Research Paper

Aging alters lipid mediator biosynthesis from polyunsaturated fatty acids in the brain of senescence-accelerated mice with age-related cognitive dysfunction

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Abstract This study aimed to clarify whether long-chain polyunsaturated fatty acid (LCPUFA) metabolism in the brain that causes an age-related decline of cognitive function changes with aging. The senescence-accelerated mice-prone 10 (SAMP10) is an animal model used in studies related to aging. Young (two-month-old) mice and aged (nine-month-old) mice were fed control diet for 12 weeks. Age-related changes in lipid mediator profiles in the cortex and hippocampus were assessed by comprehensive lipidomics, using liquid chromatography-tandem mass spectrometry after memory examination. The expression of fatty acid metabolism-related mRNAs was evaluated in the hippocampus. Free arachidonic acid (ARA) levels were extremely high among all detected free fatty acids, being approximately 8-fold higher than those of free DHA in the cortex and hippocampus of both young and aged mice. Level of ARA-derived metabolites was extraordinarily high compared to that of other PUFA-derived metabolites, whereas that of DHA-derived metabolites was very low in the cortex and hippocampus of both mice. Aging decreased ARA-derived metabolites in the hippocampus, regardless of the biosynthetic pathway, such as cyclooxygenase, 5-lipoxygenase, 12/15-lipoxygenase, cytochrome P450, and non-enzymatic autoxidation. DHA-derived metabolites in the hippocampus were also decreased by age. There was no significant difference in mRNA expression of the various enzymes related to metabolite biosynthesis between young and aged mice. The cognitive function of SAMP10 was impaired due to aging. Based on analyzing a board range of LCPUFA-derived metabolites, we propose that age-related decrease in LCPUFA metabolism in the hippocampus may be involved in the impaired cognitive function in SAMP10 mice.

Key words: senescence-accelerated mice, polyunsaturated fatty acids, lipid mediator, brain

Introduction

Long-chain polyunsaturated fatty acids (LCPUFAs) are the main constituents of biomembranes. They consist of two series, namely n-6 and n-3. The major n-6 LCPUFA is arachidonic acid (ARA), while the major n-3 LCPUFAs are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). They have 20−22 carbons with 4−6 double bonds, and play important roles in physiological homeostasis, including the modulation of membrane fluidity, membrane enzyme activities, and physiological responses[1,2].

Some of these responses are induced by the formation of

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lipid mediators from LCPUFAs themselves. These lipid mediators have been shown to exert a range of biological effects. The 2-series prostaglandins and 4-series leukotrienes, biosynthesized from ARA, are well-known lipid mediators that are generally involved in the initiation of acute inflammation. On the other hand, lipid mediators derived from EPA and DHA, such as the 3-series prostaglandins, 5-series leukotrienes, resolvins, protectin, and maresin, exhibit anti-inflammatory effects. The biosynthesis of LCPUFA-derived lipid mediators is mainly evoked by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes. Lipid mediators are also partially produced through non-enzymatic autoxidation mechanisms[3,4]. Recently, some studies have shown new physiological functions of several metabolites. For instance, 18-hydroxyeicosapentaenoic acid derived from EPA inhibits proinflammatory activation of cardiac fibroblasts[5], and DHA-derived protectin D1 (PD1) remarkably attenuates influenza virus replication and improves the survival and pathology of severe influenza in mice[6].

LCPUFAs are the major components of brain phospholipids and their levels in humans[7] and animals[8] have been reported to decrease with aging. DHA/EPA or ARA supplementation has been shown to improve the cognitive function in healthy elderly humans[9,10], and ameliorate the reduced cognitive function in aged animals as well[11,12]. Various mechanisms, such as synaptic plasticity, neurogenesis, and membrane fluidity have been shown to be associated with this effect in aged animals[2,11]. A few metabolites, such as PGE2 and PD1, have been shown to improve brain function[13,14], though the relationship between lipid metabolites and brain function still remains unclear. Moreover, non-reported study has examined the changes in LCPUFA metabolite profiles in the brain with age-related impairment of cognitive function.

The current study aimed to clarify changes in LCPUFA metabolism in the brain, which may be involved in an age-related decline of cognitive function. SAMP10 strain was selected as a model of age-related neurodegeneration, exhibiting age-related cerebral atrophy due to the loss and perikaryal shrinkage of cortical neurons[15]. Aged SAMP10 mice have a more deteriorated cognitive function than SAMR1 control mice. In the present study, we investigated 137 molecular species of free fatty acids and metabolites by comprehensive lipidomics systems using liquid chromatography-tandem mass spectrometry (LC-MS/MS), and mRNA expression-associated metabolism of LCPUFA in the brain of young and aged SAMP10 mice.

Materials and Methods

Animals and diets

All protocols for animal procedures were approved by the Ethics Committee of Animal Experiment in accordance with the Internal Regulations on Animal Experiments at Suntory, which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as amended in 2 June 2017). Male SAMP10 mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). They were housed individually in polycarbonate cages with paper bedding, which was changed once a week. The facility was maintained under specific pathogen-free conditions at a temperature of 23±2°C and humidity of 55±10%, with a 12-h light/dark cycle (light was switched on at 7:00). Before the experiment, mice were acclimated to the facility for more than a week. The mice had free access to water and feed; we used the latter based on AIN-93M containing 4% fat with modified lipid compositions. The lipids consisted of palm oil, soybean oil, and linseed oil (SHOWA KOSAN CO., LTD., Minato-ku, Tokyo, Japan). The fatty acid compositions of the diet are shown in Table 1. All the diets were stored at 4°C and changed twice a week to prevent oxidation. After more than 1 week of acclimation period, SAMP10 mice aged 2 or 9 months were fed the diet for 12 weeks, and we measured weight once a week and observed general appearance twice a week.

Gas–liquid chromatography (GC) for fatty acid composition

Lipids in the diet were extracted and purified by the

Table 1. Fatty acid composition of the diet

FA (AIN93M contained 4% fat)	(%)
Palmitic acid (PA)	26.9
Stearic acid (SA)	4.3
Oleic acid (OA)	31.2
Linoleic acid (LA)	22.5
α-Linolenic acid (ALA)	11.3
Dihomo-γ-linolenic acid (DGLA)	0.0
Arachidonic acid (ARA)	0.0
Eicosapentaenoic acid (EPA)	0.0
Docosahexaenoic acid (DHA)	0.0
Others	3.7

method of Folch et al[16]. Phospholipids, which were obtained using thin-layer chromatography (hexane : diethyl ether=7 : 3), were methylesterified by incubating in methanolic HCl at 50˚C for 3 h, extracted with hexane, and subjected to capillary gas−liquid chromatography (Agilent 7890B; Agilent Technologies, Santa Clara, CA) using an SP-2330 column (30 m×0.32 mm×0.2 μm; Supelco, Bellefonte, PA) with He (at 30 cm/s) as the carrier. The column temperature was initially 180˚C for 2 min and then increased to 220˚C at a rate of 2˚C/min.

LC-MS/MS analysis for lipidomics in brain samples

Brain tissues were homogenized on ice with methanol, and the extracted lipids were purified in solid-phase using Sep-Pak C18 3 cc Vac cartridges (Waters) with deuterium-labeled internal standards (IS; AA-d_8, 15-HETE-d_8, 14,15-EET-d_{11}, PGE2-d_4, and LTB4-d_4, each at a final concentration of 10 pg/μL), excluding the sample that one hippocampus of the aged mice could not be extracted lipids. Lipidomic analysis was performed using a UPLC system coupled with a quadrupole/linear ion trap MS (QTRAP5500; SCIEX, Framingham, MA), as described previously[1,17]. LC separation was performed using a reversed-phase column [ACQUITY UPLC BEH C18 column (1.0×150 mm, 1.7 μm particle size; Waters, Milford, MA)]. Samples were eluted with a mobile phase composed of 0.1 vol% acetic acid in water and acetonitrile/methanol (4 : 1, v/v) (73 : 27) for 5 min, and ramped to 30 : 70 after 15 min, to 20 : 80 after 25 min, and held there for 8 min, ramping to 0 : 100 after 35 min, and held there for 10 min with flow rates of 70 μL/min (0−30 min), 80 μL/min (30−33 min), and 100 μL/min (33−45 min). MS/MS analyses were conducted in negative ion mode, and 137 molecular species of free fatty acids and metabolites were identified (Supplement Table 1) and quantified by selected reaction monitoring (SRM). SRM transitions, declustering potential, entrance potential, collision energy, collision cell exit potential, and retention time for all the analytes and internal standards are described previously[18]. Compounds were quantified using stable internal standards. Extraction yield and matrix recovery were determined using IS and quantified with calibration curves. The lipid compounds were grouped into 4 types, (group 1) free fatty acids, (group 2) monohydrides and epoxides, (group 3) diols, leukotrienes, thromboxanes, lipoxines, and resolvins, and (group 4) prostaglandins and others, and they were corrected with ARA-d_8, 15-HETE-d_8, LTB4-d_4, and PGE2-d_4, respectively.

RNA sequencing

Total RNA was isolated from brain tissues using ISOGEN (Nippon Gene, Tokyo, Japan) and purified using the RNeasy Mini kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions, as previously described[19]. The integrity of isolated RNA was determined using a bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA integrity numbers (RIN) of all samples were identified higher than 8.6 and five samples were selected in order of higher RIN value for library preparation and sequencing, for each young and aged mouse. The mRNA library preparation was based on SureSelect Strand-Specific RNA Library Prep for Illumina Multiplexed Sequencing (Agilent Technologies, version C.0, December 2014). RNA-seq libraries were sequenced on a HiSeq 1500 DNA Sequencer (Illumina, San Diego, CA, USA) in a 50-base single-end read mode, as described previously[20]. The sequence reads were mapped to the mouse genome (NCBI version 37) using TopHat2 (version 2.0.8) and botwie2 (version 2.1.0) with default parameters, and gene annotation was provided based on NCBI RefSeq. Transcript abundances were estimated using Cufflinks (version 2.1.1), which was run with the same reference annotation as TopHat2 to generate FPKM (fragments per kilobase per million mapped reads) values for known gene models.

Memory examination

We evaluated the cognitive function of mice from both age groups using the Morris water maze test and the step-through test, which were modified from previously described methods[21], after 12 weeks of feeding.

The Morris water maze pool consisted of a circular plastic water tank, 90 cm in diameter and 60 cm in depth. The inside of the pool was painted black, and it was filled with water to a height of 40 cm at approximately 22˚C. The pool was divided into quadrants and a clear platform (8 cm in diameter) was submerged 1 cm below the water level in one of the pool quadrants; there were many cues in the internal pool, which could be used by the mouse for spatial localization. These cues were held constant throughout the test. Mice were first provided spatial learning trials for 4 days. They were monitored via a video camera mounted overhead, and the latency to escape onto the hidden platform was automatically recorded using a video tracking system

in a trial. Each mouse was allowed to rest on the platform for 10 s after locating it. If it could not locate the platform within 120 s, the trial was terminated, and researchers placed it on the platform for 10 s. Mice received three trials per day from different start locations for a total of 4 days. After the final training trial, mice were subjected to a single 120 s probe test on day 5. The platform was removed, and time spent in target quadrant, the distance moved, and swimming speed were measured by a video tracking system. After a trial, the mouse was dried, and returned to its house cage.

One day after Morris water maze test, the step-through type passive avoidance test was carried out. The apparatus consisted of two compartments separated by a sliding door. One of the compartments was well-lit, and the other was darkened. The mouse was placed in the former compartment for 30 s. In an acquisition trial, the door to the dark compartment was opened, and the time before the mouse entered the dark compartment was recorded. Subsequently, the mouse was placed inside the dark compartment, the door was closed, and a slight foot-shock was delivered (0.2 mA for 1 s). The mouse was then removed and returned to its home cage. Next, in a retention trial, the mouse was once again placed in the well-lit compartment, and the latency to re-enter the dark compartment was recorded up to a maximum of 300 s. After each trial, the apparatus was cleaned.

Brain tissues

After we conducted two behavioral assays and the mice were euthanized by exsanguination under anesthesia with isoflurane, and brain tissues (cortex and hippocampus) were immediately harvested thereafter. Samples were immediately stored at −80°C until analysis.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). Data are shown as the mean±standard error. All data were checked for normality of distribution and equality of variance using the Shapiro−Wilk test; homogeneity of variance was tested using Levene's test. Comparisons between the young and aged mice were performed using an unpaired Student's *t*-test or a non-parametric Mann−Whitney *U* test. For all tests, *P* values less than 0.05 were considered statistically significant.

Results

Free fatty acid levels

We performed targeted lipidomic analysis on the cortex and hippocampus of young (5 months old) and aged (12 months old) SAMP10 mice after intake of the control diet for 12 weeks. There was no difference in body and brain weights between the young and aged mice (Table 2). Levels of free PA, SA, and ARA were high in both the cortex and hippocampus of both the young and aged mice, compared with other FAs. Level of free ARA was approximately 8-fold higher than that of free DHA. There was no

Table 2. Body weight and brain weight of young and aged mice

	Young (*n*=8) Mean±SE	Aged (*n*=7) Mean±SE
Body weight (g)	29.7±0.9	27.3±1.2
Brain weight (mg)	248±5	246±10

There was no significant difference between the young and aged mice for any of the variables (as per unpaired *t*-test).

Table 3. Free FA levels in the cortex and hippocampus

pg/mg tissue	Cortex		Hippocampus	
	Young (*n*=8) Mean±SE	Aged (*n*=7) Mean±SE	Young (*n*=8) Mean±SE	Aged (*n*=6) Mean±SE
PA	12164±952	11909±381	11501±712	11608±1417
POA	305±29	264±18	309±22	231±29
SA	17235±822	17900±338	19998±678	19887±2326
OA	1347±87	1289±55	1816±125	1403±88*
LA	124±6	124±11	173±10	133±13*
ALA	ND	5±3	7±3	ND
GLA	2±1	1±1	5±0	2±1
SDA	ND	ND	ND	ND
DGLA	66±7	52±4	106±14	89±14
MA	51±7	46±5	105±12	95±8
ETA	ND	ND	ND	ND
ARA	10161±285	9561±487	14255±621	12988±611
EPA	40±4	43±8	64±6	48±5
AdA	115±8	122±11	198±20	154±17
DPA(n3)	33±3	33±4	52±5	40±4
DPA(n6)	15±2	15±1	22±3	22±4
DHA	1442±146	1380±156	1976±162	1475±147

Significant differences were observed between the young and aged mice ($P<0.05$, *Mann−Whitney test). PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; SDA, Stearidonic acid; DGLA, dihomo- γ-linolenic acid; MA, mead acid; ETA, eicosatetraenoic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; AdA, adrenic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid

difference in the levels of free fatty acids in the cortex across the young and aged mice. Levels of free OA and LA in the hippocampus were significantly lower in the aged mice than in the young ones (Table 3).

Metabolite levels

A total of 40 metabolites, derived from LA, GLA, DGLA, ARA, EPA, and DHA, were identified in the targeted lipidomics. While the level of ARA-derived metabolites was high compared to that of other PUFA-derived metabolites, the level of n-3 PUFA-derived metabolites, such as DHA or EPA, was low in both the cortex and hippocampus. There was no difference in the levels of metabolites in the cortex between the young and aged mice (Fig. 1A). Levels of ARA- and DHA-derived metabolites in the hippocampus were significantly lower in the aged mice than in the young ones (Fig. 1B).

Figure 2 shows a comparison of the levels of ARA-derived metabolites across a variety of fatty-acid metabolizing enzymes or auto-oxidation. Levels of ARA-derived metabolites in COX and CYP pathways in the cortex tended to be lower in the aged mice (33.9±10.2 and 11.0±1.4 pg/mg tissue, respectively) than in the young ones (43.5±4.4 pg/mg tissue; Mann−Whitney: P=0.089 and 15.0±1.5 pg/mg tissue; unpaired t-test: P=0.070, respectively). Moreover, levels of these in the hippocampus were significantly lower in the aged mice (30.0±5.0 and 8.5±1.4 pg/mg tissue, respectively) than in the young ones (52.1±8.5 pg/mg tissue; Mann−Whitney: $P<0.05$ and 17.4±1.9 pg/mg tissue; unpaired t-test: $P<0.05$, respectively). Levels of ARA-derived metabolites in the 5-LOX and 12/15-LOX pathways were lower in the aged mice (21.3±1.5 and 92.1±8.5 pg/mg

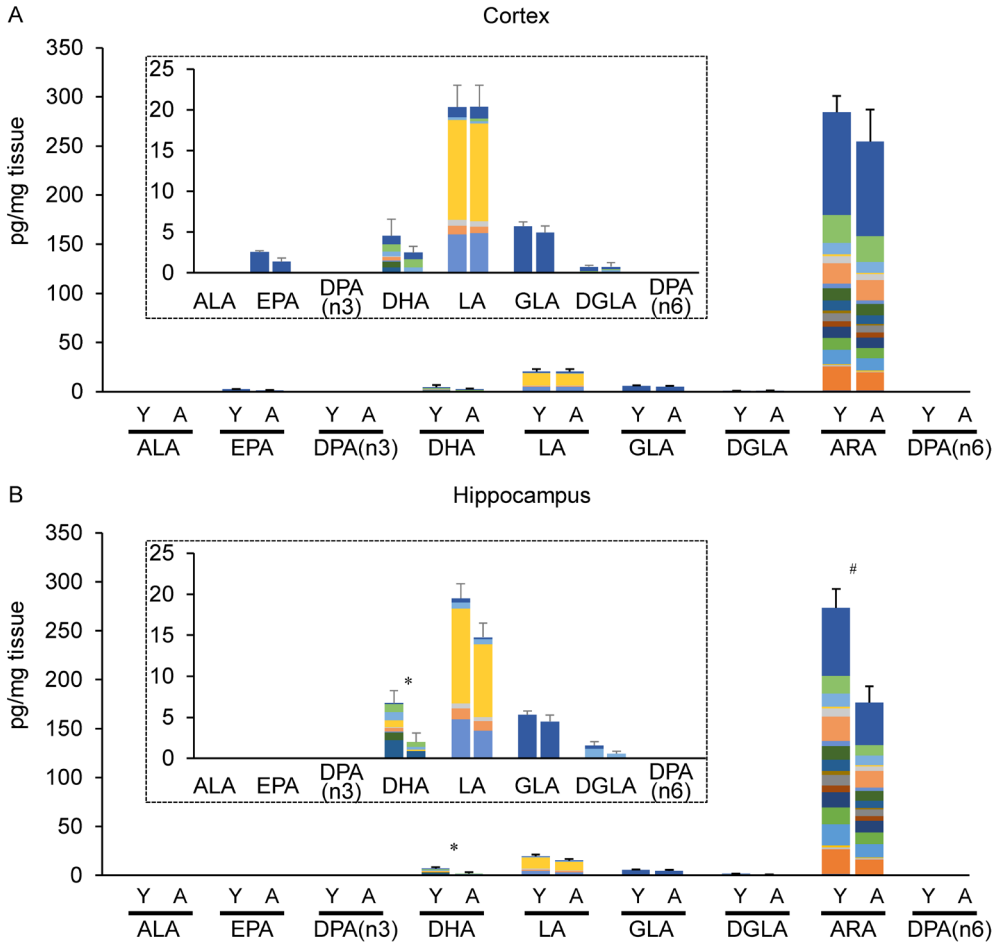

Fig. 1. Age-related changes in metabolite profiles in the cortex and hippocampus.
The total amounts of metabolites derived from ALA, EPA, DPA (n3), DHA, LA, GLA, DGLA, ARA, and DPA (n6) are presented. Inset shows an enlargement of the small range. Data are presented as the mean±standard error (cortex (A); young mice (n=8), aged mice (n=7), hippocampus (B); young mice (n=8), aged mice (n=6)). There was a significant difference between the young (Y) mice and aged (A) mice ($P<0.05$, $^{\#}$unpaired t-test; *Mann−Whitney test). ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; ARA, arachidonic acid

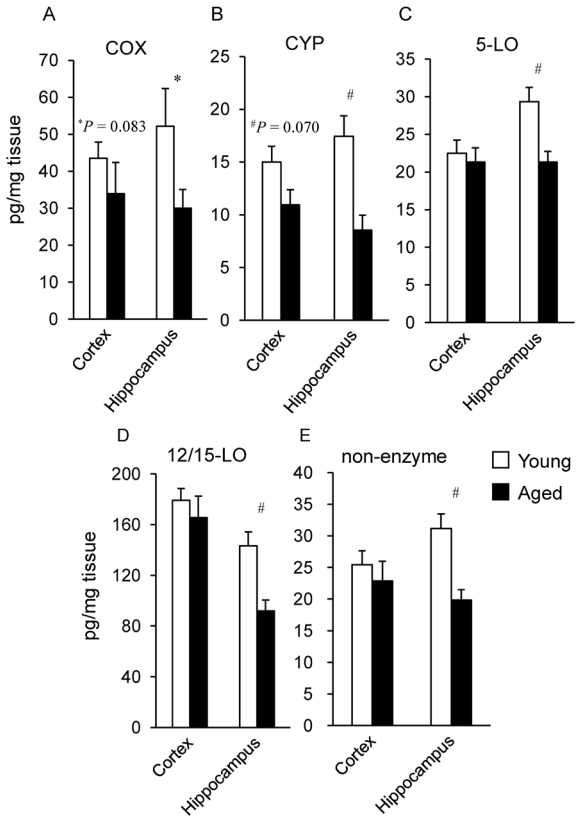

Fig. 2. Age-related changes in the levels of ARA-derived metabolites synthesized by enzymes and by non-enzymatic autoxidation in the cortex and hippocampus.

The total amount of PGE2, PGD2, PGF2a, 15-keto-PGE2, 15-deoxy-PGJ2, 6-keto-PGF1a, TXB2, and 12-HHTrE metabolized by COX (A). The total amount of 5,6-DHT, 8,9-DHT, 11,12-DHT, 14,15-DHT, 8,9-EET, 11,12-EET, and 14,15-EET metabolized by CYP (B). The total amount of LTB4, LTB4-20OH, LTD4, 5-HETE, and 5-oxo-ETE metabolized by 5LO (C). The total amount of LXA4, LXB4, 5,15-diHETE, 8,15-diHETE, HxA3, HxB3, 12-HETE, 15-HETE, 12-oxo-ETE, and 15-oxo-ETE metabolized by 12/15LO (D). The total amount of 8-HETE, 9-HETE, 11-HETE, 16-HETE, 17-HETE, 18-HETE, 19-HETE, and 20-HETE synthesized by non-enzymatic autooxidation (E). Data are presented as the mean± standard error (cortex; young mice (n=8), aged mice (n=7), hippocampus; young mice (n=8), aged mice (n=6)). There was a significant difference between the young mice and aged mice ($P<0.05$, $^{\#}$unpaired t-test; *Mann−Whitney test). COX, cyclooxygenase; LO, lipoxygenase; CYP, cytochrome P450

tissue, respectively) than in the young ones (29.4±1.9 and 143.2±11.1 pg/mg tissue; unpaired t-test: $P<0.05$, respectively) in the hippocampus. Levels of these in the cortex were not different between the young (22.5±1.7 and 179.0 ±9.6 pg/mg tissue, respectively) and the aged mice (21.3± 1.8 and 165.4±17.1 pg/mg tissue, respectively). Levels of ARA-derived metabolites in non-enzymatic autoxidation was lower in the aged mice (19.8±1.7 pg/mg tissue) than in the young ones (31.1±2.3 pg/mg tissue; unpaired t-test: $P<0.05$) in the hippocampus. Levels of that in the cortex were not different between the young (25.4±2.2 pg/mg tissue) and the aged mice (22.9±3.1 pg/mg tissue).

A total of 19 metabolites derived from ARA were identified in both the cortex and hippocampus. Most of the ARA-derived metabolites were significantly lower in the hippocampus of aged mice than in that of young ones, such as thromboxane B2 (0.7±0.2 and 1.9±0.3 pg/mg tissue), 12-heptadecatrienoic acid (12.9±1.9 and 21.6±3.3 pg/mg tissue), 5-hydroxyeicosatetraenoic acid (HETE; 11.7±0.8 and 15.9±1.0 pg/mg tissue), 5-oxo-eicosatetraenoic acid (oxo-ETE; 9.6±0.7 and 13.4±0.9 pg/mg tissue), 12-oxo-ETE (10.4±1.3 and 18.4±2.1 pg/mg tissue), 15-HETE (17.2 ±1.6 and 25.0±1.7 pg/mg tissue), 15-oxo-ETE (44.1±4.5 and 69.1±7.6 pg/mg tissue), Hepoxilin B3 (9.9±2.2 and 17.1±1.9 pg/mg tissue), 8,9-epoxyeicosatrienoic acid (EET; 1.0±0.6 and 4.0±0.6 pg/mg tissue), 11,12-EET (3.1±0.3 and 5.2±0.5 pg/mg tissue), 14,15-EET (4.5±0.5 and 8.3± 0.9 pg/mg tissue), 11-HETE (7.3±0.7 and 11.6±1.0 pg/mg tissue), and 16-HETE (1.0±0.2 and 1.8±0.1 pg/mg tissue). There was no difference in ARA-derived metabolites in the cortex between the young and aged mice (Fig. 3, Supplement Table 2). The typical LC-MS/MS chromatograms of ARA-derived metabolites, which changed in aging, are shown in Supplement Fig. 1.

mRNA expression of metabolism-related enzymes

The expression of mRNAs related to fatty acid-metabolizing enzymes is shown in Fig. 4. There was hardly any difference in mRNA expression between the young and aged mice, excluding four mRNAs. *Gpx4*, *Cyp2d22*, and *Cyp2d26* mRNAs were significantly higher in the aged mice than in the young ones, whereas *Agpat5* mRNA was significantly lower in the aged mice than in the young ones. The rates of change were all small.

Evaluation of behavior

We first performed the Morris Water maze test. The escape latency to find the submerged platform was significantly higher in the aged mice than in the young ones during the training days, excluding day 3 (Fig. 5A). In probe trial, the durations spent in the target quadrant were 37.9±9.8 and 20.7±10.3 s in the young and aged mice,

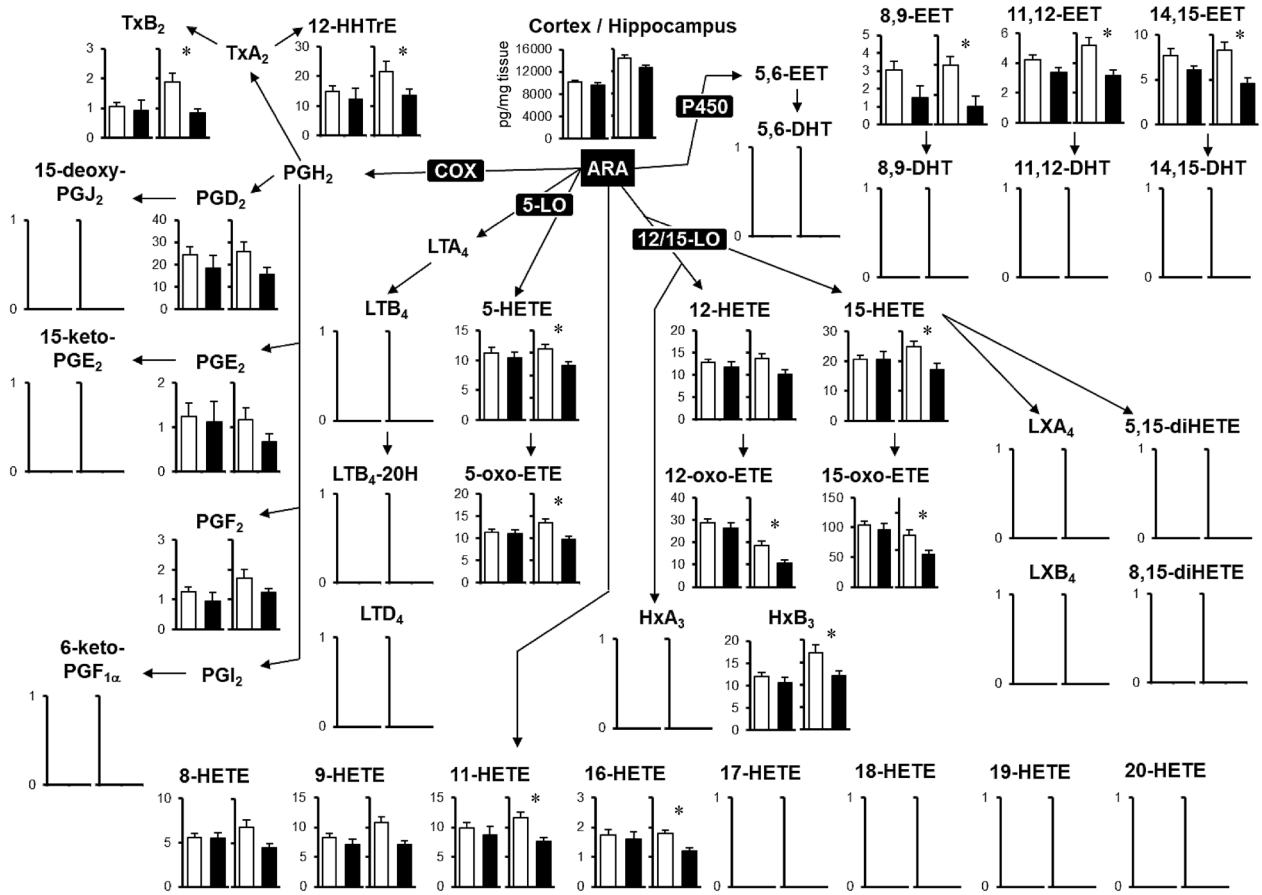

Fig. 3. Age-related changes in the levels of metabolites derived from ARA in the cortex and hippocampus.
Data are presented as the mean±standard error (cortex; young mice (n=8), aged mice (n=7), hippocampus; young mice (n=8), aged mice (n=6)). There was a significant difference between the young and aged mice (P<0.05, *Mann−Whitney test). COX, cyclooxygenase; LO, lipoxygenase; CYP, cytochrome P450

respectively, which did not differ significantly (Fig. 5B). The swimming speed, except in the target quadrant, did not differ across the young and aged mice (Fig. 5C). The distance moved was significantly shorter in the aged mice than in the young ones (Fig. 5D). We next performed the step-through test. In acquisition trial, there was no difference in latency time between the young mice (17±3 s) and aged ones (14±3 s). In retention trial, however, the latency time was lower in the aged mice (200±42 s) than in the young ones (288±13 s, Mann−Whitney, P<0.05; Fig. 5E).

Discussion

In the present study, the levels of metabolites biosynthesized from various fatty acids clearly indicated the difference in the metabolism of LCPUFAs, such as DHA and ARA, in the hippocampus of young and aged SAMP10 mice, but not in the cortex (Fig. 1). The results quantitatively confirmed the metabolic levels related to DHA and ARA in the hippocampus of aged mice to be approximately one-third and two-thirds of that in the young mice, respectively. As far as we know, this is the first study quantifying the decrease in DHA- and ARA-derived metabolites in the hippocampus with age by a broad-targeted analysis of metabolites.

Metabolism of n-6 PUFA may occur selectively, since the levels of n-6 free fatty acids, such as ARA, were higher than those of n-3 free fatty acids in both the young mice and the aged ones. The levels of free ARA were clearly higher than those of other PUFAs (Table 3), and various metabolites derived from ARA were produced in the brain of SAMP10 mice (Fig. 3). ARA-derived metabolites were generated regardless of the type of enzymes, and metabolites derived from nonenzymatic oxidation were also detected in previous studies; PGE2[14], LXA4, LTB4[22], and 12-HETE[23] were identified in the brain tissue. Moreover, in the cortex of SAMP8 mouse, COX metabolites, LOX metabolites, and products of nonenzymatic oxidation were detected in a previous study[24]. Several metabolites play

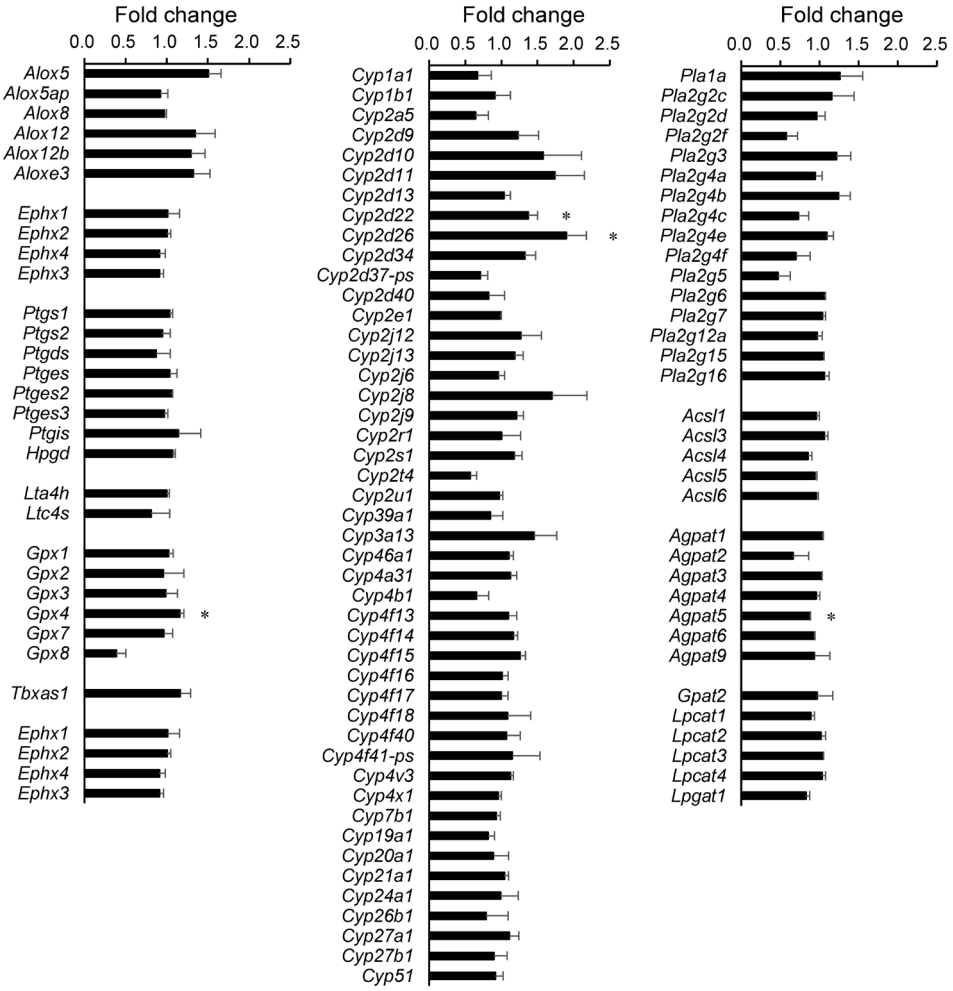

Fig. 4. Age-related changes in mRNA expression associated with fatty acid metabolizing enzymes in the hippocampus.
Data are presented as the mean of fold change (young mice (n=5), aged mice (n=5)). Significant differences were observed between the young mice and aged mice ($P<0.05$, *unpaired t-test).

important roles in neural function, such as spatial learning, synaptic plasticity (PGE2)[14], and resolution of inflammation (LXA4)[14], and act as second messengers in synaptic transmission and neuroprotection (12-HETE, 15-HETE)[25,26]. However, whether other metabolites derived from ARA play roles in cognitive function remains unclear.

ARA-derived metabolites were synthesized by each enzyme, such as COX, 5-LOX, 12/15-LOX, and CYP, as well as by non-enzymatic autoxidation, in both the cortex and hippocampus. Levels of these metabolites in the hippocampus decreased with age, regardless of the biosynthetic pathway, and the metabolites from COX or CYP in the cortex also decreased with age (Fig. 2). Although the levels of ARA-derived metabolites in the hippocampus of aged mice were significantly lower, they did not change with age in the cortex (Fig. 3). The metabolism of LA, as an n-6 PUFA, did not show a decrease with age, either in the hippocampus or in the cortex (Fig. 1, Supplement Table 3). The cortex and hippocampus are interconnected areas of the brain, supporting basic cognitive functions that are important for the formation and retrieval of declarative memories. Metabolism of LCPUFA in the brain is probably affected by aging, especially in the hippocampus. A previous study demonstrated no difference in 5-HETE, 8-HETE, 9-HETE, 11-HETE, 15-HETE, PGF2a, and PGD2 between the young and aged mice, and PGE2 and TXB2 in the cortex of aged SAMP8 mice were higher than those in young SAMP8 mice[24]. Overexpression of amyloid-beta (Aβ) was observed in the aged SAMP8 mice[27], and SAMP10 mouse served as a unique model of age-dependence with inherited cerebral atrophy and cognitive dysfunction[15]. The mechanism of aging might be different between SAMP10 and SAMP8, for example, depending on whether there is an increase in Aβ accumulation in the brain. Future studies are

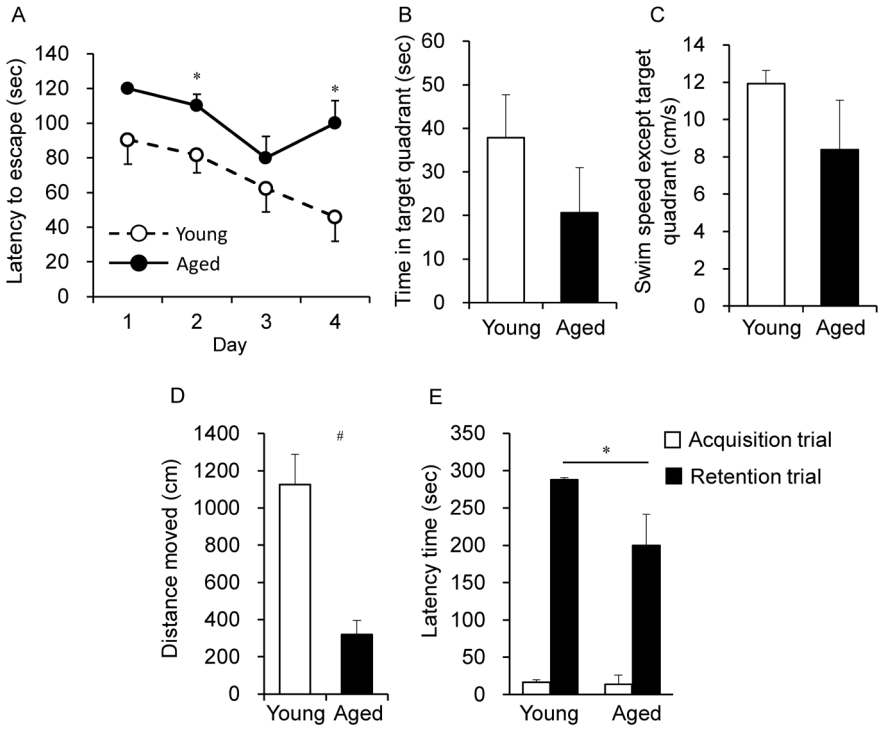

Fig. 5. Age-related changes in Morris water maze test and passive avoidance response test in SAMP10.
Morris water maze test: escape latency to find the hidden platform per group in three trials over 4 consecutive days (A). Time spent in target quadrant (B), swimming speed (C), and distance moved (D) in the probe test. The step-through type passive avoidance test: The latency time to enter the dark chamber in an acquisition trial or in a retention trial (E). Data are presented as the mean± standard error (young mice (n=8), aged mice (n=7)). There was a significant difference between the young and aged mice ($P<$ 0.05, $^{\#}$unpaired t-test; *Mann−Whitney test).

expected to reveal the effects of aging on the metabolism of LCPUFA in the brain of the wildtype mouse and/or other species.

The greater decrease in DHA-derived metabolite biosynthesis in aged hippocampus might be influenced by the very less free DHA available in the brain. The level of free DHA was approximately one-eighth-fold lower than that of free ARA, although both DHA and ARA are the main fatty acid components in the brain. In the human orbitofrontal cortex, compositions of DHA and ARA in phospholipids were approximately 15% and 10%, respectively[7]. Moreover, compositions of DHA and ARA in the hippocampus of rat have been shown to be approximately 15% and 13.5%[8], whereas those in the brain of SAMP8 mouse were 19.3% and 10.8%, respectively[21]. In this study, fatty acid composition in the hippocampus could not be tested owing to the limited volume of tissue; however, in the previous study, we did confirm their composition in the hippocampus of SAMP10 mouse to be similar to that in SAMP8 mouse[28]. Thus, DHA is the most abundant LCPUFA in the brain, probably implying its important role as a component of cell membrane by maintaining its distribution in phospholipids. DHA-derived metabolites, such as PD1[13] and Maresin[29], have also been reported to be useful in maintaining brain function; however, they were under the detection limit in the resting state of the hippocampus and cortex. The presence of a small amount of DHA-derived metabolite might have an effect on cognitive function and the decrease in biosynthesis of these metabolites with age in the hippocampus might affect brain function.

Since LCPUFA metabolism became less active in the aged hippocampus, mRNA expression levels of LCPUFA-metabolizing enzymes were evaluated by comprehensive transcriptome analysis. The results showed no significant difference in mRNA expression across various enzymes related to metabolite biosynthesis, excluding only four mRNAs (Fig. 4). Decreased specific enzyme activity or abnormal translation of enzyme genes in the hippocampus may occur by aging. Thus, the molecular mechanisms underlying downregulated LCPUFA metabolism in the aged hippocampus still remain to be elucidated.

Cognitive function in aged SAMP10 mice was impaired

compared to that in young mice, since the latency of aged mice was lower than that of young mice in the step-through test (Fig. 5). This result was in reasonable agreement with a previous study[15]. On the other hand, the result of Morris water maze test, which considers motor activity, may not have been properly evaluated, since there was a significant difference in the distance moved by the young mice and aged mice. The result of the step-through test was thought to mainly reflect hippocampal function[30]. We therefore propose that active LCPUFA metabolism in the hippocampus may play important roles in maintaining the cognitive function of the brain. The dietary ARA or DHA was previously shown to increase ARA-derived or DHA-derived metabolites in the brain[18]. Further, diets including DHA or ARA increased their corresponding levels in the hippocampus[8]. Future studies of LCPUFA supplementation would reveal the causal relationship between the reduced LCPUFA metabolism in the hippocampus and the cognitive function of aged brain.

In conclusion, the present study analyzed a broad range of metabolites derived from LCPUFA in the brain to determine the effects of aging on the metabolism. We believe that this study is expandable further to clarify the role of LCPUFA in brain function. Based on our findings, we propose that age-related decrease in LCPUFA metabolism in the hippocampus may be involved in the impaired cognitive function in SAMP10 mice.

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Conflict of Interest

All other authors have no conflicts of interest.

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