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LOSS OF RAR-RELATED ORPHAN RECEPTOR ALPHA (ROR α) SELECTIVELY LOWERS DOCOSAHEXAENOIC ACID IN DEVELOPING CEREBELLUM

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Abstract

Deficiency in retinoid acid receptor-related orphan receptor alpha (ROR α) of *staggerer* mice results in extensive granule and Purkinje cell loss in the cerebellum as well as in learned motor deficits, cognition impairments and perseverative tendencies that are commonly observed in autistic spectrum disorder (ASD). The effects of ROR α on brain lipid metabolism associated with cerebellar atrophy remain unexplored. The aim of this study is to examine the effects of ROR α deficiency on brain phospholipid fatty acid concentrations and compositions. *Staggerer* mice (*Rora*^{sg/sg}) and wildtype littermates (*Rora*^{+/+}) were fed n-3 polyunsaturated fatty acids (PUFA) containing diets *ad libitum*. At 2 months and 7 or more months old, brain total phospholipids fatty acids were quantified by gas chromatography-flame ionization detection. In the cerebellum, all fatty acid concentrations were reduced in 2 months old mice. Since total fatty acid concentrations were significantly different at 2-month-old, we examined changes in fatty acid composition. The composition of ARA was not significantly different between genotypes; though DHA composition remained significantly lowered. Despite cerebellar atrophy at >7-months-old, cerebellar fatty acid concentrations had recovered comparably to wildtype control. Therefore, ROR α may be necessary for fatty acid accretions during neurodevelopment. Specifically, the effects of ROR α on PUFA metabolisms are region-specific and age-dependent.

Keywords

Cerebellum; Nuclear Receptor; Autistic Disorder; Phospholipids; Development

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Introduction

Staggerer mice were first reported in 1962 as spontaneous mutant offspring of stock obese mice with ataxic gait, hypotonia, body imbalance and tremor associated with predominant cerebellar cortex degeneration [1]. The *staggerer* cerebellum exhibit folia with small fissures, indistinct lamination of cerebellar cortex, thin molecular layer and absence of granular layer [1–3]. Developmental atrophy of *staggerer* cerebellum begins at birth with accelerated degeneration of Purkinje and granule cells [4, 5]. Purkinje cells of *staggerer* cerebellum are small, scarce, and devoid of dendritic branchlet spines which contribute to a histologically ectopic scatter pattern as compared to a monolayer phenotype [1, 6, 7]. *Staggerer* mutation impairs early differentiation, maturation and survival of Purkinje cells, but does not affect neurogenesis [4, 8–12]. In contrast to earlier rates of Purkinje cell loss in developing cerebellum of 3-month-old male heterozygous *staggerer* mouse as compared to female [8], sex effects on Purkinje cell size and loss were not observed in homozygous *staggerer* mouse [13]. The intrinsic defect of *staggerer* Purkinje cell leads to deficits in innervation with granule cells; thereby reduces the rate of granule cell precursor proliferation leading to increased apoptosis and the consequent absence of granular layer [2, 3, 6, 14]. Similarly, in hippocampal dentate gyrus, it was reported that *staggerer* mutation affects neuron differentiation and maturation but not neurogenesis [15].

Staggerer mutation was traced by positional cloning to an intragenic deletion of retinoic acid-related orphan nuclear receptor alpha, ROR α [16]. Comparative analyses had demonstrated similar abnormal cerebellar atrophy and Purkinje cell cytology between *staggerer* mutant mice and ROR α knock-out mice; further providing evidence that loss of ROR α function is the cause of *staggerer* characteristics [13, 17]. ROR α expressions in murine and human tissues are ubiquitous [16, 18, 19]. In the brain, ROR α expression is highest in cerebellum and thalamus followed by moderate-to-low expressions in olfactory bulbs and cortex, and undetectable expressions in striatum and hippocampus [15, 20, 21]. Specifically in the cerebellum, ROR α is highly expressed in Purkinje cells followed by weaker expression in stellate and basket cells [16, 22, 23]; while granule cells do not express ROR α [22]. In addition to neuronal expression of ROR α , glial expression of ROR α is restricted to astrocytes [24]. Genetic ablation of neuronal and astrocytic ROR α expression induced cell-autonomous and non-cell-autonomous regression of dendritic arbor and loss of branchlet spines and functional synapses; thereby leading to impairment of motor coordination [25–27]. In contrast to Purkinje cells, ROR α -expressing stellate and basket cells are unaffected by ROR α deficiency [22]. ROR α -expressing thalamic neuronal cells and retinal ganglion cells are also unaffected in *staggerer* mutants [22]. In olfactory bulbs, *staggerer* mutation reduces the number of neurons and interneurons in mitral cells as well as the astrocyte network in granular layer [28]. Therefore, degeneration of ROR α -expressing cells are region-dependent and cell-restrictive.

ROR α is implicated in neuropsychiatric disorders including autism spectrum disorders (ASD), bipolar disorder (BD) and major depressive disorder (MDD) [29–40]. Specifically, neuroanatomical abnormalities in patients with autism including loss of Purkinje cells, reduced cerebellar volume and interrupted cerebello-thalamo-cortical pathways resembles that of *staggerer* mice [41]. ROR α expression and protein are reduced in lymphoblastoid cell

lines and postmortem cerebellum of autistic patients, respectively [29, 42]. Furthermore, ROR α transcriptionally regulates neuronal genes associated with ASD [30]. Even though the canonical functions of the cerebellum consist of balance control and motor coordination, emerging evidence supports additional cerebellar roles in emotional processing and cognition [43–47]. In addition to pronounced impairment in gait and balance, *staggerer* mice exhibits significant deficits in learned motor tasks [48]. *Staggerer* mice also exhibit deficiencies in cognitive behaviors. *Staggerer* mice commit significantly more errors in exploration and spatial learning tasks [49, 50]. While passive avoidance learning is unaffected in *staggerer* mice, deficits in active avoidance learning due to impairments in reversal training may explain the perseverative tendencies in *staggerer* mice [49, 51]. Therefore, *staggerer* may be a novel functionally relevant model of ASD.

Polyunsaturated fatty acids (PUFA), including omega-6 (n-6) and omega-3 (n-3) fatty acids, are critical for early brain development [52–55]. Particularly, the predominant species, arachidonic acid (ARA; n-6 PUFA) and docosahexaenoic acid (DHA; n-3 PUFA) [56, 57], are enriched for neuronal growth, synaptogenesis, neuronal survival and modulation of neurotransmitters [58–60]. Even though abnormal lipid metabolism in human autistic brains has not been investigated, emerging evidence has demonstrated abnormal lipid metabolism in plasma biomarkers [61, 62]. Nevertheless, PUFA interventions and modulations in brain phospholipid fatty acid composition may alleviate autistic-like cognitive and social behaviors [63–65]. Interestingly, *staggerer* mice exhibit aberrant lipid metabolism. *Staggerer* serum cholesterol and triglycerides are reduced due to lower expressions of apolipoprotein A-I and C-III, respectively [66, 67]. Moreover, ROR α mediates the regulation of lipoprotein homeostasis [68]. Hepatic expressions of peroxisome proliferator-activated receptor- γ , coactivator 1 (*PGC-1 α*) and *lipin1* are increased in *staggerer* mice which suggests that ROR α may regulate mitochondrial fatty acid oxidation [69]. ROR α is essential in repressing proliferators-activated receptor- γ (PPAR γ)-mediated lipogenic gene expressions in liver; thereby repressing diet-induced hepatic steatosis [70]. However, the effects of ROR α deficiency on brain lipid metabolism are unknown. The aim of this study is to examine the effects of ROR α deficiency on brain phospholipid fatty acid concentrations and compositions.

Methods

Animals and Diet

All procedures were approved and performed in accordance with the policies set by the Institutional Animal Care and Use Committee at the Boston Children's Hospital and National Institute on Alcohol Abuse and Alcoholism (NIAAA). *Rora* heterozygous *staggerer* (*Rora*^{+/sg}) mice were obtained from The Jackson Laboratory (stock number: 002651; Bar Harbor, ME, USA) and bred to generate homozygous ROR α -deficient *staggerer* (*Rora*^{sg/sg}) and wildtype littermates. No exclusion criteria were pre-determined. Mice were housed in pie-shaped cages with 2–5 companions. Wildtype mice were heavier in weight as compared to *Rora*^{sg/sg} mice (2-month-old wildtype = 25g; 2-month-old *Rora*^{sg/sg} = 17g; >7-month-old wildtype = 31g; >7-month-old *Rora*^{sg/sg} = 22g). Mice had *ad libitum* access to custom-designed diet (Diet 1: no EPA and DHA) from Dyets Inc. (Bethlehem, PA, USA) or Prolab

Isopro RMH 3000 chow (Diet 2: with EPA and DHA) from LabDiet (St. Louis, MO) and water. The fat compositions of diet 1 and diet 2 were 16.5% and 14.3% (g of fat/kg of diet), respectively (Table 1). To investigate the effects of ROR α mutation and sex on cerebellar phospholipid fatty acid concentrations and composition, a total of 23 three-month-old mice on diet 1 were selected for analysis (Male wildtype = 5; male *Rora*^{sg/sg} = 7; female wildtype = 5; female *Rora*^{sg/sg} = 5). To investigate the effects of age and brain region on ROR α -mediated changes in brain phospholipid fatty acid concentration and composition, a total of 23 mice on diet 2 were selected for analysis (2-month-old wildtype = 6; 2-month-old *Rora*^{sg/sg} = 7; >7-month-old wildtype = 5; >7-month-old *Rora*^{sg/sg} = 5). On the days of euthanization, mice were anesthetized with intraperitoneal injection of ketamine/xylazine (100–120 mg/kg ketamine and 10 mg/kg xylazine) followed by cervical dislocation. Brains from both sexes were collected for primary endpoint of total phospholipids fatty acid analysis. Cerebellum of 3-month-old *Rora*^{sg/sg} and wildtype littermate were collected; while 2-month-old and >7-month-old *Rora*^{sg/sg} and wildtype littermate brains were dissected into prefrontal cortex, cortex, striatum, hippocampus and cerebellum. Serum was collected from 2-month-old mice.

Lipid Extraction

Brain and serum total lipids were extracted by chloroform:methanol:0.88% KCl (2:1:0.75 by vol.) developed by Folch, Lees and Sloane Stanley. Total phospholipids were isolated by thin-layer chromatography (TLC) using TLC G-plates (EMD Chemical, Gibbstown, NJ, purchase year: 2017) and authentic standards (Avanti, Alabaster, AL, purchase year: 2017). Mixture of heptane:diethyl ether:glacial acetic acid (60:40:2 by vol.) was used for separation of neutral lipids including total phospholipids. Band corresponding to total phospholipids was visualized under UV light after spraying with 0.1% 8-anilino-1-naphthalene sulfonic acid. Brain total phospholipid bands and serum total lipids were collected into test tubes with known quantities of internal standards, heptadecanoic acids (17:0) and n-3 docosatrienoic acid (22:3n-3). Fatty acids are converted into fatty acid methyl esters (FAME) with 14% boron trifluoride-methanol at 100°C for one hour. FAME were analyzed and quantified by gas chromatography-flame ionization detection (GC-FID).

Gas Chromatography-Flame Ionization Detection

FAME were analyzed using an Agilent 7890A gas chromatograph coupled with a flame ionization detector (Agilent Technologies, Inc., Santa Clara, CA). Highly efficient, 15-meter DB-FFAP capillary column was used for FAME separation (0.1 mm ID x 0.1 μ m film thickness). Samples were injected with 60:1 split ratio. The injector and detector ports were set at 250°C. FAME were eluted using a temperature program set initially at 150°C for 0.25 minutes, followed by increases at 10°C/minute to 200°C for 5 minutes and finally at 10°C/minute to 245°C for 20 minutes to complete the run. The carrier gas was hydrogen with a constant flow rate of 40 ml/minute. Peaks were identified by retention times of authentic FAME standards (Nu-Chek-Prep., Elysian, MN). Area under the curve of each identified FAME peak are collected blindly by laboratory technician who were not provided with no information on the samples. The concentration of each fatty acid from brain total phospholipids and serum total lipids was quantified by peak comparison to the internal standards (17:0 and 22:3n-3) and expressed as nmol/g brain and nmol/ml, respectively, with

no blinding. Fatty acid compositions were calculated as percentage of individual fatty acid concentrations to the total fatty acid concentrations of analyzed phospholipid class. Composition data were expressed as mol%. Total fatty acid concentrations were calculated by the sum of all quantified fatty acids.

Statistics

Data are expressed as means $\pm$ SD. No sample size calculation was performed *a priori*. Based on pilot data, a 50% difference between *Rora*^{sg/sg} and wildtype littermate was estimated. Therefore, power analysis for two-sided test with α of 0.05 and desired power of 0.8 estimated sample size of three per group. The effect of genotype and sex on brain total fatty acid concentrations and compositions were determined by two-way ANOVA analysis (GraphPad Prism 8.0.1). Sex effect were not significant; thereby male and female samples were pooled to test the effect of genotype on brain phospholipid fatty acid concentrations and compositions at different age and brain regions. Means were compared by unpaired, two-tailed *t*-tests by Microsoft Excel 365 ProPlus. Statistical significance was set at $P < 0.05$.

Results

The effects of genotype and sex on cerebellar fatty acid concentrations

In accordance to literature, cerebellum atrophy was observed in *Staggerer* (*Rora*^{sg/sg}) mice. At 3-month-old, there was a significant genotype effect on cerebellar weight (Figure 1A). Male *Rora*^{sg/sg} cerebellum was $78 \pm 12\%$ lower as compared to wildtype littermate (*Rora*^{+/+}). Female *Rora*^{sg/sg} cerebellum was $70 \pm 7.6\%$ lower as compared to *Rora*^{+/+} mice. There was no significant effect of sex on cerebellar weights (Figure 1A).

Fatty acid concentrations of predominant species of saturated (SFA), monounsaturated (MUFA), omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA) were affected by genotype but not by sex (Figure 1B). Since total fatty acid concentration was significantly different between genotype (*Rora*^{+/+} = 238410 ± 9480 nmol/g brain; *Rora*^{sg/sg} = 308513 ± 85165 nmol/g brain; $p = 0.02$) (Supplementary Table 1), fatty acid compositions were calculated. After adjustment, significant main effect of genotype remained for oleic acid (18:1n-9) and docosahexaenoic acid (22:6n-3; DHA) (Figure 1C). Oleic acid composition in cerebellar total lipids was $14 \pm 5.3\%$ higher in *Rora*^{sg/sg} as compared to *Rora*^{+/+} (*Rora*^{sg/sg} = 22 ± 1.3 mol%; *Rora*^{+/+} = 19 ± 0.63 mol%). DHA composition in cerebellar total lipids was $37 \pm 19\%$ lower in *Rora*^{sg/sg} as compared to *Rora*^{+/+} (*Rora*^{sg/sg} = 8 ± 2.5 mol%; *Rora*^{+/+} = 13 ± 1.5 mol%).

Serum fatty acid concentration and brain weight

Fatty acid analysis of serum from 2-months-old mice demonstrated that majority of serum fatty acids did not exhibit significant changes between *Rora*^{sg/sg} and *Rora*^{+/+}. However, serum palmitate (16:0) and linoleic acid (18:2n-6) in *Rora*^{sg/sg} were significantly higher in the serum as compared to *Rora*^{+/+} (Table 2).

Even with addition of EPA and DHA in the diet, cerebellum atrophy was pronounced in *Rora^{sg/sg}* mice. *Rora^{sg/sg}* cerebellum weight was lower by $82 \pm 5.2\%$ at an earlier age of 2 months as compared to control. While cerebellum weight increased with age, at 7 months or older, *Rora^{sg/sg}* cerebellum was still $55 \pm 22\%$ lower than *Rora^{+/+}* (Figure 2). No significant changes were detected between *Rora^{sg/sg}* and *Rora^{+/+}* in other analyzed regions including prefrontal cortex, cortex, striatum and hippocampus (Figure 2).

Cerebellum phospholipid fatty acid profile

At 2-months-old, cerebellar phospholipid fatty acid concentrations were lower in *Rora^{sg/sg}* as compared to *Rora^{+/+}*. Major species of SFA including palmitate (16:0) and stearate (18:0) were lower in *Rora^{sg/sg}* mice by $75 \pm 5.6\%$ (*Rora^{sg/sg}*, 7111 ± 1565 nmol/g brain; *Rora^{+/+}*, 28054 ± 2584 nmol/g brain) and $73 \pm 4.7\%$ (*Rora^{sg/sg}*, 6320 ± 1110 nmol/g brain; *Rora^{+/+}*, 23744 ± 2270 nmol/g brain) as compared to age-control *Rora^{+/+}* (Table 3). Major species of MUFA, oleic acid, also followed the same trend with $63 \pm 7\%$ (*Rora^{sg/sg}*, 8339 ± 1585 nmol/g brain; *Rora^{+/+}*, 22636 ± 2669 nmol/g brain) lower in *Rora^{sg/sg}* mice as compared to age-control *Rora^{+/+}* (Table 3). Predominant species of PUFA including ARA and DHA were both significantly lower in *Rora^{sg/sg}* mice by $69 \pm 9.3\%$ (*Rora^{sg/sg}*, 2193 ± 652 nmol/g brain; *Rora^{+/+}*, 7000 ± 1024 nmol/g brain) and $86 \pm 4.6\%$ (*Rora^{sg/sg}*, 2252 ± 732 nmol/g brain; *Rora^{+/+}*, 16063 ± 2772 nmol/g brain) as compared to *Rora^{+/+}*, respectively (Table 2). This translated to a collectively lower levels of SFA, MUFA, n-6 PUFA and n-3 PUFA in *Rora^{sg/sg}* mice by 73 ± 5.2 , 64 ± 7 , 65 ± 12 , and $85 \pm 4.6\%$ as compared to age-control wildtype littermate, respectively.

Since the size of cerebellum was significantly different between genotype, we calculated fatty acid composition to account for weight differences and to provide further insights to the relationships between fatty acids in phospholipid membrane. Composition analyses demonstrated that major species of SFA were significantly lower in *Rora^{sg/sg}* mice as compared to *Rora^{+/+}*; however, minor longer chain fatty acids (>20 carbon chain length) were significantly higher in *Rora^{sg/sg}* mice as compared to *Rora^{+/+}* (Table 3). In contrast to the lowering of MUFA concentrations, MUFA compositions were significantly higher in *Rora^{sg/sg}* mice as compared to *Rora^{+/+}* (Table 3). In the case of PUFA, the compositions of minor species of n-6 PUFA were significantly higher in *Rora^{sg/sg}* mice as compared to *Rora^{+/+}*. However, the composition of predominant species of n-6 PUFA, ARA, did not significantly change; even though concentration was significantly lower (Table 3). The compositions of minor species of n-3 PUFA were significantly higher in *Rora^{sg/sg}* mice as compared to *Rora^{+/+}*. In accordance to concentration analysis, the composition of the predominant species of n-3 PUFA, DHA, was $51 \pm 10\%$ (*Rora^{sg/sg}*, 6.9 ± 1.4 mol%; *Rora^{+/+}*, 14.1 ± 1.1 mol%) lower in *Rora^{sg/sg}* mice as compared to *Rora^{+/+}* (Table 3).

In contrast to 2-months-old mice, fatty acid concentrations did not differ between >7-months-old *Rora^{sg/sg}* and wildtype mice (Table 3). However, there were two significant changes in fatty acid composition in 24:1n-9 (*Rora^{sg/sg}*, 1.8 ± 0.45 mol%; *Rora^{+/+}*, 1.1 ± 0.31 mol%) and DHA (*Rora^{sg/sg}*, 6.1 ± 3.6 mol%; *Rora^{+/+}*, 12.3 ± 1.5 mol%). The consistent lowering of DHA composition in adolescent and adult mice may indicate a biologically essential role for ROR α in the regulations of phospholipid DHA metabolism.

Cortex phospholipid fatty acid profiles

At 2-months-old and >7-months-old, cortical fatty acid concentrations of major species in four classes of fatty acids did not significantly differ between *Rora*^{sg/sg} and wildtype mice. Lowering of minor species of SFA and MUFA were found in *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} littermates. At 2-months-old and >7-months-old, 20:0 was significantly lower; whereas 22:0 was lower at 2-months-old and 24:0 was lower at >7-months-old in *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} (Table 4). At 2-months-old, 20:1n-9 and 24:1n-9 were significantly lower in *Rora*^{sg/sg} mice as compared to *Rora*^{+/+}; while at >7-month-old, only 22:1n-9 exhibited significant lowering in *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} (Table 4). In contrast to concentrations, fatty acid composition demonstrated that while absolute quantities of fatty acid did not differ, there were major changes to compositions of major species of fatty acid in the phospholipid membrane. This included a significantly higher composition of major SFA species, 16:0, in 2-month-old *Rora*^{sg/sg} mice (31 ± 0.96 mol%) as compared *Rora*^{+/+} (29.6 ± 1.1 mol%). This observation continued in >7-months-old cortex. Similarly, a change in 20:0 concentration also translated to a significant but negligible lowering in 20:0 compositions at both age (Table 4). Lowering of MUFA composition was found to compensate for higher palmitate composition. 18:1n-9, 20:1n-9 and 24:1n-9 were significantly lower in 2-months-old *Rora*^{sg/sg} mice as compared to *Rora*^{+/+}; yet at >7-months-old only 18:1n-9 was significantly lower. PUFA concentration and composition were not affected in the cortex with an exception of 0.03 mol% lowering of 20:2n-6 composition at 2-months-old.

No significant difference was found in prefrontal cortex SFA concentrations at both ages. Nevertheless, composition of 16:0 was significantly higher in *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} at both ages (Table 5). Fatty acid concentrations of minor species of long-chain omega-9 MUFA (n-9) including 20:1n-9, 22:1n-9 and 24:1n-9 were significantly lower by $33 \pm 8.9\%$, $35 \pm 12\%$ and $44 \pm 20\%$, respectively, in 2-months-old *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} (Table 5). These lowering were translated in composition in addition to a significant lowering of 18:1n-9 composition (Table 5). In contrast, >7-months-old *Rora*^{sg/sg} mice had significantly lower levels of 18:1n-9 (*Rora*^{sg/sg}, 19837 ± 1018 nmol/g brain; *Rora*^{+/+}, 22194 ± 1327 nmol/g brain) and 20:1n-9 (*Rora*^{sg/sg}, 1240 ± 165 nmol/g brain; *Rora*^{+/+}, 1691 ± 187 nmol/g brain) as compared to *Rora*^{+/+} which directly translated to compositional differences between >7-months-old *Rora*^{sg/sg} and *Rora*^{+/+} mice (Table 5). In contrast to cortex, prefrontal cortex exhibited significant changes to n-6 PUFA concentrations. At 2-months-old, 20:2n-6 concentration was significantly lower (*Rora*^{sg/sg}, 181 ± 7 nmol/g brain; *Rora*^{+/+}, 201 ± 6 nmol/g brain); while ARA (*Rora*^{sg/sg}, 10468 ± 467 nmol/g brain; *Rora*^{+/+}, 11754 ± 773 nmol/g brain) and 22:4n-6 (*Rora*^{sg/sg}, 2656 ± 153 nmol/g brain; *Rora*^{+/+}, 3109 ± 219 nmol/g brain) were significantly lower at >7-months-old *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} (Table 5). Even though n-6 PUFA concentrations were lowered, compositions of n-6 PUFA were not affected except for a statistically significant 0.18 mol% lowering of 22:4n-6 in >7-months-old *Rora*^{sg/sg} mice.

Striatum and hippocampus phospholipid fatty acid profiles

Striatal phospholipid fatty acid concentration did not differ between *Rora*^{sg/sg} and *Rora*^{+/+} mice (Table 6). Palmitate (16:0) compositions were higher in *Rora*^{sg/sg} mice as compared to

Rora^{+/+} at both ages (Table 6). Stearate (18:0) composition was only significantly lower in >7-month-old *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} (Table 6). Slight lowering of 20:1n-9 in *Rora*^{sg/sg} striatum as compared to *Rora*^{+/+} were observed at both ages (Table 6). PUFA compositions were largely unaffected. However, 20:2n-6 and 18:3n-3 (ALA) compositions were significantly lower in 2-months-old *Rora*^{sg/sg} mice as compared to *Rora*^{+/+} (Table 6). Since these PUFA species are less accreted in brain phospholipids, the biological significance of these lowering may be inconsequential.

Similarly, hippocampal phospholipid fatty acid concentration did not differ between *Rora*^{sg/sg} and *Rora*^{+/+} mice (Table 7). Fatty acid composition of 2-months-old mice also did not significantly differ between genotype (Table 7). At >7-months-old mice, small but significantly lower compositions of 18:0 and 20:2n-6 were observed in *Rora*^{sg/sg} mice as compared to *Rora*^{+/+}. N-3 PUFA compositions were not significantly different between genotype at either age group (Table 7).

Discussion

This study is the first to investigate the effects of ROR α deficiency on fatty acid metabolism in the brain. *Staggerer* mice brain fatty acid metabolism was differentially affected among brain regions which may be dependent on regional expressions of ROR α . Extensive atrophy of developing cerebellum was accompanied by extensive lowering of all fatty acid species concentrations. Despite lower levels of fatty acids in developing cerebellum of *staggerer* mutants, ROR α deficiency does not appear to impede accretions of fatty acids in adulthood. By 7-month-old, fatty acid concentrations and compositions were indistinguishable between genotypes except for DHA, even though cerebellar weight at 7-month-old was still significantly smaller than wildtype. This implied that while all fatty acids may be initially low in developing *staggerer* cerebella, ROR α may not affect the ability of cerebellum to compensate low concentrations via accelerated accretion of affected fatty acids between 2 months and 7 months of age. In fact, at 3 months old, *staggerer* cerebellum accreted significantly higher SFA, MUFA and PUFA in total phospholipids as compared to wildtype providing evidence that ROR α deficiency delays fatty acid accretions during critical periods of development. The function of ROR α on fatty acid metabolism may be associated with the temporal expressions of ROR α during lifespan which warrants future investigations.

In contrast to other fatty acids, DHA concentrations remained significantly reduced in *staggerer* cerebellum as compared to wildtype at different ages and with the two different diets examined in this study. Moreover, the lowering of cerebellar DHA concentration in *staggerer* mice as compared to wildtype were statistically similar between sexes; even though ROR α expression is differentially regulated by male and female hormones and may regulate the transcription of aromatase [31, 71]. DHA concentrations were reduced in *staggerer* cerebellar phospholipids of both sexes that were consuming diets without preformed EPA and DHA. This reduction in DHA concentration was not compensated by increasing levels of n-6 docosapentaenoic acid (n-6 DPA) but rather increases in other n-6 PUFA including ARA. This suggests an aberrant regulation of fatty acid accretions in cerebellum and that the lowering of DHA concentrations was not the result of n-3 PUFA deprivation. Furthermore, the reduction of DHA concentration in *staggerer* cerebellar

phospholipids was not rescued by supplementation of EPA and DHA in the diet; thus, implying that ROR α may have a direct or indirect effect on accretion of DHA to brain phospholipids and/or loss of DHA from brain phospholipids. PUFA metabolism was unaffected in regions with low and undetectable expression of ROR α . While saturated fatty acids, MUFA, and minor species of n-6 PUFA concentrations and compositions were significantly affected in cortex and prefrontal cortex, the small effect sizes are likely biologically insignificant. Collectively, these findings suggest that ROR α has a selective and targeted effect on DHA metabolism in cerebellum across ages. Therefore, future investigations should characterize phospholipid DHA metabolism via lipid kinetic study and profiles of DHA metabolism genes in *staggerer* mutants to better understand the findings of this study [72].

This research has implications for supplementing n-3 PUFA in humans throughout different stages of the lifespan. Fish oil is a common supplement used by families with individuals affected by ASD [73]. It is predominantly composed of two n-3 PUFA, eicosapentaenoic acid (EPA) and DHA. Dietary deficiency of n-3 PUFA during development had been shown to impair cognitive performance and prepulse inhibition (PPI) as well as increase in depressive-like and anxiety-like behaviors in rodents [74–79]. Furthermore, in environmental exposure models for ASD in rodents, n-3 PUFA or DHA supplementation were effective in reversing impairments in social interactions and elevation in social anxiety induced by maternal immune activation or exposure of food allergen during development [63, 80, 81]. Similarly, genetic models of ASD, including BTBR and serotonin transporter (SERT) knockout, demonstrated that pre- and postnatal n-3 PUFA or DHA supplementation may increase social interest and social preference for novel mice over objects as compared to mice fed n-3 PUFA deprived diets [82, 83]. The improvements in social behaviors were observed in conjunction with higher DHA composition in the brain [82–84]. Therefore, dietary DHA intervention shows promising results for the prevention of the core behaviors associated with ASD in different animal models.

Despite findings in animal models, the efficacy of DHA or n-3 PUFA supplementation for improvements of ASD symptoms in humans remain inconclusive. A meta-analysis of case control cohorts found that DHA and EPA levels in blood of ASD children aged 12 and under were selectively lower with no changes to total n-3 PUFA concentrations as compared to typically developing children, despite no differences in reported dietary intakes of n-3 PUFA between children with and without ASD [85]. The current randomized controlled trials are limited to small sample sizes, short duration of treatment, ambiguous biomarkers of fatty acid intake and recruitment of mostly ASD children with mild symptomology. However, it is also important to note that trials reporting increase in blood DHA after supplementation also failed to observe improvements of social behavior in children with ASD [86, 87]. Therefore, it is plausible that children with ASD may have dysfunction in fatty acid metabolism in the brain where increased level in blood may not be able to compensate for accelerated metabolisms of n-3 PUFA in autistic brain. Our study provide evidence in mice that a diet containing EPA and DHA may yield serum DHA level (4.4–5.2 mol% of total serum fatty acids) similarly to that of n-3 PUFA supplemented children (2.7–5.5%). Further, deficiency of ROR α may selectively and negatively affect the metabolism of DHA in the brain either by slowing incorporation of DHA into brain phospholipids and/or accelerating loss of DHA

from brain phospholipids; thereby resulting in an observed reduction of brain phospholipid DHA in half. ROR α expression was reduced in a subset of ASD patients with severe language impairment [42]. Of the six RCT, the Mankad trial was conducted in nonverbal ASD children [88]. N-3 PUFA supplementation in this population did not alter plasma fatty acid composition like the *staggerer* mice and no improvement in social behaviors were observed; thus, providing additional evidence that gene deficiency may affect efficacy of dietary n-3 PUFA supplementation on aberrant social behaviour due to the role of gene on n-3 PUFA metabolism in the brain. Future studies should aim to investigate the role of ROR α on social interaction behavior in rodent models. In addition, future studies should confirm cerebellar expressions of ROR α in nonverbal autistic patients and associate expressions with brain phospholipid lipidome of postmortem autistic brains.

While the present findings support a role of ROR α in fatty acid metabolisms, there are limitations to our study. The variability of brain fatty acid concentrations from older age mice was greater than that of younger age mice. This is most likely due to the variability in the age of the samples collected for >7-months-old group. Gait impairment and stunted growth affected the survival of *staggerer* mice to adulthood; thereby, sample size was reduced for *staggerer* mice of older age. Hence observations of older *staggerer* mice should be confirmed with a larger sample size. In addition, while this study examined fatty acid concentrations and compositions in total phospholipids, it is possible that there may be phospholipid class specific modulations that may be masked by total phospholipid analyses.

To our knowledge, this study is the first to examine the effects of an ASD associated gene on fatty acid metabolism in the brain. Deficiency in ROR α may lower fatty acid concentration during neurodevelopment and delay fatty acid accretions during lifespan. Furthermore, ROR α deficiency selectively lower DHA concentration in developing cerebellum across lifespan. The effect of ROR α on fatty acid metabolism appears to be brain region-specific and age-dependent. The effects of ASD associated genes such as ROR α on DHA metabolisms in the brain may explain the lack of efficacy of n-3 PUFA supplementation in children with ASD as compared to other psychiatric disorders. It is important to examine fatty acid metabolism in other genetic models for ASD to further understand the effects of gene-lipid interactions in the etiology and pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations:

RORα	retinoid acid-related orphan receptor alpha
ALA	alpha-linolenic acid

ASD	autism spectrum disorder
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid

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Figure 1.

The effects of genotype and sex on total fatty acid concentrations and compositions of cerebellum from 3-month-old mice. A) Brain weights of dissected cerebellum for fatty acid quantification. B) Fatty acid concentrations of predominant species of saturated (16:0 and 18:0), monounsaturated (18:1n-9), n-6 (20:4n-6; AA) and n-3 polyunsaturated fatty acids (22:6n-3; DHA). C) Fatty acid compositions of predominant species of saturated (16:0 and 18:0), monounsaturated (18:1n-9), n-6 (20:4n-6) and n-3 polyunsaturated fatty acids (22:6n-3). Two-way ANOVA with *post hoc* Bonferroni multiple comparison test were performed. Statistical significance was set a $p < 0.05$. C)

Figure 2.

Brain weights of dissected brain regions for fatty acid quantification. 2-month-old $n = 4-5$; >7-month-old $n = 4-7$. * $p < 0.01$; ** $p < 0.0001$. Cereb, cerebellum; PFC, prefrontal cortex; Cor, cortex; Str, striatum; Hippo, hippocampus.

Table 1.Dietary fatty acid compositions (mean $\pm$ SD; n = 5)

FA	mg FA/g diet (mol% diet composition)	
	Diet 1: No EPA and DHA	Diet 2: With EPA and DHA
10:0	3 (2.5) $\pm$ 0.6 (0.1)	0 (0) $\pm$ 0 (0)
12:0	25 (21) $\pm$ 4.7 (0.3)	0 (0) $\pm$ 0 (0)
14:0	10 (8.4) $\pm$ 1.8 (0.08)	0.73 (1.2) $\pm$ 0.14 (0.02)
16:0	8.8 (7.4) $\pm$ 1.6 (0.06)	11 (18) $\pm$ 0.11 (0.08)
18:0	8.3 (6.9) $\pm$ 1.5 (0.1)	4.4 (7.1) $\pm$ 0.048 (0.45)
20:0	0.31 (0.26) $\pm$ 0.06 (0.005)	0.19 (0.30) $\pm$ 0.006 (0.009)
22:0	0.40 (0.33) $\pm$ 0.07 (0.007)	0.13 (0.21) $\pm$ 0.004 (0.005)
24:0	0.19 (0.16) $\pm$ 0.03 (0.006)	0.10 (0.16) $\pm$ 0.006 (0.01)
16:1n-7	0.062 (0.1) $\pm$ 0.009 (0.004)	0.88 (1.4) $\pm$ 0.13 (0.016)
18:1n-9	34 (28) $\pm$ 6.3 (0.3)	18 (29) $\pm$ 0.13 (0.2)
18:1n-7	0.51 (0.4) $\pm$ 0.08 (0.02)	1.10 (1.8) $\pm$ 0.019 (0.025)
20:1n-9	0.16 (0.13) $\pm$ 0.03 (0.003)	0.32 (0.51) $\pm$ 0.007 (0.013)
22:1n-9	0 (0) $\pm$ 0 (0)	0.023 (0.04) $\pm$ 0.004 (0.007)
24:1n-9	0.064 (0.05) $\pm$ 0.01 (0.003)	0.06 (0.10) $\pm$ 0.004 (0.006)
18:2n-6	28 (23) $\pm$ 5.1 (0.2)	21 (33) $\pm$ 0.17 (0.31)
18:3n-6	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)
20:2n-6	0.015 (0.01) $\pm$ 0.001 (0.002)	0.18 (0.28) $\pm$ 0.004 (0.006)
20:3n-6	0 (0) $\pm$ 0 (0)	0.04 (0.06) $\pm$ 0.005 (0.009)
20:4n-6	0 (0) $\pm$ 0 (0)	0.15 (0.25) $\pm$ 0.008 (0.012)
22:4n-6	0.027 (0.02) $\pm$ 0.005 (0.004)	0.05 (0.08) $\pm$ 0.005 (0.008)
22:5n-6	0 (0) $\pm$ 0 (0)	0.040 (0.06) $\pm$ 0.002 (0.003)
18:3n-3	1.8 (1.5) $\pm$ 0.3 (0.01)	2 (3.1) $\pm$ 0.02 (0.034)
20:5n-3	0 (0) $\pm$ 0 (0)	0.61 (1.0) $\pm$ 0.028 (0.043)
22:5n-3	0 (0) $\pm$ 0 (0)	0.13 (0.21) $\pm$ 0.007 (0.01)
22:6n-3	0 (0) $\pm$ 0 (0)	0.81 (1.3) $\pm$ 0.072 (0.11)
Total SFA	56 (47) $\pm$ 10 (0.3)	17 (27) $\pm$ 0.16 (0.12)
Total MUFA	35 (29) $\pm$ 6 (0.3)	21 (33) $\pm$ 0.14 (0.2)
Total n-6 PUFA	28 (23) $\pm$ 5 (0.2)	21 (34) $\pm$ 0.14 (0.3)
Total n-3 PUFA	1.8 (1.5) $\pm$ 0.3 (0.01)	4 (5.6) $\pm$ 0.094 (0.13)

N denotes individual food pellet sampled for analysis.

Table 2.

Serum total fatty acids concentrations and compositions of 2-month-old *staggerer* mice (*Rora*^{sg/sg}) and wildtype littermate (*Rora*^{+/+}) (mean ± SD; *Rora*^{sg/sg} n = 4, *Rora*^{+/+} n = 5)

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/ml (mol%)	<i>Staggerer</i> (<i>Rora</i> ^{sg/sg}) nmol/ml (mol%)
12:0	1.0 (0.023) ± 0.21 (0.004)	8.9 (0.17) ± 6.1 (0.10)
14:0	23 (0.54) ± 5.3 (0.12)	22 (0.43) ± 5.3 (0.062)
16:0	1063 (25) ± 150 (1.1)	1310 (26) ± 122 [*] (0.62)
18:0	462 (11) ± 57 (1.0)	629 (12) ± 133 (1.5)
20:0	7.5 (0.18) ± 2.4 (0.053)	10 (0.20) ± 2.0 (0.056)
22:0	5.8 (0.14) ± 3.1 (0.070)	9.0 (0.18) ± 3.5 (0.085)
24:0	4.0 (0.10) ± 0.7 (0.021)	5.1 (0.10) ± 1.4 (0.035)
16:1n7	87 (2.0) ± 20 (0.32)	75 (1.5) ± 13 (0.16)
18:1n9	597 (14) ± 92 (1.2)	618 (12) ± 68 (0.28)
18:1n7	79 (1.9) ± 11 (0.31)	73 (1.4) ± 11 (0.090)
20:1n9	15 (0.36) ± 3.5 (0.080)	15 (0.30) ± 3 (0.091)
22:1n9	3.5 (0.083) ± 0.56 (0.010)	3.7 (0.072) ± 0.23 (0.0028)
24:1n9	15 (0.35) ± 1.9 (0.0086)	16 (0.31) ± 2.5 (0.024)
18:2n6	1172 (28) ± 73 (1.6)	1580 (31) ± 121 ^{**} (1.9 [*])
18:3n6	8.0 (0.19) ± 2.2 (0.036)	7.8 (0.15) ± 1.3 (0.031)
20:2n6	11 (0.25) ± 1.8 (0.047)	11 (0.21) ± 2.6 (0.032)
20:3n6	44 (1.0) ± 8.3 (0.11)	38 (0.74) ± 4.9 (0.10)
20:4n6	305 (7.1) ± 57 (0.68)	305 (6.0) ± 29 (0.36)
22:4n6	5.3 (0.13) ± 0.53 (0.012)	5.4 (0.11) ± 1.8 (0.036)
22:5n6	6.5 (0.15) ± 1.8 (0.048)	7.6 (0.15) ± 2.8 (0.043)
18:3n3	30 (0.72) ± 3.2 (0.12)	37 (0.73) ± 3.9 (0.060)
20:5n3	61 (1.4) ± 6.7 (0.050)	80 (1.5) ± 14 (0.15)
22:5n3	19 (0.45) ± 5.4 (0.10)	20 (0.39) ± 3.5 (0.076)
22:6n3	224 (5.2) ± 45 (0.66)	227 (4.4) ± 24 (0.46)
Total FA	4248 ± 468	5113 ± 492 [*]

Statistical significance:

^{*}
 $p < 0.05$

^{**}
 $p < 0.01$.

N denotes number of animals.

Cerebellum total phospholipid fatty acid concentration and composition at 2 months old and >7 months old (mean $\pm$ SD; 2 month-old n = 5/group, >7 month-old, n = 4-7/group).

Table 3.

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%)	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%)	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%)	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%)
	2 month-old	2 month-old	>7 month-old	>7 month-old
14:0	241 (0.2) $\pm$ 69 (0.05)	98 (0.3) $\pm$ 43 ^{**} (0.07)	225 (0.2) $\pm$ 174 (0.1)	170 (0.2) $\pm$ 111 (0.01)
16:0	28054 (25) $\pm$ 2584 (0.6)	7111 (22) $\pm$ 1565 ^{***} (1.4 ^{##})	29390 (25) $\pm$ 8064 (1.5)	26649 (26) $\pm$ 14357 (11)
18:0	23744 (21) $\pm$ 2270 (0.4)	6320 (20) $\pm$ 1110 ^{***} (0.7 ^{##})	23420 (20) $\pm$ 6459 (0.9)	26847 (27) $\pm$ 14482 (14)
20:0	520 (0.5) $\pm$ 29 (0.05)	266 (0.8) $\pm$ 73 ^{***} (0.1 ^{##})	440 (0.4) $\pm$ 119 (0.1)	1049 (1.0) $\pm$ 607 (0.6)
22:0	354 (0.3) $\pm$ 35 (0.06)	196 (0.6) $\pm$ 42 ^{***} (0.1 ^{##})	343 (0.3) $\pm$ 74 (0.07)	841 (0.8) $\pm$ 486 (0.5)
24:0	494 (0.4) $\pm$ 69 (0.1)	271 (0.9) $\pm$ 58 ^{***} (0.2 ^{##})	426 (0.4) $\pm$ 165 (0.1)	977 (1.0) $\pm$ 581 (0.5)
16:1n-7	617 (0.5) $\pm$ 78 (0.07)	246 (0.8) $\pm$ 64 ^{***} (0.1 [#])	689 (0.6) $\pm$ 152 (0.07)	617 (0.5) $\pm$ 616 (0.3)
18:1n-9	22636 (20) $\pm$ 2669 (0.6)	8339 (26) $\pm$ 1585 ^{***} (1.7 ^{###})	23924 (21) $\pm$ 4551 (2.2)	24894 (20) $\pm$ 24692 (13)
18:1n-7	5989 (5.3) $\pm$ 618 (0.09)	1904 (5.9) $\pm$ 411 ^{***} (0.5)	5796 (5.1) $\pm$ 1073 (0.6)	4941 (4.0) $\pm$ 4814 (2.5)
20:1n-9	3145 (2.8) $\pm$ 423 (0.09)	1050 (3.3) $\pm$ 333 ^{***} (0.9)	3483 (3.1) $\pm$ 973 (0.9)	4449 (3.5) $\pm$ 4459 (2.2)
22:1n-9	239 (0.2) $\pm$ 11 (0.02)	102 (0.3) $\pm$ 29 ^{***} (0.09 [#])	194 (0.2) $\pm$ 53 (0.04)	309 (0.3) $\pm$ 323 (0.2)
24:1n-9	839 (0.7) $\pm$ 71 (0.1)	410 (1.3) $\pm$ 122 ^{***} (0.4 [#])	1258 (1.1) $\pm$ 374 (0.3)	2255 (1.8) $\pm$ 1886 (0.4 [#])
18:2n-6	905 (0.8) $\pm$ 87 (0.06)	505 (1.6) $\pm$ 632 (2.0)	773 (0.7) $\pm$ 148 (0.06)	398 (0.4) $\pm$ 376 (0.2)
18:3n-6	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)
20:2n-6	309 (0.3) $\pm$ 22 (0.03)	77 (0.2) $\pm$ 30 ^{***} (0.06)	181 (0.2) $\pm$ 34 (0.03)	189 (0.1) $\pm$ 214 (0.1)
20:3n-6	516 (0.5) $\pm$ 37 (0.03)	183 (0.6) $\pm$ 47 ^{***} (0.06 [#])	450 (0.4) $\pm$ 93 (0.02)	352 (0.3) $\pm$ 344 (0.2)
20:4n-6	7000 (6.1) $\pm$ 1024 (0.3)	2193 (6.7) $\pm$ 652 ^{***} (1.3)	7552 (6.5) $\pm$ 2515 (0.8)	5791 (4.8) $\pm$ 5565 (3.1)
22:4n-6	1384 91.2) $\pm$ 234 (0.08)	606 (1.9) $\pm$ 197 ^{***} (0.4 [#])	1791 (1.5) $\pm$ 691 (0.3)	2088 (1.7) $\pm$ 2006 (1.1)
22:5n-6	82 (0.07) $\pm$ 23 (0.03)	32 (0.1) $\pm$ 28 [*] (0.09)	75 (0.06) $\pm$ 75 (0.05)	61 (0.06) $\pm$ 56 (0.04)
18:3n-3	73 (0.06) $\pm$ 29 (0.02)	29 (0.09) $\pm$ 5 [*] (0.02)	68 (0.06) $\pm$ 18 (0.01)	115 (0.1) $\pm$ 74 (0.03)
20:5n-3	79 (0.07) $\pm$ 10 (0.004)	46 (0.1) $\pm$ 23 [*] (0.07)	62 (0.05) $\pm$ 15 (0.01)	56 (0.07) $\pm$ 48 (0.07)

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) 2 month-old	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) 2 month-old	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) >7 month-old	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) >7 month-old
22:5n-3	221 (0.2) ± 22 (0.02)	97 (0.3) ± 20 ^{***} (0.04 ^{###})	236 (0.2) ± 71 (0.01)	233 (0.2) ± 253 (0.1)
22:6n-3	16063 (14) ± 2772 (1.1)	2252 (6.88) ± 732 ^{***} (1.5 ^{###})	14393 (12) ± 4675 (1.5)	7607 (6.1) ± 7324 (3.6 [#])
Total FA	113503 ± 12518	32332 ± 6287 ^{***}	115166 ± 27116	110888 ± 74438

Statistical significance for fatty acid concentration:

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$.

Statistical significance for fatty acid composition:

$p < 0.05$

$p < 0.01$

$p < 0.001$.

N denotes number of animals.

Cortex total phospholipid fatty acid concentration and composition at 2 months old and >7 months old (mean $\pm$ SD; 2 month-old n = 5-6/group, >7 month-old, n = 5-6/group).

Table 4.

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%)	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%)	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%)	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%)
	2 month-old	2 month-old	>7 month-old	>7 month-old
14:0	287 (0.2) $\pm$ 88 (0.03)	319 (0.2) $\pm$ 64 (0.02)	243 (0.1) $\pm$ 25 (0.01)	244 (0.2) $\pm$ 56 (0.02)
16:0	43203 (30) $\pm$ 9591 (1.1)	47516 (31) $\pm$ 9561 (1.0) [#]	45132 (27) $\pm$ 7236 (1)	44841 (29) $\pm$ 7898 (0.6) [#]
18:0	32960 (23) $\pm$ 6852 (0.6)	34892 (23) $\pm$ 6549 (0.8)	36768 (22) $\pm$ 5787 (0.1)	33483 (21) $\pm$ 5571 (0.5)
20:0	284 (0.2) $\pm$ 51 (0.03)	222 (0.1) $\pm$ 38 [*] (0.03) [#]	330 (0.2) $\pm$ 43 (0.03)	257 (0.2) $\pm$ 29 ^{**} (0.01) [#]
22:0	261 (0.2) $\pm$ 57 (0.06)	193 (0.1) $\pm$ 23 [*] (0.03)	347 (0.2) $\pm$ 175 (0.1)	231 (0.2) $\pm$ 90 (0.08)
24:0	301 (0.2) $\pm$ 76 (0.08)	236 (0.2) $\pm$ 54 (0.06)	277 (0.2) $\pm$ 83 (0.05)	167 (0.1) $\pm$ 46 [*] (0.05)
16:1n-7	944 (0.7) $\pm$ 163 (0.04)	1164 (0.8) $\pm$ 287 (0.08) [#]	1111 (0.7) $\pm$ 200 (0.05)	991 (0.6) $\pm$ 162 (0.03)
18:1n-9	23266 (16) $\pm$ 4108 (0.4)	23276 (15) $\pm$ 4298 (0.3) ^{##}	29139 (17) $\pm$ 4834 (0.4)	26017 (17) $\pm$ 4309 (0.3) ^{##}
18:1n-7	5534 (3.8) $\pm$ 958 (0.1)	5580 (3.7) $\pm$ 1061 (0.2)	6252 (3.7) $\pm$ 1006 (0.09)	5835 (3.7) $\pm$ 981 (0.07)
20:1n-9	1491 (1.0) $\pm$ 207 (0.2)	992 (0.7) $\pm$ 159 ^{**} (0.08) ^{##}	1460 (0.8) $\pm$ 1165 (0.6)	760 (0.5) $\pm$ 565 (0.5)
22:1n-9	157 (0.1) $\pm$ 30 (0.03)	138 (0.09) $\pm$ 39 (0.02)	159 (0.1) $\pm$ 22 (0.01)	133 (0.09) $\pm$ 12 [*] (0.01)
24:1n-9	550 (0.4) $\pm$ 100 (0.1)	325 (0.2) $\pm$ 63 ^{**} (0.05) [#]	1180 (0.7) $\pm$ 444 (0.3)	793 (0.5) $\pm$ 193 (0.2)
18:2n-6	800 (0.6) $\pm$ 150 (0.06)	880 (0.6) $\pm$ 173 (0.05)	867 (0.5) $\pm$ 126 (0.05)	743 (0.5) $\pm$ 150 (0.03)
18:3n-6	28 (0.02) $\pm$ 37 (0.03)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)
20:2n-6	228 (0.2) $\pm$ 32 (0.02)	200 (0.1) $\pm$ 46 (0.02) [#]	180 (0.1) $\pm$ 8 (0.01)	158 (0.1) $\pm$ 26 (0.01)
20:3n-6	631 (0.4) $\pm$ 124 (0.03)	645 (0.4) $\pm$ 119 (0.03)	677 (0.4) $\pm$ 80 (0.03)	632 (0.4) $\pm$ 134 (0.02)
20:4n-6	12090 (8.3) $\pm$ 2303 (0.3)	12543 (8.3) $\pm$ 2004 (0.3)	15123 (9.0) $\pm$ 2395 (0.2)	14058 (8.9) $\pm$ 2586 (0.3)
22:4n-6	2768 (1.9) $\pm$ 532 (0.04)	2864 (1.9) $\pm$ 452 (0.1)	3831 (2.3) $\pm$ 578 (0.09)	3619 (2.3) $\pm$ 681 (0.1)
22:5n-6	254 (0.2) $\pm$ 53 (0.02)	290 (0.2) $\pm$ 47 (0.01)	267 (0.2) $\pm$ 95 (0.03)	268 (0.2) $\pm$ 67 (0.02)
18:3n-3	34 (0.02) $\pm$ 38 (0.02)	44 (0.03) $\pm$ 19 (0.01)	74 (0.04) $\pm$ 38 (0.01)	63 (0.04) $\pm$ 28 (0.01)
20:5n-3	52 (0.03) $\pm$ 30 (0.02)	37 (0.03) $\pm$ 24 (0.02)	58 (0.03) $\pm$ 12 (0.004)	68 (0.04) $\pm$ 20 (0.01)
22:5n-3	234 (0.2) $\pm$ 39 (0.03)	274 (0.2) $\pm$ 43 (0.02)	313 (0.2) $\pm$ 64 (0.02)	308 (0.2) $\pm$ 56 (0.02)
22:6n-3	19092 (13) $\pm$ 3067 (0.8)	19487 (13) $\pm$ 3537 (1.1)	24406 (15) $\pm$ 3065 (0.6)	23482 (15) $\pm$ 4842 (0.9)

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) 2 month-old	<i>Staggerer</i> (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) 2 month-old	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) >7 month-old	<i>Staggerer</i> (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) >7 month-old
Total FA	145421 ± 27811	152117 ± 27698	168193 ± 25911	157152 ± 26668

Statistical significance for fatty acid concentration:

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$.

Statistical significance for fatty acid composition:

$p < 0.05$
$p < 0.01$
$p < 0.001$.

N denotes number of animals.

Prefrontal cortex total phospholipid fatty acid concentration and composition at 2 months old and >7 months old (mean $\pm$ SD; 2 month-old n = 4- 5/group, >7 month-old, n = 4-5/group).

Table 5.

FA	Wildtype (<i>Kora^{+/+}</i>) nmol/g brain (mol%)	Staggerer (<i>Kora^{sg/sg}</i>) nmol/g brain (mol%)	Wildtype (<i>Kora^{+/+}</i>) nmol/g brain (mol%)	Staggerer (<i>Kora^{sg/sg}</i>) nmol/g brain (mol%)
	2 month-old	2 month-old	>7 month-old	>7 month-old
14:0	234 (0.2) $\pm$ 20 (0.01)	230 (0.2) $\pm$ 29 (0.02)	138 (0.1) $\pm$ 46 (0.03)	126 (0.1) $\pm$ 17 (0.02)
16:0	37034 (27) $\pm$ 1677 (0.5)	36268 (28) $\pm$ 1666 (0.4) [#]	34753 (27) $\pm$ 2010 (0.5)	34235 (29) $\pm$ 1047 (0.5) ^{##}
18:0	29586 (22) $\pm$ 1337 (0.2)	27736 (21) $\pm$ 1568 (0.07)	28019 (22) $\pm$ 2411 (0.6)	25208 (21) $\pm$ 1395 (0.2)
20:0	302 (0.2) $\pm$ 46 (0.03)	233 (0.2) $\pm$ 40 (0.02)	246 (0.2) $\pm$ 38 (0.03)	196 (0.2) $\pm$ 37 (0.02)
22:0	417 (0.3) $\pm$ 69 (0.04)	294 (0.2) $\pm$ 100 (0.07)	220 (0.2) $\pm$ 123 (0.08)	140 (0.1) $\pm$ 13 (0.01)
24:0	385 (0.3) $\pm$ 76 (0.05)	277 (0.2) $\pm$ 118 (0.09)	181 (0.1) $\pm$ 76 (0.05)	137 (0.1) $\pm$ 36 (0.02)
16:1n-7	772 (0.6) $\pm$ 37 (0.02)	793 (0.6) $\pm$ 91 (0.05)	739 (0.6) $\pm$ 101 (0.05)	722 (0.6) $\pm$ 86 (0.06)
18:1n-9	21180 (15) $\pm$ 1347 (0.3)	19273 (15) $\pm$ 1055 (0.2) [#]	22194 (17) $\pm$ 1327 (0.4)	19837 (17) $\pm$ 1018 [*] (0.1) [#]
18:1n-7	5094 (3.7) $\pm$ 192 (0.06)	4834 (3.7) $\pm$ 190 (0.1)	5091 (3.9) $\pm$ 267 (0.1)	4796 (4) $\pm$ 157 (0.06)
20:1n-9	1381 (1) $\pm$ 124 (0.05)	933 (0.7) $\pm$ 124 ^{**} (0.08) ^{###}	1691 (1.3) $\pm$ 187 (0.09)	1240 (1) $\pm$ 165 ^{**} (0.09) ^{##}
22:1n-9	127 (0.09) $\pm$ 16 (0.01)	88 (0.07) $\pm$ 16 ^{**} (0.01) ^{##}	109 (0.08) $\pm$ 15 (0.01)	106 (0.09) $\pm$ 30 (0.02)
24:1n-9	728 (0.5) $\pm$ 107 (0.06)	417 (0.3) $\pm$ 152 ^{**} (0.1) ^{##}	756 (0.6) $\pm$ 230 (0.1)	515 (0.4) $\pm$ 95 (0.06)
18:2n-6	822 (0.6) $\pm$ 53 (0.06)	826 (0.6) $\pm$ 59 (0.03)	663 (0.5) $\pm$ 51 (0.03)	571 (0.5) $\pm$ 22 [*] (0.01)
18:3n-6	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)
20:2n-6	201 (0.1) $\pm$ 6 (0.01)	181 (0.1) $\pm$ 7 ^{**} (0.01)	232 (0.2) $\pm$ 40 (0.04)	265 (0.2) $\pm$ 35 (0.02)
20:3n-6	580 (0.4) $\pm$ 68 (0.04)	553 (0.4) $\pm$ 70 (0.03)	472 (0.4) $\pm$ 49 (0.02)	451 (0.4) $\pm$ 26 (0.01)
20:4n-6	12881 (9.4) $\pm$ 677 (0.2)	12179 (9.4) $\pm$ 724 (0.2)	11754 (9.1) $\pm$ 773 (0.3)	10468 (8.8) $\pm$ 467 [*] (0.1)
22:4n-6	3111 (2.3) $\pm$ 273 (0.1)	2838 (2.2) $\pm$ 204 (0.09)	3109 (2.4) $\pm$ 219 (0.1)	2656 (2.2) $\pm$ 153 ^{**} (0.03) [#]
22:5n-6	266 (0.2) $\pm$ 17 (0.02)	272 (0.2) $\pm$ 24 (0.02)	204 (0.2) $\pm$ 31 (0.02)	186 (0.2) $\pm$ 18 (0.01)
18:3n-3	56 (0.04) $\pm$ 6 (0.01)	44 (0.03) $\pm$ 15 (0.01)	30 (0.02) $\pm$ 11 (0.01)	28 (0.02) $\pm$ 8 (0.01)
20:5n-3	53 (0.04) $\pm$ 14 (0.01)	51 (0.04) $\pm$ 8 (0.01)	50 (0.04) $\pm$ 2 (0.003)	52 (0.04) $\pm$ 9 (0.01)
22:5n-3	248 (0.2) $\pm$ 27 (0.01)	262 (0.2) $\pm$ 23 (0.01)	241 (0.2) $\pm$ 42 (0.02)	222 (0.2) $\pm$ 12 (0.01)
22:6n-3	21371 (16) $\pm$ 772 (0.4)	20704 (16) $\pm$ 1523 (0.3)	18460 (14) $\pm$ 1431 (0.5)	17232 (14) $\pm$ 876 (0.4)

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) 2 month-old	Slagger (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) 2 month-old	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) >7 month-old	Slagger (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) >7 month-old
Total FA	136829 ± 6392	129286 ± 7450	129353 ± 8609	119390 ± 5422

Statistical significance for fatty acid concentration:

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$.

Statistical significance for fatty acid composition:

$p < 0.05$
$p < 0.01$
$p < 0.001$.

N denotes number of animals.

Table 6.

Striatum total phospholipid fatty acid concentration and composition at 2 months old and >7 months old (mean $\pm$ SD; 2 month-old n = 5/group, >7 month-old, n = 4/group).

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%)	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%)	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%)	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%)
	2 month-old	2 month-old	>7 month-old	>7 month-old
14:0	420 (0.2) $\pm$ 241 (0.02)	543 (0.2) $\pm$ 230 (0.03)	327 (0.1) $\pm$ 84 (0.04)	319 (0.1) $\pm$ 45 (0.02)
16:0	49559 (23) $\pm$ 25193 (0.8)	57610 (25) $\pm$ 20717 (0.6) [#]	67305 (22) $\pm$ 9427 (0.8)	70536 (24) $\pm$ 4799 (0.3) [#]
18:0	45251 (21) $\pm$ 23135 (0.3)	49286 (21) $\pm$ 17590 (0.2)	65504 (22) $\pm$ 11587 (0.3)	60972 (21) $\pm$ 3805 (0.3) ^{##}
20:0	847 (0.4) $\pm$ 509 (0.07)	816 (0.3) $\pm$ 301 (0.03)	1003 (0.3) $\pm$ 227 (0.02)	875 (0.3) $\pm$ 93 (0.03)
22:0	695 (0.4) $\pm$ 279 (0.2)	749 (0.3) $\pm$ 198 (0.1)	721 (0.2) $\pm$ 203 (0.03)	672 (0.2) $\pm$ 33 (0.02)
24:0	838 (0.4) $\pm$ 449 (0.1)	915 (0.4) $\pm$ 337 (0.09)	763 (0.3) $\pm$ 206 (0.03)	629 (0.2) $\pm$ 57 (0.03)
16:1n-7	1321 (0.6) $\pm$ 627 (0.07)	1459 (0.6) $\pm$ 549 (0.08)	1910 (0.6) $\pm$ 367 (0.06)	1948 (0.7) $\pm$ 224 (0.06)
18:1n-9	42965 (20) $\pm$ 22545 (0.9)	44732 (19) $\pm$ 16612 (0.7)	52549 (17) $\pm$ 28992 (8.6)	63803 (22) $\pm$ 3963 (0.5)
18:1n-7	9832 (4.6) $\pm$ 5036 (0.2)	10253 (4.4) $\pm$ 3748 (0.2)	26601 (8.8) $\pm$ 26489 (8.8)	13389 (4.5) $\pm$ 848 (0.1)
20:1n-9	4594 (2.1) $\pm$ 2733 (0.4)	3902 (1.6) $\pm$ 1579 (0.2) [#]	7732 (2.6) $\pm$ 1715 (0.1)	6751 (2.3) $\pm$ 402 (0.1) [#]
22:1n-9	535 (0.2) $\pm$ 410 (0.09)	548 (0.2) $\pm$ 276 (0.08)	562 (0.2) $\pm$ 103 (0.01)	523 (0.2) $\pm$ 21 (0.01)
24:1n-9	1744 (0.9) $\pm$ 788 (0.3)	1608 (0.7) $\pm$ 500 (0.1)	3136 (1) $\pm$ 841 (0.2)	2679 (0.9) $\pm$ 233 (0.1)
18:2n-6	1144 (0.5) $\pm$ 560 (0.04)	1252 (0.5) $\pm$ 445 (0.05)	1412 (0.5) $\pm$ 328 (0.03)	1216 (0.4) $\pm$ 45 (0.04)
18:3n-6	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)
20:2n-6	320 (0.1) $\pm$ 169 (0.01)	289 (0.1) $\pm$ 104 (0.02) [#]	625 (0.2) $\pm$ 284 (0.07)	773 (0.3) $\pm$ 64 (0.01)
20:3n-6	1045 (0.5) $\pm$ 575 (0.04)	1212 (0.5) $\pm$ 449 (0.02)	1173 (0.4) $\pm$ 224 (0.02)	1172 (0.4) $\pm$ 58 (0.02)
20:4n-6	17106 (8.0) $\pm$ 8752 (0.2)	19443 (8.4) $\pm$ 6771 (0.4)	24249 (8.1) $\pm$ 3999 (0.2)	24018 (8.1) $\pm$ 1400 (0.1)
22:4n-6	5532 (2.6) $\pm$ 2975 (0.1)	6043 (2.6) $\pm$ 2230 (0.1)	7809 (2.6) $\pm$ 958 (0.2)	7969 (2.7) $\pm$ 462 (0.09)
22:5n-6	305 (0.1) $\pm$ 169 (0.02)	330 (0.1) $\pm$ 109 (0.01)	285 (0.1) $\pm$ 27 (0.01)	293 (0.1) $\pm$ 25 (0.01)
18:3n-3	144 (0.07) $\pm$ 73 (0.01)	95 (0.04) $\pm$ 47 (0.01) ^{##}	120 (0.04) $\pm$ 26 (0.005)	147 (0.05) $\pm$ 65 (0.02)
20:5n-3	144 (0.06) $\pm$ 91 (0.01)	161 (0.07) $\pm$ 64 (0.01)	177 (0.06) $\pm$ 38 (0.01)	213 (0.07) $\pm$ 52 (0.02)
22:5n-3	516 (0.2) $\pm$ 322 (0.04)	643 (0.3) $\pm$ 272 (0.05)	646 (0.2) $\pm$ 72 (0.04)	657 (0.2) $\pm$ 39 (0.02)
22:6n-3	28088 (13) $\pm$ 14133 (0.7)	31264 (14) $\pm$ 10834 (0.4)	35985 (12) $\pm$ 5308 (0.7)	36030 (12) $\pm$ 3033 (0.5)

FA	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) 2 month-old	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) 2 month-old	Wildtype (<i>Rora</i> ^{+/+}) nmol/g brain (mol%) >7 month-old	Staggerer (<i>Rora</i> ^{sg/sg}) nmol/g brain (mol%) >7 month-old
Total FA	212946 ± 108699	233154 ± 83436	300596 ± 50877	295584 ± 17432

Statistical significance for fatty acid concentration:

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$.

Statistical significance for fatty acid composition:

$p < 0.05$
$p < 0.01$
$p < 0.001$.

N denotes number of animals.

Hippocampus total phospholipid fatty acid concentration and composition at 2 months old and >7 months old (mean $\pm$ SD; 2 month-old n = 5/group, >7 month-old, n = 4-5/group).

Table 7.

FA	Wildtype (<i>Kora^{+/+}</i>) nmol/g brain (mol%)	Staggerer (<i>Kora^{sg/sg}</i>) nmol/g brain (mol%)	Wildtype (<i>Kora^{+/+}</i>) nmol/g brain (mol%)	Staggerer (<i>Kora^{sg/sg}</i>) nmol/g brain (mol%)
	2 month-old	2 month-old	>7 month-old	>7 month-old
14:0	82 (0.2) $\pm$ 18 (0.05)	76 (0.2) $\pm$ 8 (0.03)	107 (0.2) $\pm$ 38 (0.06)	100 (0.1) $\pm$ 13 (0.03)
16:0	11387 (27) $\pm$ 4556 (1)	8570 (28) $\pm$ 917 (0.9)	18179 (26) $\pm$ 8273 (1.2)	18625 (26) $\pm$ 3568 (1)
18:0	9663 (23) $\pm$ 3738 (1.1)	7085 (23) $\pm$ 754 (1.5)	15746 (23) $\pm$ 6585 (0.5)	15803 (22) $\pm$ 2838 (0.4) [#]
20:0	101 (0.3) $\pm$ 24 (0.05)	93 (0.3) $\pm$ 24 (0.09)	150 (0.2) $\pm$ 36 (0.07)	159 (0.2) $\pm$ 53 (0.05)
22:0	93 (0.2) $\pm$ 34 (0.04)	93 (0.3) $\pm$ 38 (0.2)	137 (0.2) $\pm$ 64 (0.05)	135 (0.2) $\pm$ 40 (0.04)
24:0	109 (0.3) $\pm$ 33 (0.1)	101 (0.3) $\pm$ 41 (0.2)	127 (0.2) $\pm$ 46 (0.05)	120 (0.2) $\pm$ 36 (0.05)
16:1n-7	252 (0.6) $\pm$ 128 (0.09)	184 (0.6) $\pm$ 40 (0.2)	499 (0.7) $\pm$ 248 (0.1)	425 (0.6) $\pm$ 76 (0.02)
18:1n-9	6879 (17) $\pm$ 2645 (0.5)	5312 (17) $\pm$ 836 (1.2)	12591 (19) $\pm$ 5049 (1.6)	12933 (18) $\pm$ 2564 (0.9)
18:1n-7	1534 (3.6) $\pm$ 674 (0.1)	1152 (3.7) $\pm$ 170 (0.09)	2519 (3.7) $\pm$ 1050 (0.3)	2648 (3.7) $\pm$ 491 (0.08)
20:1n-9	462 (1.1) $\pm$ 146 (0.2)	335 (1.1) $\pm$ 85 (0.2)	1030 (1.6) $\pm$ 346 (0.5)	988 (1.4) $\pm$ 341 (0.3)
22:1n-9	55 (0.1) $\pm$ 20 (0.05)	50 (0.2) $\pm$ 13 (0.04)	77 (0.1) $\pm$ 30(0.04)	131 (0.2) $\pm$ 46 (0.07)
24:1n-9	177 (0.5) $\pm$ 28 (0.1)	180 (0.6) $\pm$ 56 (0.2)	486 (0.7) $\pm$ 222 (0.3)	542 (0.8) $\pm$ 205 (0.2)
18:2n-6	247 (0.6) $\pm$ 121 (0.05)	204 (0.7) $\pm$ 28 (0.08)	380 (0.6) $\pm$ 170 (0.07)	355 (0.5) $\pm$ 86 (0.03)
18:3n-6	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)	0 (0) $\pm$ 0 (0)
20:2n-6	65 (0.2) $\pm$ 26 (0.01)	46 (0.2) $\pm$ 7 (0.03)	74 (0.1) $\pm$ 22 (0.02)	60 (0.08) $\pm$ 9 (0.01) [#]
20:3n-6	197 (0.5) $\pm$ 89 (0.06)	150 (0.5) $\pm$ 38 (0.06)	295 (0.4) $\pm$ 121 (0.09)	254 (0.3) $\pm$ 80 (0.06)
20:4n-6	3906 (9.3) $\pm$ 1630 (0.7)	2813 (9) $\pm$ 547 (0.8)	6415 (9.1) $\pm$ 3192 (1.2)	6791 (9.4) $\pm$ 1449 (0.2)
22:4n-6	867 (2.1) $\pm$ 352 (0.2)	644 (2) $\pm$ 153 (0.3)	1622 (2.3) $\pm$ 754 (0.3)	1847 (2.6) $\pm$ 440 (0.2)
22:5n-6	71 (0.2) $\pm$ 60 (0.09)	60 (0.2) $\pm$ 14 (0.05)	100 (0.2) $\pm$ 41 (0.05)	133 (0.2) $\pm$ 9 (0.05)
18:3n-3	15 (0.03) $\pm$ 21 (0.04)	8 (0.02) $\pm$ 11 (0.03)	13 (0.01) $\pm$ 18 (0.02)	27 (0.03) $\pm$ 32 (0.04)
20:5n-3	6 (0.01) $\pm$ 12 (0.03)	5 (0.02) $\pm$ 11 (0.04)	22 (0.03) $\pm$ 25 (0.03)	29 (0.03) $\pm$ 37 (0.04)
22:5n-3	93 (0.2) $\pm$ 27 (0.07)	77 (0.2) $\pm$ 13 (0.02)	143 (0.2) $\pm$ 64 (0.02)	137 (0.2) $\pm$ 25 (0.02)
22:6n-3	5495 (13) $\pm$ 2781 (1.7)	3947 (13) $\pm$ 1110 (2.4)	7949 (11) $\pm$ 4018 (2)	9447 (13) $\pm$ 1985 (0.7)
Total FA	41756 $\pm$ 16998	31187 $\pm$ 4260	68662 $\pm$ 29678	71687 $\pm$ 13869

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Statistical significance for fatty acid concentration:

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$.

Statistical significance for fatty acid composition:

$p < 0.05$
$p < 0.01$
$p < 0.001$.

N denotes number of animals.