ##: $p < 0.01$ vs. Control, *: $p < 0.05$, **: $p < 0.01$ vs. MeHg §: trace or not detected.

Table 2. Plasma chemical parameters of mice fed experimental diets.

	Control	MeHg	MeHg + GTE	MeHg + FO	MeHg + GTE + FO
GPT KU	9 ± 1	8 ± 1	11 ± 1	9 ± 0	11 ± 1
GOT KU	25 ± 1	32 ± 5	30 ± 4	24 ± 1	27 ± 3
GOT/GPT	2.66 ± 0.11	2.98 ± 0.18	2.39 ± 0.10 *	2.66 ± 0.09	2.37 ± 0.18 *
UN (mg/dL)	24 ± 1	21 ± 1 ##	23 ± 1	21 ± 1	24 ± 1 *
UA (mg/dL)	2.16 ± 0.09	1.62 ± 0.08 ##	1.79 ± 0.11	1.62 ± 0.11	1.43 ± 0.09

#: $p < 0.05$, ##: $p < 0.01$ vs. Control, *: $p < 0.05$, **: $p < 0.01$ vs. MeHg (n = 10 – 11).

Table 3. Plasma, brain and liver chemical parameters of mice fed experimental diets.

	Control	MeHg	MeHg + GTE	MeHg + FO	MeHg + GTE + FO
TBARS					
Plasma ($\mu\text{M/mL}$)	24 ± 1	27 ± 1	23 ± 2	25 ± 1	26 ± 1
Brain ($\mu\text{M/mg protein}$)	1.1 ± 0.1	$0.8 \pm 0.1 \text{ ##}$	1.0 ± 0.1	$1.2 \pm 0.1 \text{ **}$	$1.0 \pm 0.0 \text{ *}$
Liver ($\mu\text{M/mg protein}$)	1.9 ± 0.1	2.0 ± 0.1	$1.6 \pm 0.0 \text{ **}$	$2.6 \pm 0.1 \text{ **}$	2.2 ± 0.1
PAO					
Plasma ($\mu\text{M/mL}$)	243 ± 5	$200 \pm 7 \text{ ##}$	212 ± 7	213 ± 6	$239 \pm 7 \text{ **}$
Brain ($\mu\text{M/mg protein}$)	32.2 ± 0.7	$34.9 \pm 0.6 \text{ #}$	$25.8 \pm 0.9 \text{ **}$	$27.2 \pm 1.2 \text{ **}$	$31.4 \pm 0.8 \text{ *}$
Liver ($\mu\text{M/mg protein}$)	2.56 ± 0.03	2.60 ± 0.02	2.54 ± 0.01	2.58 ± 0.04	$2.68 \pm 0.01 \text{ *}$
Ascorbic acid					
Plasma ($\mu\text{g/mL}$)	2.14 ± 0.19	1.77 ± 0.10	1.67 ± 0.11	1.59 ± 0.10	1.50 ± 0.06
Brain ($\mu\text{g/g}$)	442 ± 20	447 ± 17	414 ± 10	$375 \pm 9 \text{ **}$	$397 \pm 12 \text{ *}$
Liver ($\mu\text{g/g}$)	20 ± 1	18 ± 1	18 ± 1	17 ± 1	17 ± 1
GSH					
Brain (nM/g)	2098 ± 44	2017 ± 37	1823 ± 31	2009 ± 49	2058 ± 97
Liver ($\mu\text{M/g}$)	8.1 ± 0.2	8.5 ± 0.3	8.4 ± 0.2	8.7 ± 0.4	8.8 ± 0.3

#: $p < 0.05$, ##: $p < 0.01$ vs. Control *: $p < 0.05$, **: $p < 0.01$ vs. MeHg (n = 10 – 11).

Fig. 4. Antioxidative enzyme activities in the liver and brain of mice fed experimental diets.

#: $p < 0.05$, ##: $p < 0.01$ vs. Control *: $p < 0.05$, **: $p < 0.01$ vs. MeHg.

GR activities were observed in the MeHg-exposed group compared with the control group. Although no significant differences in GR activity were observed, catalase activity was significantly lower among GTE-, FO-, and GTE + FO-treated groups than in the MeHg-exposed group. Finally, G6PDH activity was significantly lower in the GTE-treated group than in the MeHg-exposed group.

Discussion

A previous study demonstrated the effects of MeHg exposure on neurological function in animals and humans (Farina *et al.*, 2011), in which, MeHg-exposed mice failed to significantly reduce the number of entries into blind alleys or the distance and time to exit between trials 1 and 2. In contrast, the control group was significantly quicker to exit and made significantly fewer mistakes in the second trial. These observations indicate that MeHg treatment leads to the deterioration of learning ability in mice. In contrast, the performances of the GTE + FO-treated group in the maze test were not significantly different from those in the control group. However, MeHg-exposed mice treated with GTE or FO showed significant reductions in maze parameters between trials 1 and 2. In a previous study, simultaneous intake of FO and catechin (a component of GTE) was associated with excellent searching abilities compared with either supplement alone (Shirai and Suzuki, 2004a). In agreement, the GTE + FO-treated group in the present study also achieved low maze parameters, indicating that GTE + FO supplementation maintains excellent searching abilities against MeHg-mediated neurological impairments. Some reports indicate that GTE and/or FO intake could improve brain function in animal and human studies (Su, 2010; Innis, 2007; Rendeiro *et al.*, 2012; Kakuda, 2011; Giunta *et al.*, 2010), further suggesting that GTE and/or FO consumption could prevent MeHg-mediated deterioration of brain function.

Abib *et al.* (2011) demonstrated that epigallocatechin-3-gallate (EGCG) may attenuate cadmium toxicity by preventing lipid oxidation. Several studies have revealed that GTE and n-3 PUFA reduce oxidative stress in the brain (Andrade and Assunção, 2012; Rendeiro *et al.*, 2012; Innis, 2007; Su, 2010). However, brain TBARS concentrations were significantly reduced in the MeHg-exposed group, but were not significantly reduced in the GTE-, FO-, and GTE + FO-treated groups. In contrast, brain PAO levels were significantly higher in the MeHg-exposed group than in the control group, and were significantly lower in the GTE-, FO-, and GTE + FO-treated groups. Brain catalase and GR activities were significantly higher in the MeHg-exposed group than in the control group. These high activities may be linked with the high PAO activity in the MeHg-exposed group. In our previ-

ous study, brain catalase activity was significantly higher in mice fed a diet containing 2 ppm MeHg than in the control animals, though brain TBARS concentrations did not differ between these groups (Shirai *et al.*, 2012). These observations imply that MeHg exposure leads to oxidative stress without increasing brain TBARS. In contrast, significantly lower brain catalase activity was observed in GTE-, FO-, and GTE + FO-treated groups than in the MeHg-exposed group, suggesting that MeHg-mediated oxidative stress is ameliorated by these dietary supplements, both alone and in combination. Ascorbic acid concentrations were significantly lower in the brains of FO- and FO + GTE-treated groups than in those of the MeHg-exposed group. Our previous observations indicated that dietary FO lowered brain ascorbic acid concentrations in mice on a high lard diet (Shirai *et al.*, 2012). Therefore, brain ascorbic acid concentrations do not appear to be affected by MeHg exposure, further indicating that GTE and/or FO intake could prevent MeHg-mediated deterioration of brain function by reducing oxidative stress.

Proper liver function is critical for the elimination of deleterious compounds. However, no significant differences in plasma GOT and GPT activities were observed between the experimental diet groups of this study, and MeHg exposure did not remarkably affect TBARS concentrations compared with the control group. However, liver GR and G6PDH activities were significantly greater in the MeHg-exposed group than in the control group, implying oxidative stress in these animals. Nonetheless, dietary supplementation with GTE, FO, or GTE + FO significantly lowered catalase and GR activities in the MeHg-exposed group. Furthermore, supplementation with GTE significantly lowered TBARS levels and increased G6PDH activity compared with the MeHg-exposed group. These observations suggest that GTE intake may suppress MeHg-associated oxidative stress in the liver. However, GTE intake did not result in significant changes in plasma GOT or GPT activity, though the ratio of GOT to GPT was significantly lower than that in the MeHg-exposed group. This represented a slight increase in GPT activity among the GTE fed mice. The GTE group had slightly less fatty tissue than the MeHg-exposed group, regardless of the high G6PDH activity. Because G6PDH contributes to antioxidant and energy metabolism, these data suggest that GTE may promote energy metabolism during exposure to MeHg. Subsequent enhancement of physiological stress on the liver may result in increased GPT activity in GTE-treated mice.

Catechins have been shown to chelate Cd and accelerate its excretion (Abib *et al.*, 2011). Hence, it was assumed that GTE intake may reduce Hg accumulation in tissues. In this study, GTE and/or FO intake prevented Hg accumulation in muscle tissues. While both GTE and FO groups had

insignificantly lower body and fat tissue weights than the MeHg-exposed group, combined supplementation with GTE and FO significantly reduced body and fat tissue weights compared with those of the MeHg-exposed group. Previous reports have indicated that dietary GTE and FO can reduce body weight by increasing fat oxidation (Rains *et al.*, 2011; Martínez-Victoria and Yago, 2012; Buckley and Howe, 2010; Sae-Tan *et al.*, 2011). Previously, we showed that FO intake also reduced Hg accumulation in muscle and led to loss of body weight in MeHg-exposed mice (Shirai *et al.*, 2012). Greener *et al.* (1983) indicated that most of the MeHg was associated with a high molecular weight lipoprotein (HDL) fraction in vitro, and Yun *et al.* (2013) indicated that the mercury-containing protein in the human plasma is human serum albumin. GTE intake possibly suppresses Hg accumulation by the same mechanism. Taken together, these data suggest that fish oil and/or GTE intake may release mercury-containing HDL from muscle by promoting fat oxidation, thereby excreting MeHg from the body. However, Se concentrations in muscle were significantly increased by dietary FO and FO + GTE in MeHg-exposed mice. Some studies have implied that Se and related components can ameliorate MeHg toxicity and enhance its excretion (de Freitas *et al.*, 2009). In this study, exposure to MeHg significantly lowered muscle Se concentrations, but FO intake significantly restored muscle Se concentrations in these mice. Hence, Se may play an important role in reducing MeHg toxicity and accumulation. However, some reports demonstrated that Se consumption may increase the percentage of n-3 PUFAs in tissues (Pappas *et al.*, 2006a, 2006b; Haug *et al.*, 2007). Hence, FO-mediated increases in muscle Se may also be related to antioxidant metabolism in muscle. On the other hand, Hg and Se concentrations in the brain did not differ significantly between experimental diet groups.

In this study, dietary GTE and/or FO appeared to improve learning ability regardless of Hg accumulation in the brain. This positive effect of GTE and FO intake may reflect protection against MeHg-mediated oxidative damage. Furthermore, it is reported that EGCG protects neurons by suppressing production of NADPH oxidase-mediated reactive oxygen species and NADPH-d/nNOS expression, and that FO improves the fluidity of synaptic membranes (Wei *et al.*, 2011; He *et al.*, 2011; Suzuki *et al.*, 1998; Yang *et al.*, 2011; Hashimoto *et al.*, 2006). Furthermore, Grotto *et al.* (2011) agree that the protective effects of FO during MeHg exposure may be related to its anti-inflammatory effects. These mechanisms may have contributed to the present observations of improved brain functions in MeHg-exposed mice.

Our data demonstrate that GTE intake attenuates MeHg toxicity and Hg accumulation in muscle, presumably by sup-

pressing oxidative stress and promoting fat metabolism. The Japanese frequently eat fish with green tea. While this dietary combination may have health advantages, high fish consumption elevates exposure to MeHg. It is likely that other foods also reduce the risk of MeHg toxicity and Hg accumulation. Ouédraogo *et al.* (2011) suggested that Hg bioaccessibility from fish can be modified by cooking and by coingestion of tea and coffee. Therefore, it is difficult to estimate the risks of excessive fish eating. Nonetheless, it is clear that dietary fish and green tea have health benefits. Moreover, some studies have shown that simultaneous ingestion of fish and green tea promotes health more effectively than either alone (Giunta *et al.*, 2010; Shirai and Suzuki, 2004a, 2004b, 2008), suggesting that GTE could be consumed to maximize the health benefits of fish.

Acknowledgments This study was supported by a Grant from the Ministry of Agriculture, Forestry and Fisheries (Development of new technology on regulatory science: No. 2027).

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