JLR RESEARCH ARTICLE

Maternal DHA intake in mice increased DHA metabolites in the pup brain and ameliorated MeHg-induced behavioral disorder

Ami Oguro[1]*, Taichi Fujiyama[1], Yasuhiro Ishihara[2], Chisato Kataoka[3], Megumi Yamamoto[3], Komyo Eto[3], Yoshihiro Komohara[4], Susumu Imaoka[5], Toshihide Sakuragi[6,7], Mayumi Tsuji[6], Eiji Shibata[8], Yaichiro Kotake[1], and Takeshi Yamazaki[9]

[1]Graduate School of Biomedical and Health Sciences, and [2]Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan; [3]National Institute for Minamata Disease, Kumamoto, Japan; [4]Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; [5]Department of Biomedical Sciences, School of Biological and Environmental Sciences, Kwansei Gakuin University, Hyogo, Japan; [6]Department of Environmental Health, School of Medicine, and [7]Department of Obstetrics and Gynecology, School of Medicine, University of Occupational and Environmental Health, Fukuoka, Japan; [8]Department of Obstetrics and Gynecology, Dokkyo Medical University, Tochigi, Japan; and [9]Program of Life and Environmental Sciences, Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan

Abstract Although pregnant women's fish consumption is beneficial for the brain development of the fetus due to the DHA in fish, seafood also contains methylmercury (MeHg), which adversely affects fetal brain development. Epidemiological studies suggest that high DHA levels in pregnant women's sera may protect the fetal brain from MeHg-induced neurotoxicity, but the underlying mechanism is unknown. Our earlier study revealed that DHA and its metabolite 19,20-dihydroxydocosapentaenoic acid (19,20-DHDP) produced by cytochrome P450s (P450s) and soluble epoxide hydrolase (sEH) can suppress MeHg-induced cytotoxicity in mouse primary neuronal cells. In the present study, DHA supplementation to pregnant mice suppressed MeHg-induced impairments of pups' body weight, grip strength, motor function, and short-term memory. DHA supplementation also suppressed MeHg-induced oxidative stress and the decrease in the number of subplate neurons in the cerebral cortex of the pups. DHA supplementation to dams significantly increased the DHA metabolites 19,20-epoxydocosapentaenoic acid (19,20-EDP) and 19,20-DHDP as well as DHA itself in the fetal and infant brains, although the expression levels of P450s and sEH were low in the fetal brain and liver. DHA metabolites were detected in the mouse breast milk and in human umbilical cord blood, indicating the active transfer of DHA metabolites from dams to pups. These results demonstrate that DHA supplementation increased DHA and its metabolites in the mouse pup brain and alleviated the effects of MeHg on fetal brain development. Pregnant women's intake of fish containing high levels of DHA (or DHA supplementation) may help prevent MeHg-induced neurotoxicity in the fetus.

Supplementary key words Brain Lipids • Cytochrome P450 • Omega-3 fatty acids • Pregnancy • Toxicology • docosahexaenoic acid (DHA) • dihydroxydocosapentaenoic acids (DHDPs) • soluble epoxide hydrolase (sEH) • methylmercury • neurotoxicity

Docosahexaenoic acid (DHA) is a major ω-3 polyunsaturated fatty acid (PUFA) that is abundant in the brain and essential for neuronal development and optimal cognitive health (1). DHA is esterified into membrane phospholipids which regulate membrane flexibility and fluidity, and DHA regulates synaptic integrity and plasticity in the brain. Free DHA produced by phospholipase A2 acts as a signal molecule by activating several receptors, and it is also converted into various bioactive metabolites known as docosanoids (2). It has been shown that DHA intake can alleviate the symptoms of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease (3). DHA is also transferred from mothers to fetuses and infants via the placenta and breast milk, and an epidemiological study revealed that DHA intake by mothers increases the psychomotor development index of their children (4).

DHA is abundant in fish, but because methylmercury (MeHg) is also concentrated in fish and shellfish through the food chain, a high consumption of fish by pregnant women raises concerns about MeHg neurotoxicity in the fetal brain. Such adverse effects on fetal brain development can occur because a complex formed between MeHg and cysteine allows MeHg to

*For correspondence: Ami Oguro, aoguro@hiroshima-u.ac.jp.

 https://doi.org/10.1016/j.jlr.2023.100458

pass through the blood-brain barrier and the placenta (5, 6), enabling the transfer of MeHg from mother to fetus. Indeed, the concentration of MeHg in umbilical cord blood has been shown to be significantly greater than that in maternal blood (7), suggesting that fetuses are at higher risk of MeHg toxicity than their mothers. Thus, the consumption of fish by pregnant women has a beneficial effect on fetal brain development via DHA supplementation while simultaneously conferring a risk of MeHg-induced neurotoxicity in the fetal brain. However, a cohort study conducted in the Republic of Seychelles showed that among the 20-month-old children of mothers with lower levels of ω-3 PUFAs in their blood, the MeHg concentrations in the mothers' hair were negatively correlated with the psychomotor development index of the children (8). A cohort study performed in the Faroe Islands revealed that 7-year-olds' short-term recall on the California Verbal Test was decreased with higher MeHg concentrations in their mothers' cord blood, and this relationship became stronger after adjustment for the ω-3 PUFA concentrations (9). These findings suggest that a maternal intake of ω-3 PUFAs such as DHA can attenuate MeHg-induced neurotoxicity in the fetal brain, but the underlying mechanisms have not been established.

An earlier study by our group demonstrated that DHA can reduce the MeHg-induced neurotoxicity in human SH-SY5Y cells and mouse primary neuronal cells by activating retinoid X receptor and thereby inducing antioxidant genes (10), suggesting that DHA is neuroprotective against MeHg. In addition to DHA, the DHA metabolite 19,20-dihydroxydocosapentaenoic acid (19,20-DHDP) also had a neuroprotective effect against MeHg. DHA is known to be metabolized to various bioactive metabolites by lipoxygenase, cyclooxygenase-2, or cytochrome P450s (P450s). The lipoxygenase-mediated metabolites, which are known as protectins (PDs), maresins, and resolvins (RvDs) are known to have neuronal protective and antiinflammatory effects (11). DHA is also metabolized to the epoxidized forms known as epoxydocosapentaenoic acids (7,8-EDP, 10,11-EDP, 13,14-EDP, 16,17-EDP, and 19,20-EDP) or metabolized to monohydroxylated forms by P450s, and EDPs are further hydrolyzed to the corresponding diols (DHDPs) by soluble epoxide hydrolase (sEH) (12). Another of our group's investigation revealed that DHA supplementation in adult rats increased the DHDP levels in the brain, although the levels of EDPs and that of DHA itself were not increased; we also observed that the production of DHDPs was important for the neuroprotective effect of DHA intake against rotenone-induced neurotoxicity (13). An increase in reactive oxygen species is the major mechanism that has been proposed to mediate MeHg-induced neurotoxicity due to the binding of MeHg to thiol groups of manganese superoxide dismutase and glutathione (14). In our above-mentioned research, we observed that 19,20-DHDP increased the expression levels of antioxidant enzymes such as SOD1 and catalase in neuronal cells (10, 13), which can be expected to be related to the neuroprotective effects of 19,20-DHDP.

In the present study, we investigated the effect of a maternal supplementation of DHA in mice on MeHg-induced neurotoxicity in the pup brain in vivo, and we analyzed the accumulation and production of DHA metabolites in the brains of the mouse fetuses and infants.

MATERIALS AND METHODS

Reagents

Methylmercury chloride was obtained from Tokyo Chemical Industry (Tokyo). DHA, DHA-d5, 7,8-EDP, 10,11-EDP, 13,14-EDP, 16,17-EDP, 19,20-EDP, 7,8-DHDP, 10,11-DHDP, 13,14-DHDP, 16,17-DHDP, and 19,20-DHDP were purchased from Cayman Chemical Co. (Ann Arbor, MI).

Animal treatment

C57BL/6J mice were purchased from SLC (Shizuoka, Japan). Ten-week-old male mice (10 weeks) were mated with 8-week-old female mice, and the vaginal plug was checked the next morning. The plugged female were randomly allocated to three experimental groups (control, MeHg, and MeHg+DHA groups), and each pregnant mouse was kept in separate cages (n = 5 for the behavioral test and for the measurements of lipid peroxidation and total Hg, n = 3 for the neuropathological analysis, and n = 4 for the liquid chromatography-mass spectrometry [LC-MS] analysis). We divided the pups randomly for each experiment. The mice were freely fed a diet (AIN-93G) containing cottonseed oil at a final concentration of 7% (w/w) with or without DHA supplementation. DHA was added to the cottonseed oil diet at a final concentration of 4% (w/w) of total fat. Methylmercury chloride was dissolved in water containing an equal amount of L-cysteine and then added to drinking water at a concentration of 4 ppm. MeHg and DHA were administered to mice from day 0 of pregnancy to postnatal day 21. The mice were maintained in a temperature-controlled animal facility with a 12-h light-dark cycle. The pups were sacrificed at postnatal day 21 for the measurement of total Hg or used for the behavioral test at postnatal day 33 and subsequently sacrificed for the measurement of lipid peroxidation. Pups from at least three different sets of parents were used as one experimental group. Control or DHA-fed female mice (n = 4) and their pups on embryonic day (E)16 and postnatal days (P)1, 7, and 14 (n = 4–6) were also sacrificed for the LC-MS analysis. Control mice at E16, P1, P3, P7, P10, P13, P21, and P56 (n = 3–5) were used for PCR and Western blotting analysis. All animal experiments were conducted in accord with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan. The Animal Care and Use Committee of Hiroshima University approved the experimental protocols (No. A22-71-2).

Behavioral analysis

On postnatal day 33, male pups (n = 6–10) and their dams (n = 4, 16–17-weeks-old) were used for the behavioral analysis.

The forelimb grip strength of each pup and dam was measured using a BIO-GS3 grip strength tester (Bioseb, Chaville, France) in accord with the manufacturer's instructions. Each grip-strength value was normalized to the mouse's body weight. A rotarod apparatus (LE8200: Panlab, Barcelona, Spain) was used to test the motor function of the mice: the mouse was placed on a rod that was rotating at 4 rpm at 0 min and accelerated to 40 rpm after 5 min of run time, and the latency time for the mouse to fall was measured. Mice were given two trials for 3 consecutive days as training. The short-term memory of each pup and dam was measured by a Y-maze test used to analyze spatial working memory. The Y-maze consists of three arms (40 cm × 4 cm × 10 cm) interconnected at 120°angles with a common neutral area (triangular neutral zone) in the center. The mouse was placed in the center and freely explored the maze for 8 min, and then the number of maze arms visited by the mouse was counted, along with the sequence: visiting an arm was defined as placement of the hindlimbs completely within the arm. Spontaneous alternation behavior was defined as entering into three different arms consecutively. The number of maximum spontaneous alternation behaviors was defined as the total number of arms entered minus 2, and the percent spontaneous alternation was calculated as (actual alternations/maximum alternations) ×100.

Lipid peroxidation

Thiobarbituric acid-reactive substance was used as an index of lipid peroxidation and measured as described (13). Briefly, the hippocampus, cortex, and cerebellum of pups on postnatal day 33 (n = 5, male) and 16- to 17-week-old dams (n = 3) were homogenized in a 1.15% KCl solution and mixed with SDS, thiobarbituric acid, butylhydroxytoluene, and acetic acid buffer. The mixture was boiled for 1 h, and the resulting product was extracted with a 1-butanol-pyridine mixed solution. The absorbance at 532 nm was measured, with 1,1,3,3-tetraethoxypropane serving as a standard.

Quantification of total Hg in the cortex, hippocampus, and cerebellum

Tissue samples of the hippocampus, cortex, and cerebellum of pups at postnatal day 21 (n = 6, male) were suspended in a 4× volume of 0.1% L-cysteine in 2M NaOH in 1.6 ml polypropylene tubes. The samples were heated to 80°C for 20–30 min and then homogenized. Total Hg in the homogenate was quantified with a direct thermal decomposition mercury analyzer (MA-3000; Nippon Instruments, Tokyo) as described (15).

Quantification of DHA and its metabolites, DHA-epoxides (EDPs) and diols (DHDPs) by LC-MS

Control or DHA-fed female mice (n = 4) and their pups on E16 and P1, P7, and P14 (n = 4–6) were used for the LC-MS analysis. Lipid extraction from the mouse whole brain, liver, plasma, and breast milk was performed according to the protocol described by Hennebelle (16). Cold methanol containing 0.1% acetic acid and 0.1% butylated hydroxytoluene was added to the frozen tissues, plasma, or breast milk, and 10 pmol DHA-d5 was added to the homogenized tissues, plasma, or breast milk. After storage overnight at −80°C, the samples were centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant was applied to 60 mg Waters Oasis HLB 3 cc cartridges (Waters, Milford, MA), which were prerinsed with 3 ml of ethyl acetate and 6 ml of methanol and preconditioned with 6 ml of wash solution containing 5% methanol and 0.1% acetic acid in ultrapure water. The columns were then washed twice with wash solution and dried under a vacuum for 20 min. Lipids were eluted with 0.5 ml of methanol and 1.5 ml of ethyl acetate in a new tube with 6 µl of 30% glycerol/methanol. The solvent was then evaporated under nitrogen, the residue was reconstituted in 100 µl of methanol, and 10 µl of sample was analyzed by an ultra-performance liquid chromatography system coupled with an Acquity TQD tandem quadrupole mass spectrometer (Waters) and a reversed-phase column (BEH C18 1.7 µm, 2.1 × 50 mm; Waters). The column temperature was kept at 40°C. Mobile phase A (20% methanol and 0.1% acetic acid) and mobile phase B (80% acetonitrile, 20% methanol, and 0.1% acetic acid) were used, and the chromatography was performed at a flow rate of 0.2 ml/min by gradient elution according to the following protocol: a linear gradient from 100% A to 60% B at 0–14 min, 60% B at 14–18 min, a linear gradient from 60% B to 100% B at 18–19 min, and 100% B at 19–26 min. The electrospray ionization source was set in negative ion mode, and the quantification of EDPs and DHDPs was performed using the multiple reaction monitoring mode with ion transition at m/z 361 > 273 (19,20-diol), 361 > 233 (16,17-diol), 361 >193 (13,14-diol), 361 > 153 (10,11-diol), 361 > 113 (7,8-diol), 343 >241 (19,20-epo), 343 > 233 (16,17-epo), 343 > 193 (13,14-epo), 343 >153 (10,11-epo), and 343 > 141 (7,8-epo).

Isolation of RNA and reverse-transcription PCR

Total RNA was extracted from the mouse whole brain with RNAiso Plus (TaKaRa Bio, Shiga, Japan) following the manufacturer's instructions and then converted to cDNA by reverse transcription. Real-time PCR was performed using a CFX Connect instrument (Bio-Rad, Hercules, CA) with TB Green Premix Ex Taq II (TaKaRa). The primers were as follows: for mouse *Cyp2a4/5*, 5′-CCTCCATGGGGCAGGTCTAT-3′ (forward) and 5′-GTCGATGAAGTCCCTTGGGG-3′ (reverse): for mouse *Cyp2c29*, 5′- ACCTCATCCCCAAGGGAACT-3′ (forward) and 5′-TAGAAGGCATCACAGCAGGG-3′ (reverse): for mouse *Cyp2e1*, 5′-GTCATCCCCAAGGGTACAGT-3′ (forward) and 5′-AGGCCTTCTCCAACACACAC-3′ (reverse): and for mouse *Ephx2*, 5′-CCAGATGATGTGTGAGCTGA-3′ (forward) and 5′ -CCTGGCTTCACTGTCACATA-3′ (reverse). The primers for mouse *Histone* were 5′-TGGTAAGGGTGG-TAAAGGC-3′ (forward) and 5′-GCT TGG TAA TGC CCT GGA TG-3′ (reverse).

Western blotting

Mouse brain and liver tissues were homogenized with 5 volumes of a 0.15 M KCl solution containing 1 mM EDTA and 0.25 mM phenylmethylsulfonyl fluoride in a glass homogenizer. Homogenates were centrifuged at 900 *g* for 10 min at 4°C, and its supernatant fraction was further centrifuged at 12,000 *g* for 30 min at 4°C. The supernatant fraction was then centrifuged at 100,000 *g* for 60 min at 4°C, and its supernatant was used as the cytosol fraction. The microsomal pellet was suspended in a solution of 0.1 M potassium phosphate buffer (pH 7.4) containing 30% glycerol, 1 mM EDTA, and 1 mM dithiothreitol. The microsomal or cytosolic fraction was subjected to 4%–20% Mini-PROTEAN® TGX Stain-Free™ Gel (Bio-Rad). Total protein was detected by UV light exposure using Vilber Bio Imaging FUSION system (Vilber, Eberhardzell, Germany) and subsequently analyzed by Western blotting with anti-rat CYP2A1 antibody (for detection of mouse CYP2A4/5), anti-rat CYP2E1 antibody, or anti-sEH antibody. These antibodies were prepared as described (17–20).

Immunohistochemical analysis

Mouse brains (from E16 and P1) were fixed with 4% paraformaldehyde overnight at 4°C and then immersed in 30% sucrose solution overnight at 4°C and embedded in O.C.T. compound (Sakura, Tokyo) at −80°C until cryosectioning. Next, 20 μm sections were obtained using a cryostat and mounted on MAS-coated glass slides. The sections were dried and washed with PBS three times and treated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. After three more washes with PBS, the sections were incubated with 1% bovine serum albumin in PBS for 1 h and incubated with anti-MAP2 antibody (Novus Biologicals, Littleton, CO) (1:200) or anti-NeuN antibody (Merck Millipore, Burlington, MA) (1:200) overnight at 4°C. The sections were then washed with PBS and incubated with the horseradish peroxidase-conjugated secondary antibody (1:300) for 1 h at room temperature. After another wash in PBS, color was developed with diaminobenzidine. The sections were washed and counterstained with hematoxylin. The number of subplate neurons were quantified by calculating the intensity of the MAP2 signal in the subplate of E16 cortex using Image J software or by a manual count of the number of NeuN-positive cells in the subplate of P1 cortex.

The sample collection of human umbilical cord blood samples

This study was conducted according to the Declaration of Helsinki and approved by the review boards of the University of Occupational and Environmental Health, and Hiroshima University (Epidemiological Research Ethics Review Board, license no. e2022-0016). Umbilical cord blood of pregnant women (n = 4) was collected at the University of Occupational and Environmental Health Hospital between May 2022 and September 2022. Plasma was separated from blood by centrifugation, and the isolated plasma was frozen and stored before use.

Statistical analysis

The statistical analysis for single comparisons between means was carried out using Student's *t* test. For multiple comparisons, we performed a one-way analysis of variance (ANOVA) followed by Holm's post-hoc test. The normality of data distribution was analyzed using the F-test or Bartlett's test. Probability (p)-values < 0.05 were considered significant.

RESULTS

DHA supplementation to pregnant mice alleviated the MeHg-induced impairments of motor activity and short-term memory of their pups

The administration of MeHg at 4 ppm in the dams' drinking water from day 0 of pregnancy to postnatal day 21 resulted in the pups' weight loss (**Fig. 1**A) and decreased grip strength (Fig. 1B) at postnatal day 33. The administration of MeHg to dams also affected pup behavior, resulting in a decreased latency time to fall from the rotarod (Fig. 1C) and to spontaneous alternation of arms on the Y-maze test (Fig. 1D), indicating that

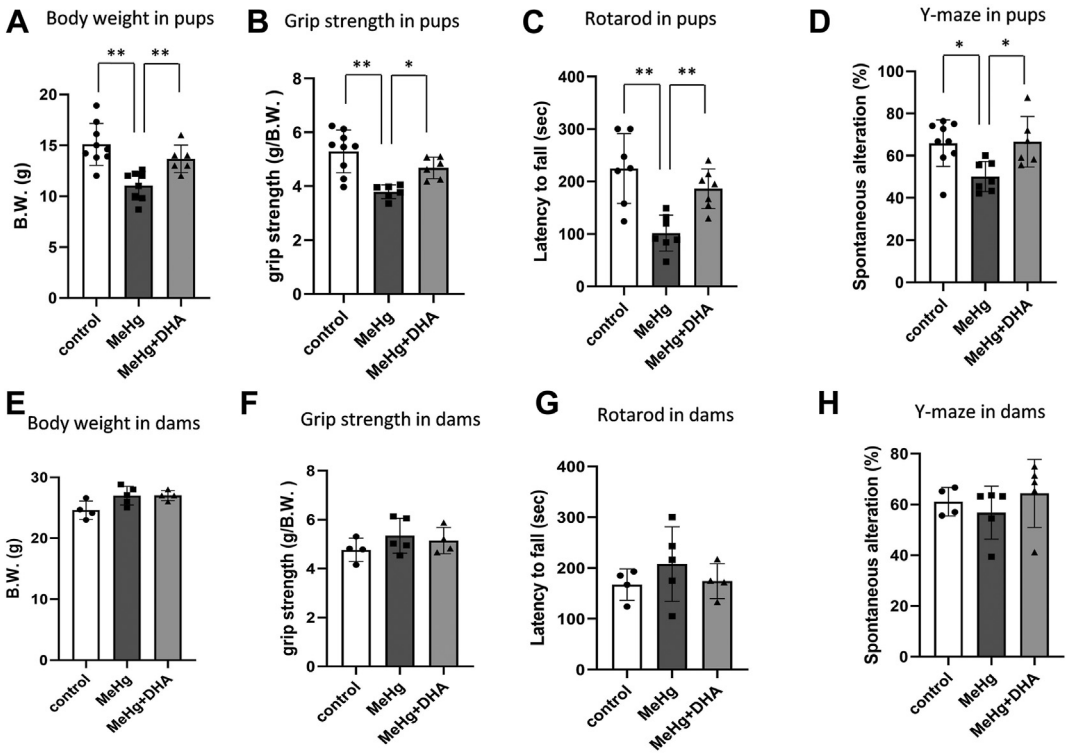

Fig. 1. Effects of maternal intake of MeHg or MeHg + DHA in mice on the motor activity and short-term memory of dams and pups. MeHg (4 ppm with drinking water) or MeHg + DHA (4% of total dietary fat) was administered to pregnant mice (n = 4–5) from day 0 of pregnancy to postnatal day 21, and the body weight (A) and grip strength (B) of the pups (male, n = 6–10) at postnatal day 33 were analyzed. The motor activity (C) and short-term memory (D) of the pups were analyzed by rotarod and Y-maze tests, respectively. The dams also underwent these behavioral tests (E–H). The results were analyzed by a one-way ANOVA followed by Holm's post hoc test. Values are the mean ± S.D. for 6–10 pups or 4–5 dams. **P* < 0.05, ***P* < 0.01. MeHg, methylmercury.

the pups' motor function and short-term memory were impaired by MeHg. On the other hand, these declines due to MeHg were not observed in the dams (Fig. 1E–H). However, the intake of DHA+MeHg suppressed the MeHg-induced impairments of body weight, grip strength, motor function, and short-term memory of the pups (Fig. 1A–D). These results indicated that the pup brain was more sensitive to MeHg than the dam brain, and maternal DHA intake was protective against MeHg-induced neurotoxicity in the pups.

Accumulation of total Hg in the pup brain by their dams' intake of MeHg and DHA

MeHg in murine maternal blood can cross the placenta and accumulate in the pup brain. We next investigated the possibility that DHA can decrease the levels of MeHg in the pup brain. The total Hg levels in the hippocampus, cortex, and cerebellum of pups were 0.6–0.8 ppm after maternal MeHg exposure, and there was no significant difference in the Hg levels in these regions (**Fig. 2**A–C). Unexpectedly however, the Hg levels in these regions of the pup brain were increased by maternal DHA supplementation. These results suggest, at least, that the beneficial effects of DHA supplementation were not caused by the decrease in the accumulation of MeHg in the pup brain.

MeHg-induced oxidative stress in the hippocampus and cortex was suppressed by maternal DHA supplementation

The major mechanism underlying the neurotoxicity of MeHg is considered to be an increase in oxidative stress (21). One of our research group's earlier studies revealed that a preincubation of DHA suppressed the MeHg-induced increase in reactive oxygen species and increased the expression levels of antioxidant enzymes in neuronal cells (10). In the cerebral cortex of the pups examined in the present study, lipid peroxidation was increased by the maternal administration of MeHg (**Fig. 3**B). However, in the pups born from dams administered MeHg together with DHA, the levels of lipid peroxidation in the hippocampus and cerebral cortex were lower than those in the group given only MeHg (Fig. 3A, B). The administration of MeHg did not significantly alter the lipid peroxidation in the brains of the dams (Fig. 3D–F). These results indicate that a maternal administration of MeHg increased the oxidative stress in their pups' brains and that a maternal intake of DHA suppressed the MeHg-induced increase in oxidative stress in their pups' brains.

Neuropathological changes of the pup cortex induced by the maternal administration of MeHg or MeHg + DHA

We next performed an immunohistochemical analysis to investigate neuropathological changes of the pup brain (E16 and P1) due to the maternal administration of MeHg or MeHg + DHA. Subplate neurons are a transient population of neurons in the immature cerebral cortex, and they are essential to the developmental assembly of cortical circuits (22). Subplate neurons have been preferentially labeled by anti-MAP2 antibody in fetuses and by anti-NeuN antibody in infants (23, 24). We observed that the maternal administration of MeHg decreased the number of subplate neurons in the pup cortex on both E16 (**Fig. 4**A, B) and P1(Fig. 4D, E) compared to the control. In addition, the maternal intake of DHA alleviated the MeHg-induced decrease in the subplate neurons of the pup brain (Fig. 4C, F). Subplate neurons have been reported to be vulnerable to oxidative stress (25), which suggests that MeHg-induced oxidative stress preferentially disrupts subplate neurons in the pup brain.

Changes in the levels of DHA and its metabolites in the brain and liver of the pups and the dams by DHA supplementation

Our earlier study demonstrated that DHA intake increased the levels of DHDPs, which are DHA metabolites, in the brain of adult rats, but not the levels of

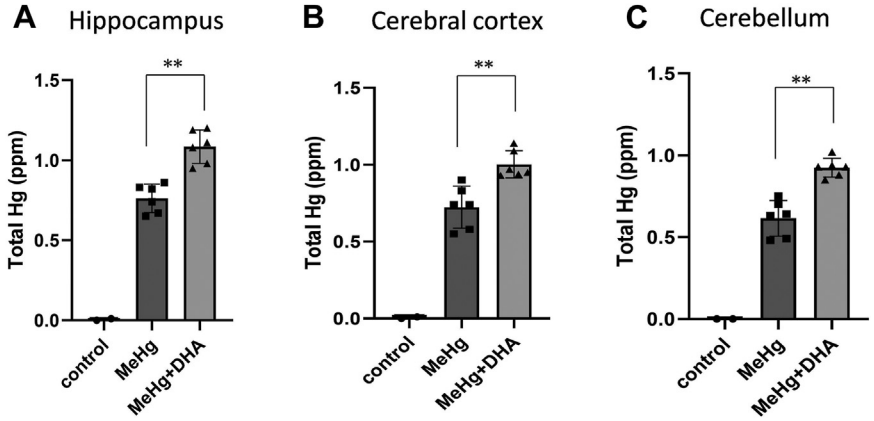

Fig. 2. MeHg levels in the hippocampus, cerebral cortex, and cerebellum of the pup brain. The total Hg concentrations in the hippocampus (A), cerebral cortex (B), and cerebellum (C) of the pups (male, n = 6) born from dams administered MeHg or MeHg + DHA were determined on postnatal day 21 with a direct thermal decomposition mercury analyzer. Data were analyzed by a one-way ANOVA followed by Holm's post hoc test. Values are the mean ± S.D. for 6 mice. $^{**}P < 0.01$. MeHg, methylmercury.

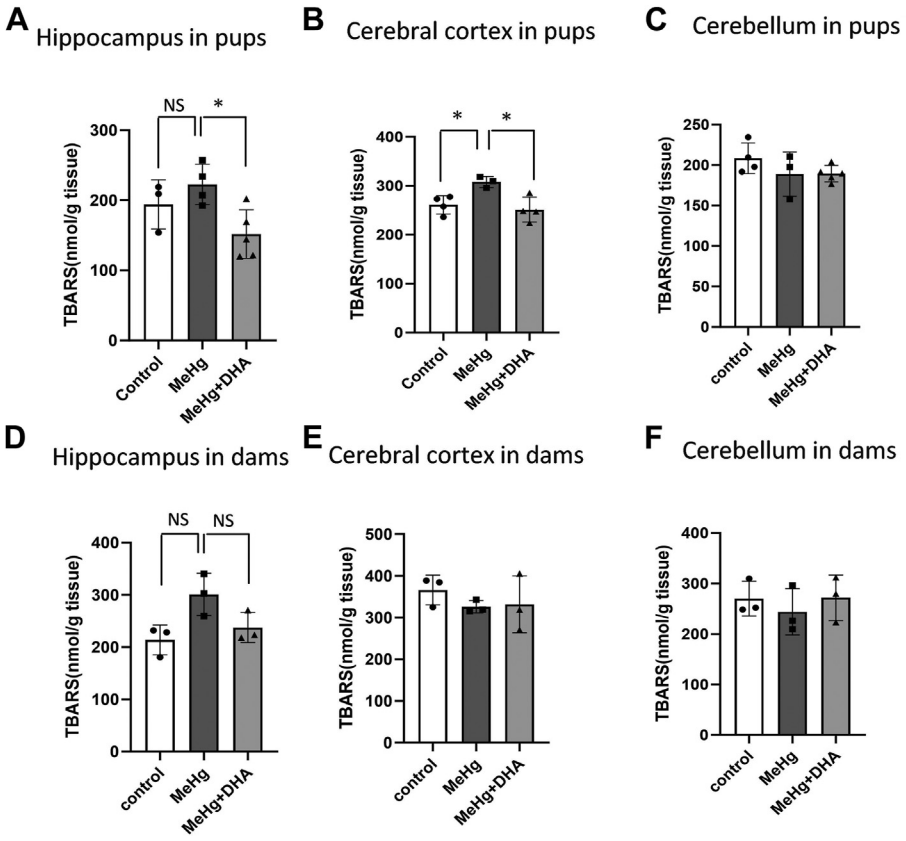

Fig. 3. Lipid peroxidation in the hippocampus, cerebral cortex, and cerebellum of the pup brain. The levels of lipid peroxide in the hippocampus (A), cerebral cortex (B), and cerebellum (C) of the pups (male, n = 3–5) at postnatal day 33 born from dams administered MeHg or MeHg + DHA were measured by a TBARS assay. Lipid peroxide levels in 16 to 17-week-old dam brains (n = 3) (D–F) were also measured. Data were analyzed using a one-way ANOVA, followed by Holm's post hoc test. Values are the mean ± S.D. for 5 pups or 3 dams. *$P < 0.05$. MeHg, methylmercury; NS, not significant; TBARS, Thiobarbituric acid-reactive substance.

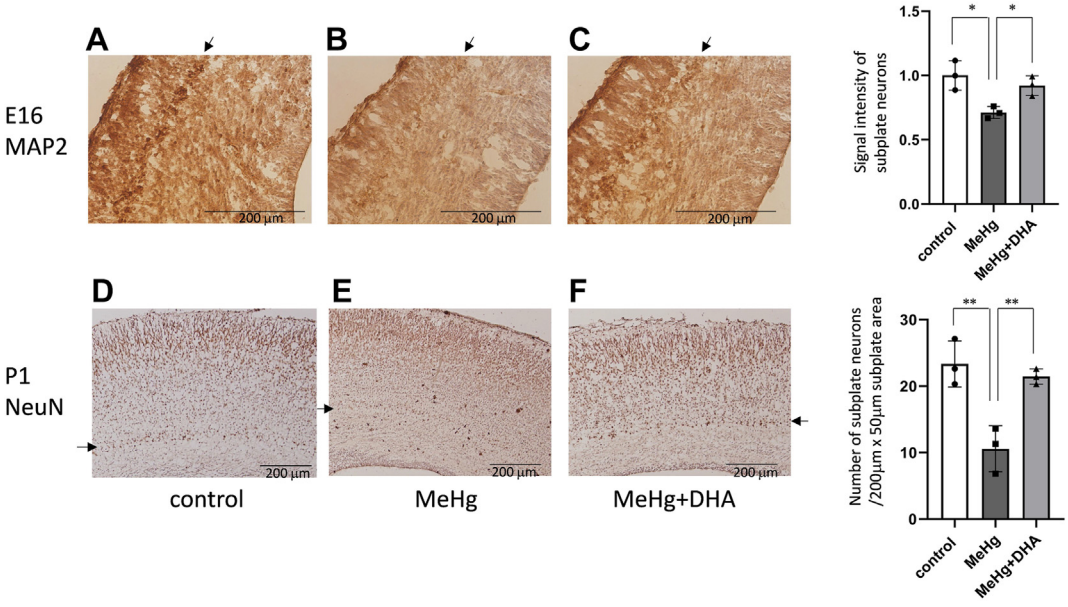

Fig. 4. Neuropathological changes of the pup brain by maternal intake of MeHg or MeHg + DHA in mice. Subplate neurons in the cortex of the pups on embryonic day 16 (A–C), and postnatal day 1 (D–F) of pups born from dams administered MeHg or MeHg + DHA were analyzed by immunohistochemical staining with anti-MAP2 antibody (E16) or anti-NeuN antibody (P1). The number of subplate neurons was quantified by calculating the intensity of the MAP2 signal in the subplate of E16 cortex by Image J software or by counting the number of NeuN-positive cells in the subplate of P1 cortex. Arrows: subplate. Values are mean ± S.D. for 3 mice. *$P < 0.05$, **$P < 0.01$. MeHg, methylmercury.

DHA itself (10). In the present study, the maternal intake of DHA by mice also did not significantly increase DHA levels in the dams' brains, and it did not increase DHA epoxides (EDPs). However, the DHA supplementation did increase the levels of three DHA diols: 19,20-DHDP, 16,17-DHDP, and 13,14-DHDP (**Fig. 5**A). 19,20-EDP and 19,20-DHDP were abundant in the brains of adult mice (Fig. 5A), which is consistent

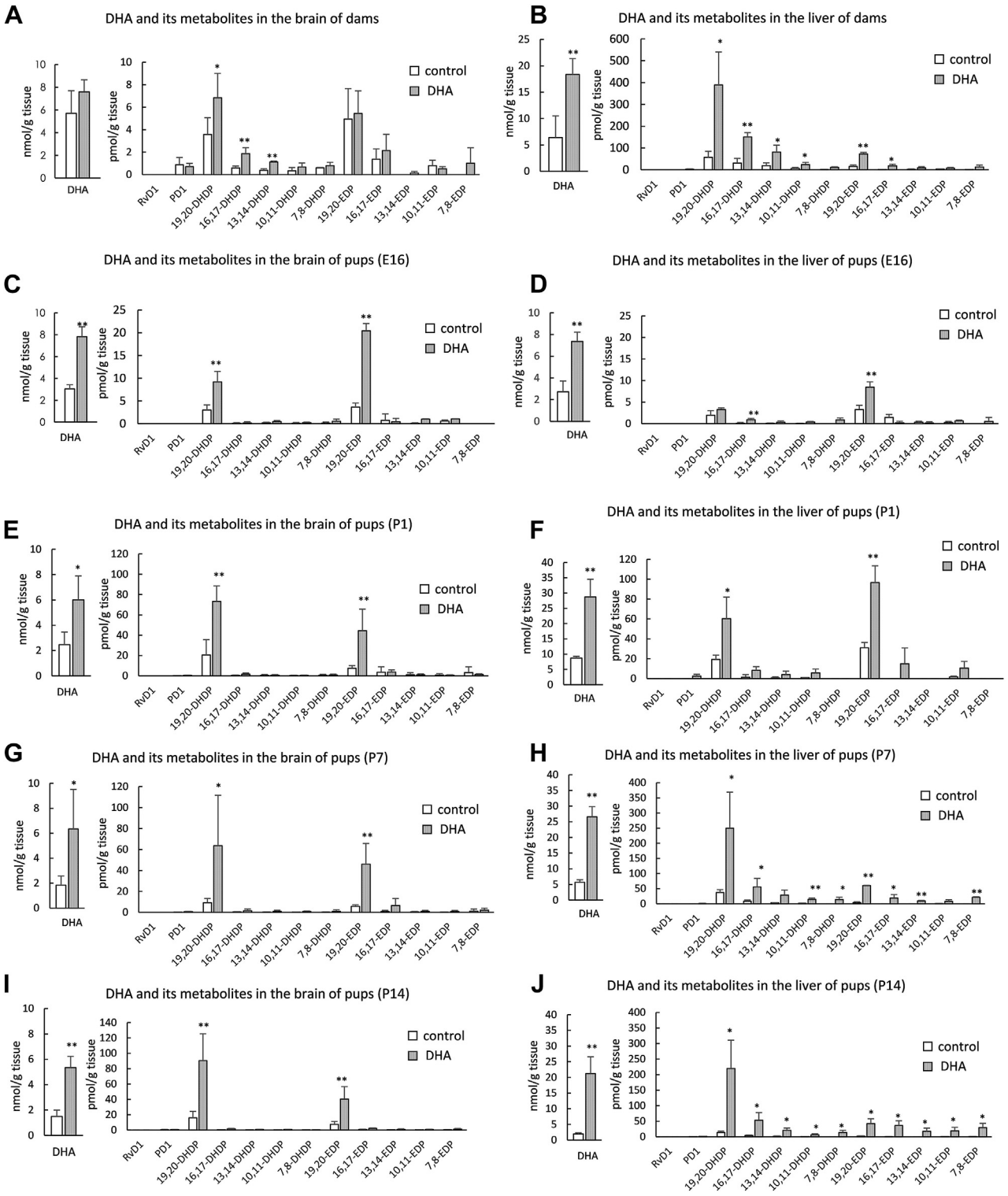

Fig. 5. Accumulation of DHA and its metabolites in the whole brain and liver by supplementation of DHA. DHA was given to pregnant mice, and the levels of DHA and its metabolites in the whole brain (A) and liver (B) of the dams were measured by LC-MS analysis. The levels of DHA and its metabolites in the whole brain (C, E, G, I) and the liver (D, F, H, J) of embryo and pups born from dams supplemented with DHA were analyzed on E16, P1, P7, and P14. Data were analyzed by a one-way ANOVA followed by Student's *t* test. Values are mean ± S.D. for 4–6 pups or 4 dams. **P* < 0.05, ***P* < 0.01.

with our study of adult rats (13). In contrast, the DHA intake by the dams significantly increased the levels of DHA and its metabolites, especially DHDPs, in their livers (Fig. 5B). ResolvinD1 (RvD1) and protectinD1 (PD1), DHA metabolites produced by cyclooxygenase and lipoxygenase, are well studied, but in our experiments, the brain levels of these metabolites were very low and were not increased by DHA intake in the brain or liver (Fig. 5A, B). In the pup brain, DHA metabolites, especially 19,20-EDP and 19,20-DHDP, were detected on embryonic day (E) 16 (Fig. 5C), postnatal day (P) 1 (Fig. 5E), P7 (Fig. 5G), and P14 (Fig. 5I) along with DHA itself, and the levels of these metabolites were higher in the infants than in the fetuses. In addition, the maternal intake of DHA increased the DHA levels in the pup brain and also significantly and selectively increased the levels of 19,20-EDP and 19,20-DHDP in the pup brain. The levels of these increased DHA metabolites per tissue weight in the brains of fetuses and infants were significantly higher than those in the dam brain, indicating that 19,20-EDP and 19,20-DHDP can selectively accumulate in the brains of the fetuses and infants to a higher level than in the dams by maternal intake of DHA. In E16 pups, maternal DHA intake slightly increased the levels of DHA metabolites in the livers (Fig. 5D), while a significant increase in the levels of DHA metabolites was observed in the brain (Fig. 5C), indicating a preferential accumulation of DHA metabolites in the fetal brain but not the liver. In the liver of pups, 19,20-EDP and 19,20-DHDP were increased by DHA intake on P1 (Fig. 5F), and the increase in the 19,20-DHDP level was significant on P7 (Fig. 5H) and P14 (Fig. 5J). The increases in DHA metabolites in the livers of P7 and P14 pups due to the maternal intake of DHA were similar to those in the livers of the dams.

Changes in the expression levels of DHA-metabolizing enzymes by aging in the mouse brain and liver

To investigate the sites at which the conversion of DHA into DHA metabolites takes place in the pups, we analyzed the expression levels of the EDP-producing enzymes P450s and DHDP-producing enzyme sEH in the brain and liver. In the brain and liver collected on E16 and P1, the mRNA and protein levels of P450s (**Fig. 6**A, B, E, F) and sEH (Fig. 6C, D, G, H) were much lower than those in adult mice (P56). These results suggest that the significant accumulation of DHA metabolites in the brain of fetuses (Fig. 5C) was not caused by the production of these metabolites from DHA in the fetal brain or liver, and they suggested the possibility that these metabolites were transferred via the placenta from dams to pups. The expression levels of these enzymes were increased by aging. In the liver, CYP2E1 protein was detected from P7 (Fig. 6F), and sEH protein was increased from P3 (Fig. 6H), suggesting that infant mice at P7 can produce EDPs and DHDPs in their liver, and thus the levels of DHA metabolites in the livers of P7 (Fig. 5H) and P14 infants (Fig. 5J) would have been similar to those of their dams (Fig. 5B).

The levels of DHA and its metabolites in mouse breast milk and human umbilical cord blood

We next examined the possibility that DHA metabolites can be transferred from mothers to fetuses and infants via umbilical cord blood and breast milk. DHA metabolites as well as DHA were detected in the plasma (**Fig. 7**A) and breast milk (Fig. 7B) of the mouse dams. While most isomers of EDPs and DHDPs were increased in the dams' plasma by DHA intake, 19,20-EDP and 19,20-DHDP were especially increased in the dams' breast milk. DHA and its metabolites were significantly concentrated in the breast milk rather than in plasma by DHA intake. DHA can also preferentially pass the placenta relative to the other fatty acids in order to deliver DHA to the fetus. We therefore also analyzed DHA metabolites in human umbilical cord blood, and we observed that EDPs and DHDPs were present in the human umbilical cord blood, with 19,20-DHDP being most abundant (Fig. 7C). These results suggested that in addition to DHA itself, DHA metabolites are also preferentially transferred from plasma to umbilical cord blood and breast milk.

DISCUSSION

Our present findings demonstrate that maternal DHA intake can alleviate the MeHg-induced impairments of motor activity and short-term memory in pups. Some reports have also shown deficits of motor activity, spatial learning, and memory by prenatal MeHg exposure in mice (26, 27). Indeed, in humans, prenatal exposure to MeHg has been related to neurodevelopmental abnormalities in children including motor dysfunction, deficiencies in vocabulary, verbal skills and memory, and other cognitive deficits (8, 28). In mice, a prenatal administration of MeHg on gestational days 12–14 was shown to have more adverse effects on locomotor activity, reference memory, and working memory compared to a similar administration on gestational days 7–9 (29), which indicates that MeHg exposure in late pregnancy may have the greatest adverse effects on the fetal brain. However, these deficits were not observed in dams in the present study. It has been shown that the concentration of MeHg in cord blood is approximately twofold higher than in maternal blood (30, 31), because an amino acid transporter (LAT1) that can transport MeHg is present on both the maternal and fetal surfaces of the syncytiotrophoblasts in both the placenta and blood-brain barrier (32). In addition, exposure to MeHg during early fetal development causes brain damage at much lower concentrations than those affecting the mature brain (33). Weight loss of the pups is also frequently used as a marker of MeHg toxicity in in vivo studies (34). In humans, a negative correlation between blood mercury

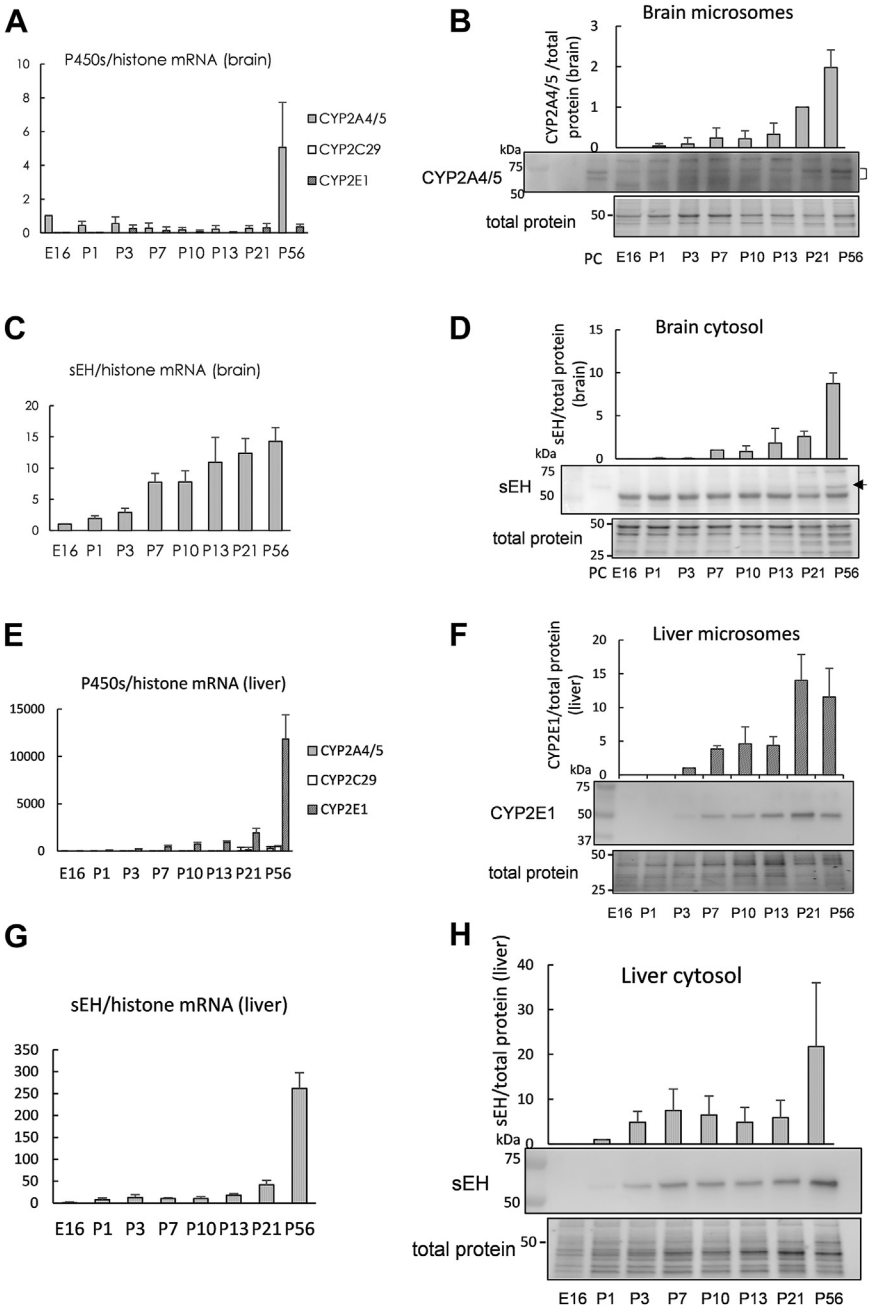

Fig. 6. Expression levels of EDP-producing P450s and sEH in the whole brain and liver of mice at each day of age. The mRNA levels of EDP-producing P450s (*cyp2a4/5*, *cyp2c29*, *cyp2e1*) (A, E) and *sEH* (C, G) in the whole brain (A, C) and the liver (E, G) were analyzed. The protein levels of CYP2A4/5 in the whole brain (B) and of CYP2E1 in the liver (F) were analyzed by Western blotting using mice tissue microsomes. The protein levels of sEH in the whole brain (D) and liver (H) were analyzed using a mouse cytosol fraction. Band intensity of Western blotting was quantified by Image J software. These samples were subjected to Mini-PROTEAN® TGX Stain-Free™ Gel, and total protein was visualized by UV light exposure. The total protein of each lane was quantified by FUSION software. Phenobarbital-treated mouse brain microsomes were used as a positive control for the detection of CYP2A4/5 in the brain, and mouse liver cytosol was used as a positive control for the detection of sEH in the brain. Values are mean ± S.D. for 3–5 mice. P450, cytochrome P450; PC, positive control; sEH, soluble epoxide hydrolase.

levels during gestation and birth weight has been observed (31, 35). MeHg can affect developing skeletal muscles to alter motor function (34). MeHg can also affect the expression of hypothalamic neuropeptides that control food intake and body weight (36). These results suggest that DHA may not only reduce MeHg-induced neurotoxicity but also extensively reduce the toxicity of MeHg in fetal development during pregnancy.

In our present experiments, although the maternal supplementation with DHA had beneficial effects on the pup brain, the Hg levels in the pup brain were also increased by DHA intake. Our prior study showed that DHA and its metabolite 19,20-DHDP did not alter the

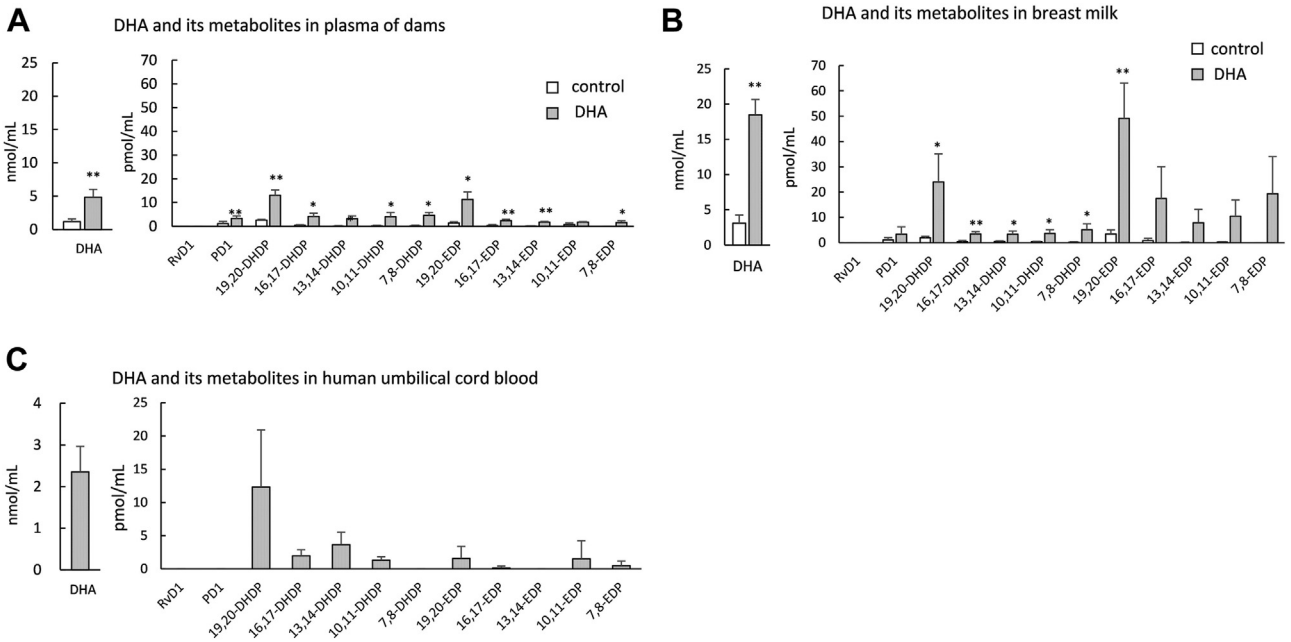

Fig. 7. Levels of DHA and its metabolites in the plasma and breast milk of mice and in human umbilical cord blood. The pregnant mice were supplemented with DHA from day 0 of pregnancy, and the levels of DHA and its metabolites in the plasma of maternal blood (A) on E16 and in the breast milk (B) on postnatal days 13–14 were analyzed by LC-MS analysis. Human umbilical cord blood (n = 5) was collected, and the levels of DHA and its metabolites in the plasma were analyzed (C). Data were analyzed by a one-way ANOVA followed by Student's *t* test. Values are mean ± S.D. for 3 mice (A and B). **P* < 0.05, ***P* < 0.01. DHDP, dihydroxydocosapentaenoic acid; EDP, epoxydocosapentaenoic acid; RvD1, resolvinD1.

cellular Hg levels in SH-SY5Y cells, although 19,20-DHDP increased the Hg efflux transporter MRP4 (10). Those results contradict the findings of an earlier study in which maternal DHA intake was found to reduce Hg levels in the pup brain (37). The administration of selenium has also been shown to confer protection against MeHg-induced neurotoxicity, but brain mercury levels were higher by coexposure to MeHg and selenium compared to those by exposure to MeHg alone (38). These data indicate that brain Hg concentrations have no significant relationships to the relative toxicity of MeHg. Indeed, the total Hg concentration has been shown to not correspond to neuropathological changes in brain regions (39). It is possible that DHA changes the permeability of MeHg through the blood-brain barrier or blood-placental barrier, but further investigations are required to clarify the mechanism.

MeHg-induced oxidative stress in the brain has been closely associated with neurological disorders (21). Maternal exposure to MeHg has been shown to decrease the brain glutathione levels in infancy (33). MeHg also inactivates manganese superoxide dismutase by binding to thiol groups of the protein (14). In the present study, an increase in oxidative stress by MeHg exposure was observed in the cerebral cortex of the pups but not in the hippocampus or cerebellum. In addition, the basal levels of oxidative stress in the cerebral cortex were higher than those in the hippocampus or cerebellum. It has been demonstrated that the basal expression levels and activities of antioxidant enzymes were higher in the mouse hippocampus than the cerebral cortex (39). Such high hippocampal levels of antioxidant enzymes might have been responsible for the resistance against MeHg-induced oxidative stress in the hippocampus of pups observed in the present study. Indeed, we observed that the maternal administration of MeHg decreased the number of subplate neurons in the cortex of the fetuses and infants. There are two autopsy reports of fetal MeHg poisoning in humans, from 1965 (Kumamoto, Japan) (40) and 1978 (Iraq) (41). In both cases, hypoplasia of neurons was observed in the cerebral cortex and cerebellum, and incomplete or abnormal migration of neuron was suggested. Subplate neurons control the radial migration of neocortical neurons and are essential for cortical development (42). A decrease in the number of subplate neurons in the immature cerebral cortex by MeHg may thus be one of the causes of hypoplasia of neurons in the cortex induced by a maternal administration of MeHg.

Our present analyses revealed that the oxidative stress in the cerebral cortex that was induced by MeHg was suppressed by the maternal intake of DHA. Our earlier studies showed that DHA intake by rats increased the expression levels of the antioxidant enzymes SOD1 and catalase in their brains (13) and that a direct addition of DHA or its metabolite 19,20-DHDP increased these genes in neuronal cells (10). We speculate that these antioxidant effects of DHA or 19,20-DHDP are thus at least partly responsible for the

observed decrease in oxidative stress in the pup brain and that the accompanying reduction of oxidative stress would lead to an alleviation of MeHg-induced neurotoxicity in the brain.

Indeed, DHA and its metabolites, including DHDPs, were significantly increased in the brains of the mouse fetuses and infants by the maternal intake of DHA. We also observed that these DHA metabolites were probably transferred from the dams to the pups via the placenta and breast milk, based on the lower expression levels of P450s and sEH in the fetuses and immediately after birth. These metabolites were indeed present in the umbilical cord blood and breast milk. Although most of the isomers of EDPs and DHDPs were increased in the dam plasma by DHA intake (Fig. 7A), 19,20-EDP and 19,20-DHDP were selectively accumulated in the brains of the fetuses (Fig. 5C). These results suggest that these two isomers can be selectively transported to the blood-placental barrier or blood-brain barrier in the fetuses. These accumulations of DHA metabolites were specifically observed in the brain but not the liver of fetuses. Further investigation of this result is necessary, including an exploration of the specific transporters for these metabolites in the fetal brain. In addition, 19,20-EDP and 19,20-DHDP were also preferentially transferred from the blood to the breast milk, suggesting a selective transport. A member of the major facilitator superfamily Mfsd2a has been identified as a transporter of DHA in the form of lysophosphatidic acid across the blood-brain barrier (43), and fatty acid transportation proteins (44) are also known to play a role in DHA transport to the brain. The ability of these transporters to carry DHA metabolites has not been established. We observed that the levels of DHA metabolites in the mouse liver on P7 were significantly increased compared to those on E16 and P1, and we contend that these increases were likely due to the increased ability to produce DHA metabolites by the increased expression levels of CYP2E1 and sEH in the liver at P7, because the pattern of DHA metabolites was similar to that of the dam liver. Lower expression levels of P450s and sEH in fetuses and increases in these expression levels by the passage of days after birth have also been described (45, 46). RvD1 and PD1 are DHA metabolites that are well known for their antiinflammatory effects, but in our present study, the contribution of these metabolites to the beneficial effects of DHA intake would have been low, because their levels in the brain were much lower. Indeed, a recent report questioned whether these metabolites exist in the tissue at sufficient levels for their bioactivity (47).

Although DHA levels in the dam plasma and liver were significantly increased by DHA intake in this study, the brain levels of DHA in the dams were not significantly increased. Similar findings were obtained in our study of rats (13) and another investigation (48). In humans, the quantities of DHA incorporated into the adult brain have been shown to be equivalent to the quantities of DHA consumed by the brain (49). Despite our finding that the DHA levels were not increased in our present experiments, DHDPs that are metabolites of DHA were increased by the dams' intake of DHA, which is consistent with our finding in adult rats. Because EDP-producing P450s and sEH (which contribute to the production of DHDPs) were expressed in the brain and the expression levels of sEH were higher than those of P450s (13), DHDPs may be abundantly produced in the mouse dam brain. Another possibility is a preferential transfer of DHDPs to the dam brain from plasma, as we observed that the levels of DHA metabolites were significantly increased in the dam plasma by DHA intake.

This study demonstrated, for the first time, that EDPs and DHDPs that are DHA metabolites were present in mouse breast milk and human umbilical cord blood. In humans, an increase in 19,20-DHDP has been shown in the plasma by supplementation with DHA or its precursor, docosapentaenoic acid (50, 51). This preferential transfer of DHA metabolites suggests their importance for fetal brain development. Our prior investigations showed that 19,20-DHDP has neuroprotective effects (10, 13). 19,20-DHDP has also been reported to exert antiapoptotic effects in astrocytes (52). The precursor of 19,20-DHDP, i.e., 19,20-EDP, has antiinflammatory effects in hippocampal neurons (50). Direct supplementation of these metabolites in pregnant mice will clarify their neuroprotective effects against MeHg-induced neurotoxicity in the brain.

In conclusion, although a maternal intake of fish has been associated with both beneficial effects of DHA and the risk of MeHg-induced neurotoxicity in the fetal brain, our present results demonstrate that DHA intake can alleviate MeHg-induced neurotoxicity in vivo due to the accumulation of DHA and its metabolites in the fetal brain. Numerous countries have established governmental guidelines to minimize the consumption of large amounts of large fish containing MeHg with the intention of reducing exposure of MeHg, particularly in pregnant women. The consumption of fish species with a high DHA content, such as salmon and sea bream, by pregnant women will reduce MeHg-induced neurotoxicity, even though such fish may contain MeHg. Supplementation with DHA or its metabolites may also be effective for reducing fetal MeHg toxicity.

Data Availability

All data are available upon request to the corresponding author (Graduate School of Biomedical and Health Sciences, Hiroshima University; aoguro@hiroshima-u.ac.jp)

Supplemental data

This article contains supplemental data.

Author contributions

A. O., Conceptualization; A. O., Y. I., C. K., M. Y., and Y. K., Methodology; A. O., T. F., C. K., M. Y., and K. E., Investigation;

A. O., Writing-Original Draft, A. O. and T. Y., Funding acquisition; Y. I., Y. K., S. I., T. S., M. T., and E. S., Resources; A. O., T. F., Y. I., C. K., M. Y., K. E., Y. K., Y. K., S. I., T. S., M. T., E. S., and T. Y., Writing-Review & Editing.

Author ORCIDs
Chisato Kataoka https://orcid.org/0000-0002-6901-0819

Funding and additional information
This study was supported in part by JSPS KAKENHI Grants-in-Aid for Scientific Research (C) (Nos. 20K07031 and 20K12182). It was also supported by a grant from the Kobayashi Foundation, a Lotte Research Promotion Grant, and funds from the Uehara Memorial Foundation, the Food Science Institute Foundation (Ryoushoku-kenkyukai) and Satake Foundation.

Conflict of interest
The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations
DHDP, dihydroxydocosapentaenoic acid; EDP, epoxydocosapentaenoic acid; MeHg, methylmercury; P450, cytochrome P450; PD1, protectinD1; RvD1, resolvinD1; sEH, soluble epoxide hydrolase.

Manuscript received February 17, 2023, and in revised form October 5, 2023. Published, JLR Papers in Press, October 12, 2023, https://doi.org/10.1016/j.jlr.2023.100458

REFERENCES

1. Bazinet, R. P., and Laye, S. (2014) Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat. Rev. Neurosci.* **15**, 771–785
2. Duan, J., Song, Y., Zhang, X., and Wang, C. (2021) Effect of omega-3 polyunsaturated fatty acids-derived bioactive lipids on metabolic disorders. *Front. Physiol.* **12**, 646491
3. Avallone, R., Vitale, G., and Bertolotti, M. (2019) Omega-3 fatty acids and neurodegenerative diseases: new evidence in clinical trials. *Int. J. Mol. Sci.* **20**, 4256
4. Shulkin, M., Pimpin, L., Bellinger, D., Kranz, S., Fawzi, W., Duggan, C., *et al.* (2018) n-3 fatty acid supplementation in mothers, preterm infants, and term infants and childhood psychomotor and visual development: a systematic review and meta-analysis. *J. Nutr.* **148**, 409–418
5. Aschner, M. (1989) Brain, kidney and liver 203Hg-methyl mercury uptake in the rat: relationship to the neutral amino acid carrier. *Pharmacol. Toxicol.* **65**, 17–20
6. Kajiwara, Y., Yasutake, A., Adachi, T., and Hirayama, K. (1996) Methylmercury transport across the placenta via neutral amino acid carrier. *Arch. Toxicol.* **70**, 310–314
7. Syversen, T., and Kaur, P. (2012) The toxicology of mercury and its compounds. *J. Trace Elem. Med. Biol.* **26**, 215–226
8. Strain, J. J., Yeates, A. J., van Wijngaarden, E., Thurston, S. W., Mulhern, M. S., McSorley, E. M., *et al.* (2015) Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: associations with child development at 20 mo of age in an observational study in the Republic of Seychelles. *Am. J. Clin. Nutr.* **101**, 530–537
9. Choi, A. L., Mogensen, U. B., Bjerve, K. S., Debes, F., Weihe, P., Grandjean, P., *et al.* (2014) Negative confounding by essential fatty acids in methylmercury neurotoxicity associations. *Neurotoxicol. Teratol.* **42**, 85–92
10. Oguro, A., Fujita, K., Ishihara, Y., Yamamoto, M., and Yamazaki, T. (2021) DHA and its metabolites have a protective role against methylmercury-induced neurotoxicity in mouse primary neuron and SH-SY5Y cells. *Int. J. Mol. Sci.* **22**, 3213
11. Serhan, C. N., Dalli, J., Colas, R. A., Winkler, J. W., and Chiang, N. (2015) Protectins and maresins: new pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. *Biochim. Biophys. Acta.* **1851**, 397–413
12. McReynolds, C., Morisseau, C., Wagner, K., and Hammock, B. (2020) Epoxy fatty acids are promising targets for treatment of pain, cardiovascular disease and other indications characterized by mitochondrial dysfunction, endoplasmic stress and inflammation. *Adv. Exp. Med. Biol.* **1274**, 71–99
13. Oguro, A., Ishihara, Y., Siswanto, F. M., Yamazaki, T., Ishida, A., Imaishi, H., *et al.* (2021) Contribution of DHA diols (19,20-DHDP) produced by cytochrome P450s and soluble epoxide hydrolase to the beneficial effects of DHA supplementation in the brains of rotenone-induced rat models of Parkinson's disease. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids.* **1866**, 158858
14. Shinyashiki, M., Kumagai, Y., Homma-Takeda, S., Nagafune, J., Takasawa, N., Suzuki, J., *et al.* (1996) Selective inhibition of the mouse brain Mn-SOD by methylmercury. *Environ. Toxicol. Pharmacol.* **2**, 359–366
15. Yamamoto, M., Yanagisawa, R., Sakai, A., Mogi, M., Shuto, S., Shudo, M., *et al.* (2021) Toxicokinetics of methylmercury in diabetic KK-Ay mice and C57BL/6 mice. *J Appl Toxicol.* **41**, 928–940
16. Hennebelle, M., Zhang, Z., Metherel, A. H., Kitson, A. P., Otoki, Y., Richardson, C. E., *et al.* (2017) Linoleic acid participates in the response to ischemic brain injury through oxidized metabolites that regulate neurotransmission. *Sci. Rep.* **7**, 4342
17. Nakamura, M., Imaoka, S., Amano, F., and Funae, Y. (1998) P450 isoforms in a murine macrophage cell line, RAW264.7, and changes in the levels of P450 isoforms by treatment of cells with lipopolysaccharide and interferon-gamma. *Biochim. Biophys. Acta.* **1385**, 101–106
18. Imaoka, S., Terano, Y., and Funae, Y. (1990) Changes in the amount of cytochrome P450s in rat hepatic microsomes with starvation. *Arch. Biochem. Biophys.* **278**, 168–178
19. Oguro, A., Fujita, N., and Imaoka, S. (2009) Regulation of soluble epoxide hydrolase (sEH) in mice with diabetes: high glucose suppresses sEH expression. *Drug Metab. Pharmacokinet.* **24**, 438–445
20. Hiroi, T., Okada, K., Imaoka, S., Osada, M., and Funae, Y. (2006) Bisphenol A binds to protein disulfide isomerase and inhibits its enzymatic and hormone-binding activities. *Endocrinology.* **147**, 2773–2780
21. Fujimura, M., and Usuki, F. (2020) Methylmercury-mediated oxidative stress and activation of the cellular protective system. *Antioxidants (Basel).* **9**, 1004
22. Allendoerfer, K. L., and Shatz, C. J. (1994) The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. *Annu. Rev. Neurosci.* **17**, 185–218
23. Barnes, A. P., Lilley, B. N., Pan, Y. A., Plummer, L. J., Powell, A. W., Raines, A. N., *et al.* (2007) LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons. *Cell.* **129**, 549–563
24. Tolner, E. A., Sheikh, A., Yukin, A. Y., Kaila, K., and Kanold, P. O. (2012) Subplate neurons promote spindle bursts and thalamocortical patterning in the neonatal rat somatosensory cortex. *J. Neurosci.* **32**, 692–702
25. Folkerth, R. D., Trachtenberg, F. L., and Haynes, R. L. (2008) Oxidative injury in the cerebral cortex and subplate neurons in periventricular leukomalacia. *J. Neuropathol. Exp. Neurol.* **67**, 677–686
26. Montgomery, K. S., Mackey, J., Thuett, K., Ginestra, S., Bizon, J. L., and Abbott, L. C. (2008) Chronic, low-dose prenatal exposure to methylmercury impairs motor and mnemonic function in adult C57/B6 mice. *Behav. Brain Res.* **191**, 55–61
27. Onishchenko, N., Tamm, C., Vahter, M., Hokfelt, T., Johnson, J. A., Johnson, D. A., *et al.* (2007) Developmental exposure to methylmercury alters learning and induces depression-like behavior in male mice. *Toxicol. Sci.* **97**, 428–437
28. Oken, E., and Bellinger, D. C. (2008) Fish consumption, methylmercury and child neurodevelopment. *Curr. Opin. Pediatr.* **20**, 178–183
29. Dore, F. Y., Goulet, S., Gallagher, A., Harvey, P. O., Cantin, J. F., D'Aigle, T., *et al.* (2001) Neurobehavioral changes in mice treated with methylmercury at two different stages of fetal development. *Neurotoxicol. Teratol.* **23**, 463–472

30. Sakamoto, M., Murata, K., Domingo, J. L., Yamamoto, M., Oliveira, R. B., Kawakami, S., *et al.* (2016) Implications of mercury concentrations in umbilical cord tissue in relation to maternal hair segments as biomarkers for prenatal exposure to methylmercury. *Environ. Res.* **149**, 282–287
31. Vigeh, M., Nishioka, E., Ohtani, K., Omori, Y., Matsukawa, T., Koda, S., *et al.* (2018) Prenatal mercury exposure and birth weight. *Reprod. Toxicol.* **76**, 78–83
32. Gaccioli, F., Aye, I. L., Roos, S., Lager, S., Ramirez, V. I., Kanai, Y., *et al.* (2015) Expression and functional characterisation of System L amino acid transporters in the human term placenta. *Reprod. Biol. Endocrinol.* **13**, 57
33. Stringari, J., Nunes, A. K., Franco, J. L., Bohrer, D., Garcia, S. C., Dafre, A. L., *et al.* (2008) Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. *Toxicol. Appl. Pharmacol.* **227**, 147–154
34. Rand, M. D., Conrad, K., Marvin, E., Harvey, K., Henderson, D., Tawil, R., *et al.* (2020) Developmental exposure to methylmercury and resultant muscle mercury accumulation and adult motor deficits in mice. *Neurotoxicology.* **81**, 1–10
35. Papadopoulou, E., Botton, J., Caspersen, I. H., Alexander, J., Eggesbo, M., Haugen, M., *et al.* (2021) Maternal seafood intake during pregnancy, prenatal mercury exposure and child body mass index trajectories up to 8 years. *Int. J. Epidemiol.* **50**, 1134–1146
36. Ferrer, B., Peres, T. V., Dos Santos, A. A., Bornhorst, J., Morcillo, P., Goncalves, C. L., *et al.* (2018) Methylmercury affects the expression of hypothalamic neuropeptides that control body weight in C57BL/6J mice. *Toxicol. Sci.* **163**, 557–568
37. Jayashankar, S., Glover, C. N., Folven, K. I., Brattelid, T., Hogstrand, C., and Lundebye, A. K. (2012) Cerebral gene expression and neurobehavioural responses in mice pups exposed to methylmercury and docosahexaenoic acid through the maternal diet. *Environ. Toxicol. Pharmacol.* **33**, 26–38
38. Sakamoto, M., Yasutake, A., Kakita, A., Ryufuku, M., Chan, H. M., Yamamoto, M., *et al.* (2013) Selenomethionine protects against neuronal degeneration by methylmercury in the developing rat cerebrum. *Environ. Sci. Technol.* **47**, 2862–2868
39. Fujimura, M., and Usuki, F. (2017) In situ different antioxidative systems contribute to the site-specific methylmercury neurotoxicity in mice. *Toxicology.* **392**, 55–63
40. Matsumoto, H., Koya, G., and Takeuchi, T. (1965) Fetal Minamata disease. A neuropathological study of two cases of intrauterine intoxication by a methyl mercury compound. *J. Neuropathol. Exp. Neurol.* **24**, 563–574
41. Choi, B. H., Lapham, L. W., Amin-Zaki, L., and Saleem, T. (1978) Abnormal neuronal migration, deranged cerebral cortical organization, and diffuse white matter astrocytosis of human fetal brain: a major effect of methylmercury poisoning in utero. *J. Neuropathol. Exp. Neurol.* **37**, 719–733
42. Ohtaka-Maruyama, C., Okamoto, M., Endo, K., Oshima, M., Kaneko, N., Yura, K., *et al.* (2018) Synaptic transmission from subplate neurons controls radial migration of neocortical neurons. *Science.* **360**, 313–317
43. Nguyen, L. N., Ma, D., Shui, G., Wong, P., Cazenave-Gassiot, A., Zhang, X., *et al.* (2014) Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature.* **509**, 503–506
44. Zhang, W., Chen, R., Yang, T., Xu, N., Chen, J., Gao, Y., *et al.* (2018) Fatty acid transporting proteins: roles in brain development, aging, and stroke. *Prostaglandins Leukot. Essent. Fatty Acids.* **136**, 35–45
45. Hart, S. N., Cui, Y., Klaassen, C. D., and Zhong, X. B. (2009) Three patterns of cytochrome P450 gene expression during liver maturation in mice. *Drug Metab. Dispos.* **37**, 116–121
46. Lee, H. T., Lee, K. I., Lin, H. C., and Lee, T. S. (2019) Genetic deletion of soluble epoxide hydroxylase causes anxiety-like behaviors in mice. *Mol. Neurobiol.* **56**, 2495–2507
47. Sinha, G. (2022) Doubt cast on inflammation's stop signals. *Science.* **376**, 565–566
48. Trepanier, M. O., Lim, J., Lai, T. K., Cho, H. J., Domenichiello, A. F., Chen, C. T., *et al.* (2014) Intraperitoneal administration of docosahexaenoic acid for 14days increases serum unesterified DHA and seizure latency in the maximal pentylenetetrazol model. *Epilepsy Behav.* **33**, 138–143
49. Umhau, J. C., Zhou, W., Carson, R. E., Rapoport, S. I., Polozova, A., Demar, J., *et al.* (2009) Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. *J. Lipid Res.* **50**, 1259–1268
50. Borsini, A., Nicolaou, A., Camacho-Munoz, D., Kendall, A. C., Di Benedetto, M. G., Giacobbe, J., *et al.* (2021) Omega-3 polyunsaturated fatty acids protect against inflammation through production of LOX and CYP450 lipid mediators: relevance for major depression and for human hippocampal neurogenesis. *Mol. Psychiatry.* **26**, 6773–6788
51. Markworth, J. F., Kaur, G., Miller, E. G., Larsen, A. E., Sinclair, A. J., Maddipati, K. R., *et al.* (2016) Divergent shifts in lipid mediator profile following supplementation with n-3 docosapentaenoic acid and eicosapentaenoic acid. *FASEB J.* **30**, 3714–3725
52. Hu, J., Bibli, S. I., Wittig, J., Zukunft, S., Lin, J., Hammes, H. P., *et al.* (2019) Soluble epoxide hydrolase promotes astrocyte survival in retinopathy of prematurity. *J. Clin. Invest.* **129**, 5204–5218