RESEARCH ARTICLE

Molecular Nutrition & Food Research
www.mnf-journal.com

Influence of Dietary Triacylglycerol Structure on the Accumulation of Docosahexaenoic Acid [22:6(n-3)] in Organs in a Short-Term Feeding Trial with Mildly Omega-3 Deficient Rats

*Amruta Kulkarni, Kaisa M. Linderborg, Ai Zhao, Heikki Kallio, Gudmundur G. Haraldsson, Yumei Zhang, and Baoru Yang**

Scope: To study the effect of positional distribution of docosahexaenoic acid (DHA) in dietary triacylglycerols (TAG) on the tissue fatty acid content and composition of mildly (n-3) deficient rats.

Methods and results: In a 5-day feeding trial, mildly (n-3) deficient rats received 360 mg daily structured TAGs: *sn*-22:6(n-3)-18:0-18:0, *sn*-18:0-18:0-22:6(n-3), *sn*-18:0-22:6(n-3)-18:0, or tristearin. A fifth group receives standard (n-3) adequate feed AIN-93G from birth till the end of the trial. The DHA-fed groups show significantly higher DHA levels in the liver and visceral fat compared to the tristearin or normal feed groups showing that the dose and the short feeding period of DHA were sufficient to restore the DHA content in the organs of (n-3) deficient rats. Feeding *sn*-1 DHA resulted in higher levels of DHA in the liver TAG compared to *sn*-3 DHA feeding, although the difference did not reach statistical significance.

Conclusion: These findings indicated a possible difference in the tissue accumulation and/or metabolic fate of DHA from the *sn*-1 and *sn*-3 positions of TAG.

1. Introduction

Docosahexaenoic acid [DHA, 22:6(n-3)] is a long-chain omega-3 polyunsaturated fatty acid [LC (n-3) PUFA] necessary for the normal growth, development, and physiology of humans. DHA is synthesized endogenously from the essential precursor α-linolenic acid [ALA, 18:3(n-3)] via a series of desaturations and elongations. However, as the conversion efficiency of the precursor ALA to DHA is low in humans,[1,2] an exogenous supply of DHA from the diet, mainly from marine sources, is typically recommended. Quantitatively, DHA is the most significant (n-3) fatty acid in the brain, and it is also predominant in the retinal rod outer segments.[3,4] DHA is required for brain and retinal development, memory formation, neuroprotection, and synaptogenesis.[5,6] Several studies in animals and humans have also indicated that DHA deficiency in the brain is associated with impaired performance in learning and cognitive development,[4] as well as several neurological disorders, including Alzheimer's, Parkinson's, schizophrenia, and depression.[7,8]

Triacylglycerols (TAGs) are the dominating form of dietary DHA in foods. During digestion, dietary TAGs are hydrolyzed by the action of lipases, releasing fatty acids from *sn*-1 and *sn*-3 positions. As a result, the *sn*-1 and *sn*-3 fatty acids are absorbed as free fatty acids, whereas *sn*-2 fatty acids are absorbed as monacylglycerols.[9,10] Due to the chiral properties of lipases, the primary (*sn*-1 and *sn*-3) positions of TAGs are not equal as targets of the action of

A. Kulkarni, K. M. Linderborg, H. Kallio, B. Yang
Food Sciences, Department of Life Technologies
University of Turku
Turku 20520, Finland
E-mail: baoru.yang@utu.fi

A. Zhao
Vanke School of Public Health
Tsinghua University
Beijing 100083, China

G. G. Haraldsson
Science Institute
University of Iceland
Reykjavik 107, Iceland

Y. Zhang
Department of Nutrition & Food Hygiene
School of Public Health, Peking University Health Science Center
Beijing 100191, China

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202300635

DOI: 10.1002/mnfr.202300635

digestive enzymes. Lingual lipase cleaved the fatty acids at the *sn*-3 position twice as fast as those at the *sn*-1 position when a racemic mixture of diacylglycerols with radiolabeled alkyl moiety at *sn*-1 and *sn*-3 positions was incubated with lipases.[9,11] Jensen et al. showed four times faster release of 16:0 and 18:1 by hydrolysis of the *sn*-3 ester bond than *sn*-1 by the lingual lipase. Dog pancreatic lipase showed stereopreference for the *sn*-3 position of TAGs and stereopreference for the *sn*-1 position in racemic diacylglycerols.[12] Due to the positional selectivity and preference of lipases, the positional distribution of LC (n-3) PUFAs in TAG molecules may affect their absorption, distribution, and tissue uptake.[13,14] Previous studies have shown improved assimilation of DHA in the liver, erythrocytes, brain,[15] and brown adipose tissue[16] from structured TAG with DHA at the *sn*-2 position as compared to fish oil with DHA distributed among three positions of TAGs in 10-week old hamsters. Postprandial studies of lymph lipids of rats, which were fed with TAG positional isomers with DHA at *sn*-2 or *sn*-3 positions and two palmitic acids at remaining positions, showed higher DHA levels in lymph in the group fed with *sn*-2 DHA.[17] However, intervention with a structured oil with DHA located primarily in the *sn*-2 position and a randomized oil with DHA evenly distributed in the TAG molecules did not result in significant differences in DHA accretion in the liver and brain.[18]

Largely due to the unavailability of enantiopure reference TAGs, the difference in bioavailability of fatty acids between *sn*-1 and *sn*-3 positions is poorly understood. To fill in the knowledge gap, our group performed two animal feeding trials using the chemoenzymatic synthesized regio- and enantiopure TAGs with DHA at *sn*-1, 2, or 3 position.[19,20] In the first trial,[21] a four-week intervention feeding was performed in rats with TAGs containing DHA in *sn*-1, 2, or 3 positions as the single source of n-3 PUFA. At the end of the intervention, the DHA content of the plasma TAG fraction was significantly higher in the *sn*-1 DHA group compared to the *sn*-3 DHA group, whereas in visceral fat the DHA content was higher in the *sn*-3 DHA group than in the *sn*-1 DHA group.[21] In the second trial, we aimed to study the impact of a short-term 5-day intervention feeding with DHA as regio- and enantiopure structured TAGs on the absorption and tissue accumulation of DHA in rats with mild (n-3) deficiency. Analysis of fecal content of DHA indicated superior absorption of DHA from the *sn*-2 position of TAG as compared to the primary *sn*-1/3 positions, but no statistically significant difference was observed between the *sn*-1 and *sn*-3 DHA.[22] In the current study, we analyzed the fatty acid composition, especially the content of DHA and other PUFAs of the (n-3) and (n-6) families, in different organs including the liver, brain, eye, kidney, testicle, and visceral fat after the short-term intervention feeding in rats. This is the first systematic study on the impact of positional distribution of DHA on the accumulation of this valuable (n-3) PUFA in a wide range of tissues and organs. The findings of the research fill in the gap in current understanding of significance of TAG molecular structures on the bioavailability, distribution, and metabolism of dietary DHA.

2. Experimental Section

The experimental protocol included ethics approval for the animal experiment from the Medical Ethics Research Board of the Peking University Health Science Center, China (Study ID LA2016043), the synthesis of regio- and enantiopure structured TAGs, the animal feeding trial, sample collection, and subsequent lipid analysis of different organ tissues of rats. Detailed information on the design of the feeding trial has been described previously.[22] Briefly, the enantiopure structured TAGs possessing DHA either in *sn*-1 or *sn*-3 positions and two stearic acid residues in the remaining positions [*sn*-22:6(n-3)-18:0-18:0 as (*sn*-1 DHA) and *sn*-18:0-18:0-22:6(n-3) as (*sn*-3 DHA)][20] and their regioisomeric TAG [*sn*-18:0-22:6(n-3)-18:0 as (*sn*-2 DHA)][23,24] were synthesized. The excellent chemical purity (>97%) of all the TAGs obtained after column chromatography purification on silica gel was established by ^{1}H (400 MHz) and ^{13}C NMR and IR spectroscopy as well as satisfactory high-resolution accurate mass spectrometry analyses.[25] The regiopurity of the *sn*-1, *sn*-2, and *sn*-3 DHA TAGs was excellent (≥98%) as was established by 400 MHz ^{1}H and ^{13}C NMR spectroscopy.[23,26] The enantiopurity of the *sn*-1 and *sn*-3 DHA TAGs was higher than 98% (>96% enantiomeric excess) as was determined by chiral HPLC measurements based on the use of two chiral columns connected to a sample recycling system.[20] After weaning (21 days after birth), 60 Sprague-Dawley (SD) rats were kept on 7 days of adaptive feeding with standard AIN-93G feed containing soybean oil as a source of (n-3) fatty acids. After the adaptive phase, the rats were then randomly divided into five groups ($n = 12$): four groups were kept on the induction phase for 4 weeks to induce a mild (n-3) deficiency by subjecting to a custom diet based on AIN-93G containing (n-3) deficient peanut oil as the only source of fatty acids, whereas, the fifth group continued on the standard AIN-93G diet ((n-3) normal diet) (Table S1, supporting information). Due to the random division, the rats within each group were likely from different litters. After the 4 week induction phase, a 5-day intervention feeding was performed on rats at the age of 57 days with four groups receiving daily 360 mg of experimental fat as *sn*-1 DHA, *sn*-2 DHA, *sn*-3 DHA (containing 120 mg of DHA per day), or tristearin (TS) embedded inside a feed pellet as the first feed ration in the morning, and thereafter the rats were provided with the remaining normal daily ration ad libitum. The fifth group continued on the standard AIN-93G diet (Normal feed group, NF) during the intervention phase. The rats were sacrificed on day 6, i.e., 62nd day after birth, and the brain, eyes, liver, testicles, kidneys, and visceral fat were collected from each rat and weighed.

2.1. Extraction of Organ Lipids, Isolation of TAGs and Phospholipids, and Preparation of Fatty Acid Methyl Esters

Lipids of the brain, eye, liver, testicle, kidney, and visceral fat were analyzed. The brains were weighed, frozen in liquid nitrogen, and homogenized using a Bio-Gen PRO200 homogenizer (PRO Scientific, USA). The homogenate was divided into two portions, and one portion was stored at −80°C for lipid analysis. Other organs collected were immediately frozen in liquid nitrogen, individually packed, and stored at −80°C until analysis. For the extraction of lipids, large organs/tissues (liver, visceral fat, testicle, and kidney) were diced into pieces on an ice bath, and representative samples were taken randomly from the organ tissues, weighed, and homogenized in methanol (10 mL of MeOH for 1 g of tissue) by using an Ultra–Turrax T 25 instrument (IKA Werke GmbH &

Co. KG, Staufen, Germany). The tissues were homogenized at four intervals of 30 s at 8000 rpm and all the homogenized samples in methanol were stored at −80°C. Eyeballs were weighed and directly used for lipid extraction.

Lipid extraction was performed in duplicates from the liver, visceral fat, testicle, kidney, and brain, whereas lipids were extracted from only one eyeball for each rat.

Total lipids from the liver, visceral fat, testicle, kidney, and brain were extracted from one aliquot of tissue homogenate and solvent (≈200 mg for brain and kidney; ≈500 mg for liver, visceral fat, and testicle) and one eyeball of each rat (≈84–145 mg) with a modified Folch method using methanol, chloroform and 0.88% potassium chloride (KCl) in milli-Q water.[27,28] All solvents used were of HPLC grade. Triheptadecanoin and dinonadecanoylphosphatidylcholine (Larodan Fine Chemicals AB, Malmö, Sweden) were used as the internal standard in the liver, while only triheptadecanoin was used in the analysis of the brain, eye, kidney, testicle, and visceral fat tissues. The samples were homogenized and centrifuged; thereafter the supernatants were collected and filtered using a funnel filter and filter paper (Whatman, Maidstone, UK). After the addition of 0.88% KCl in milli-Q-water, the mixture was then centrifuged, and the lipid-rich chloroform layer was collected. After total lipid extraction, the liver lipids were fractionated with solid-phase extraction using Sep-Pak Vac 1cc silica cartridges (Waters, Dublin, Ireland) into polar and nonpolar lipids. The nonpolar lipids rich in TAGs were eluted using diethyl ether, and the polar lipids rich in phospholipids (PL) using methanol as previously described.[27] The total lipids extracted from the brain, eye, and testicle, were methylated to form fatty acid methyl esters (FAMEs) using the acid-catalyzed method by overnight reaction in acetyl chloride and methanol at 50°C.[29] The liver TAG- and PL-rich fractions, visceral fat, and kidney lipids were methylated by using the sodium methoxide method,[30] where lipids were suspended in lipids in dry diethyl ether, followed by the addition of methyl acetate and sodium methoxide. After 5 min of reaction, the reaction was stopped by the addition of acetic acid.

2.2. Analysis of Fatty Acid Composition

The FAMEs were analyzed using gas chromatography (GC) using Nexis GC-2030 (Shimadzu, Kyoto, Japan) equipped with AOC-20i autoinjector, AOC-20s autosampler, flame ionization detector, and wall-coated open tubular column DB-23 (60 m × 0.250 mm I.D. × 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA). The carrier gas was helium, and the injection mode was splitless with an injection volume of 0.5 µL (the split was opened after 1 min). Temperatures were 270°C for the inlet and 280°C for the detector. The oven temperature program was: 130°C held for 1 min, increased 6.5°C min^{-1} to 170°C, increased 2.75°C min^{-1} to 205°C and held for 18 min, increased 30°C min^{-1} to 230°C and held for 2 min. Retention times of the external standards [Supelco 37 Component FAME mix (Supelco, St. Louis, MO, USA), GLC-68D, GLC-68D, GLC-490, GLC-566C, 22:3(n-3), 22:4(n-6), 22:5(n-6), and 11A (Nu-Check-Prep, Elysian, MN, USA)] were used in the identification of the fatty acid peaks. Quantification of the fatty acids in liver TAG-rich fraction, brain, eye, testicle, visceral fat, and kidney was based on the triheptadecanoin, and those in liver PL-rich fraction were based on dinonadecanoylphosphatidylcholine. Correction factors obtained from the external standards mentioned above were used to calculate the content and the relative percentage of individual fatty acids.

Table 1. Lipids (mg per 100 mg) extracted from the rat organs after the 5-day intervention feeding phase.

Tissue	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
Liver TAG	1.92 ± 0.68	1.74 ± 0.64	1.73 ± 0.89	2.20 ± 0.73	1.59 ± 0.39
Liver PL	3.56 ± 0.44	3.60 ± 0.47	3.61 ± 0.32	3.42 ± 0.19	3.42 ± 0.36
Brain	4.09 ± 0.36	4.04 ± 0.36	4.06 ± 0.26	4.16 ± 0.26	3.91 ± 0.15
Eye	0.64 ± 0.1[a]	0.67 ± 0.08[a]	0.68 ± 0.07[a]	0.65 ± 0.07[a]	0.56 ± 0.05[b]
Testicle	1.51 ± 0.27	1.47 ± 0.25	1.50 ± 0.19	1.51 ± 0.23	1.48 ± 0.28
Visceral fat	22.0 ± 7.2	24.6 ± 7.3	26.8 ± 6.3	26.3 ± 4.5	22.8 ± 5.0
Kidney	4.73 ± 1.29	4.53 ± 1.28	5.01 ± 1.36	4.51 ± 1.05	3.75 ± 0.72

[a)] During the 5-day intervention period, three groups received structured triacylglycerols (360 mg per day for each rat) with docosahexaenoic acid (DHA) either at *sn*-1, *sn*-2, or *sn*-3 positions and two stearic acid residues in the remaining positions (*sn*-1 DHA, *sn*-2 DHA, *sn*-3 DHA groups), and the tristearin group received tristearin (360 mg per day for each rat), in addition to the ad libitum omega-3 fatty acid deficient diet. The normal feed group received soybean oil based standard feed from the beginning until the end of the animal trial. Values are mean ± SD. $n = 12$ in each group for liver TAG, liver PL, brain, visceral fat, and eye. For rat testicle: $n = 11$ in *sn*-1 DHA group. For rat kidney: $n = 10$ in *sn*-1 DHA group, $n = 11$ in *sn*-2 DHA group, $n = 11$ in *sn*-3 DHA group and $n = 10$ in tristearin group. Values with different superscript letters in the same row indicate that the means are significantly different ($p < 0.05$). TAG: triacyglycerol, PL: phospholipid.

2.3. Data Presentation, Statistical Analysis, and Excluded Samples

The fatty acid composition of total lipids in rat organs was statistically analyzed to detect the differences between the groups. Statistical analyses were performed with the SPSS 25 program (IBM, Armonk, NY, USA). An analysis of variance (ANOVA) test was performed to compare the means either among all the groups as well as among the three DHA-fed groups. Homogenous subsets were examined by Tukey's HSD test with Bonferroni corrections for normally distributed groups. The nonparametric Kruskal–Wallis test followed by the Mann–Whitney *U* test was performed for the data that were not normally distributed. p values < 0.05 were considered statistically significant. FA composition analysis of the testicle of one rat in the *sn*-1 DHA group was excluded because of its underdeveloped appearance. Two rats each from *sn*-1 DHA and tristearin groups and one rat each from *sn*-2 DHA and *sn*-3 DHA groups were excluded from kidney lipid analysis since their lipid content was standing out from the rest of the group (>2SD away from the mean, Table S3, supporting information).

3. Results

3.1. Total Lipid Content in the Organs and Tissues

The total lipid content extracted from the organs and tissues analyzed is shown in **Table 1**. Total PL content in the liver (mean value 3.4–3.6 mg per100 mg in the five groups) was found to be

1.5–2 times higher than the total liver TAG content (1.7–2.2 mg per100 mg). Feeding structured TAGs with DHA at *sn*-1, *sn*-2, or *sn*-3 positions did not result in a significant difference among the three groups in terms of the total content of TAG and PL in the liver. Neither were differences found in total TAG and PL levels between the DHA-fed groups and the control groups (TS and NF groups). In this study, the total lipid content of the rat organs, especially visceral fat, is lower than the typical likely due to low extraction yield using the applied extraction method. No differences were found among the groups in the total amount of lipids in the visceral fat, brain, and testicle, ($p > 0.05$) whereas, the total lipid content in the eye of the NF group was significantly lower as compared to other intervention groups ($p < 0.05$).

3.2. Liver Fatty Acid Composition

Liver TAG and PL fatty acid composition of all intervention groups are presented in **Table** 2A,B respectively. A higher DHA percentage was found in the liver PL fraction compared to the TAG fraction in all intervention groups. The difference in the percentage of DHA between PL and TAG was approximately 3-fold in the DHA-fed groups, approximately 10-fold in the TS group, and 5-fold in the NF group. DHA feeding resulted in significantly higher ($p < 0.001$) content of DHA in both TAG and PL compared to the NF group and the TS group (Tables 2A-B).

Figures 1, 2 present the content (µg extractable PUFA per100 mg fresh weight of tissue) of selected PUFAs of the (n-3) and (n-6) families, respectively, of liver TAG and PL fractions. In agreement with the findings described for the percentage of fatty acids, the DHA-fed groups showed significantly higher absolute levels of extractable DHA in the TAG and PL fractions compared to the NF and the TS groups (Figure 1C). No statistical difference in the DHA level of liver TAG and PL was detected among the three DHA-fed groups as the ANOVA was nonsignificant.

During the 5-day feeding period, the DHA-fed groups and the TS group received an additional 18:0 in the fat supplement. Compared to the group fed with normal feed, the percentage of 18:0 in the PL fraction was significantly increased in the groups fed with the structured TAGs containing DHA and stearic acid and in the TS group (significantly different to *sn*-1 DHA, *sn*-2 DHA groups, and TS groups: $p < 0.001$, $p < 0.05$, and $p < 0.01$, respectively). Among the three DHA-fed groups, stearic acid (18:0) in liver PLs was found to be significantly higher *sn*-1 DHA group as compared to the *sn*-3 DHA group ($p < 0.001$).

The level of oleic acid [OA, 18:1(n-9)], linoleic acid [LA, 18:2(n-6)], and ALA in the feed influenced the proportion of these fatty acids in the liver TAG and PL fractions. The NF group received soybean oil containing approximately 60 times higher ALA and 3 times higher LA compared to peanut oil fed to other intervention groups (Table S2, supporting information). ALA and LA percentages of both TAG and PL fractions were significantly higher in the NF group compared to all other groups ($p \leq 0.001$) (Table 2A-B). Similar statistical differences were observed when absolute values of ALA and LA of liver TAG and PL fraction were compared (Figures 1A and 2A) among all experimental groups. The peanut oil-based feed consisted of a three times higher content of oleic acid (OA) compared to soybean oil-based feed (Table S2, supporting information). This difference resulted in significantly higher levels of OA in liver TAG and PL of the DHA-fed groups and the TS group compared to the NF group ($p < 0.001$) (Table 2A-B).

DHA-fed groups had higher EPA compared to the NF group in both TAG ($p > 0.05$) and PL (significantly different from *sn*-2 DHA and *sn*-3 DHA groups) of liver tissues (Table 2A and B; Figure 1B). EPA level in TAG and PL fractions was significantly lower in the TS group than in all other groups ($p < 0.05$), showing depletion of this (n-3) PUFA by the (n-3) deficient feeding (Table 2A and B; Figure 1B). Docosapentaenoic acid [DPA, 22:5(n-3)] was low in the liver both in the TAG (below 0.05%) and in the PL fractions (0.1% or less) of all groups, although DPA in liver PL was significantly lower in the DHA-fed groups compared to the TS and NF groups ($p < 0.001$) (Table 2A and B).

ARA was found to be significantly higher in the PL (28–34% of total fatty acids) than in the TAG fraction (typically 5–6%) of the liver, the difference being close to 5-fold (Tables 2A,B). In liver PL, the level of ARA was the highest in the TS group (34%), followed by the NF group (32%), being the lowest in the DHA-fed groups (around 28%) ($p < 0.001$), whereas no significant differences were found in the absolute level and percentage of ARA in liver TAG among the groups (Table 2A, Figure 2B). The absolute content of ARA in the NF group (1078 µg per 100 mg) of liver PL fraction did not differ from DHA-fed groups (995–1013 µg per 100 mg) (Figure 2B). As with other fatty acids in the (n-6) family, the levels of Docosatetraenoic acid [DTA, 22:4(n-6)] and (n-6) Docosapentaenoic acid [DPAn-6, 22:5(n-6)] were increased in both TAG and PL fractions in the (n-3) deficient TS group compared to the other groups (Tables 2A-B; Figures 2C,2D).

DHA feeding significantly increased the total (n-3) FA level of the DHA-fed groups in both liver TAG and PL compared to the TS group ($p < 0.001$). Compared to the NF group, the DHA-fed groups had a higher total level of (n-3) PUFAs in liver PL ($p < 0.001$), whereas no difference was found between the NF group and the DHA-fed groups in liver TAG (Tables 2A-B, Figure 1D). The total (n-6) PUFA and total PUFA percentages in the liver TAG and the total PUFA in the liver PL (Tables 2A-B) were significantly higher in the NF group as compared to all other intervention groups ($p < 0.001$). In contrast, a slightly different statistical pattern was found (Figure 2E), where the NF group of liver TAG and PL had higher absolute total (n-6) FA content compared to only three DHA-fed groups ($p < 0.05$ for liver TAG; for liver PL only significantly different from the *sn*-3 DHA group). The high content of OA in the peanut oil in the diet increased the total MUFA content in the liver TAGs of the DHA-fed groups (44–46%) and the TS group (49%) compared to the NF group (32%) ($p < 0.001$) (Table 2A). In liver PL, the total MUFA content (8–9%) was lower than in the TAG fraction, and only the *sn*-3 DHA group and the TS group had significantly higher MUFA compared to the NF group ($p = 0.02$, $p = 0.001$ respectively) (Table 2B). There were no statistical differences in the total (n-3), total (n-6), total PUFA, or total MUFA contents in liver TAG or PL fractions among DHA-fed groups.

3.3. Brain and Eye Fatty Acid Composition

The relative percentages of all fatty acids in the brain and eye are shown in Tables 2C–D. The average tissue content (µg per 100 mg) of extractable ALA, EPA, DHA, and total (n-3) FA, and

Table 2. A–G) Fatty acids of rat liver (TAG and PL), brain, eye, testicle, visceral fat, and kidney (% of total fatty acids) of different intervention groups.

A: Liver TAG fraction fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
12:0	0.09 ± 0.03	0.1 ± 0.04	0.11 ± 0.05	0.1 ± 0.04	0.09 ± 0.02
14:0	0.63 ± 0.1[a]	0.66 ± 0.14[a]	0.71 ± 0.12[a]	0.72 ± 0.13[ab]	0.87 ± 0.18[b]
14:1(n-5)	0.21 ± 0.4	0.16 ± 0.25	0.11 ± 0.03	0.12 ± 0.06	0.12 ± 0.03
16:0	24.77 ± 4.05	25.38 ± 2.54	26.75 ± 3.58	26.42 ± 3.73	24.01 ± 3.01
16:1(n-7)	2.41 ± 0.93[a]	2.78 ± 0.93[ab]	3.35 ± 0.98[ab]	3.21 ± 0.97[ab]	3.68 ± 1.15[b]
18:0	4.57 ± 1.84	4.7 ± 1.73	3.96 ± 1.11	3.79 ± 1.11	3.56 ± 1.83
18:1(n-9)	38.93 ± 3.68[a]	38.45 ± 3.89[a]	39.98 ± 3.27[a]	42.82 ± 2.96[a]	24.18 ± 2.33[b]
18:1(n-7)	2.03 ± 0.41[a]	2.18 ± 0.3[a]	2.33 ± 0.19[a]	2.25 ± 0.46[a]	3.72 ± 0.51[b]
18:2(n-6)	13.03 ± 3.07[a]	12.51 ± 2.14[a]	11.69 ± 3.04[a]	11.48 ± 3.18[a]	26.96 ± 6.28[b]
18:3(n-6)	0.04 ± 0.02[a]	0.03 ± 0.01[a]	0.05 ± 0.05[a]	0.03 ± 0.02[a]	0.26 ± 0.07[b]
18:3(n-3)	0.18 ± 0.06[a]	0.2 ± 0.06[a]	0.19 ± 0.09[a]	0.15 ± 0.07[a]	1.7 ± 0.49[b]
20:0	0.07 ± 0.04	0.07 ± 0.03	0.07 ± 0.04	0.05 ± 0.03	0.05 ± 0.02
20:1(n-9)	0.2 ± 0.07[a]	0.16 ± 0.05[ab]	0.14 ± 0.05[ab]	0.12 ± 0.05[ab]	0.12 ± 0.03[b]
20:2(n-6)	0.33 ± 0.12[ab]	0.31 ± 0.08[ab]	0.29 ± 0.08[ab]	0.45 ± 0.14[b]	0.29 ± 0.04[a]
20:3(n-6)	0.2 ± 0.08[a]	0.21 ± 0.07[a]	0.18 ± 0.06[a]	0.11 ± 0.05[b]	0.24 ± 0.07[a]
20:4(n-6)	5.67 ± 2.23	5.93 ± 2.15	5.07 ± 2.02	5.98 ± 1.86	6.29 ± 2.94
20:3(n-3)	0.06 ± 0.03[ab]	0.04 ± 0.02[a]	0.05 ± 0.04[ab]	0.05 ± 0.02[a]	0.08 ± 0.04[b]
20:5(n-3)	0.45 ± 0.23[a]	0.5 ± 0.13[a]	0.38 ± 0.14[a]	0.03 ± 0.02[b]	0.38 ± 0.13[a]
22:0	0.02 ± 0.02	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02
22:1(n-9)	0.05 ± 0.04	0.16 ± 0.38	0.04 ± 0.03	0.03 ± 0.02	0.06 ± 0.07
22:2(n-6)	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.02[ab]	0.04 ± 0.02[a]	0.01 ± 0.01[b]
22:4(n-6)	0.17 ± 0.05[ab]	0.15 ± 0.04[a]	0.15 ± 0.07[a]	0.25 ± 0.12[b]	0.28 ± 0.06[c]
22:5(n-6)	0.23 ± 0.15[a]	0.18 ± 0.05[a]	0.16 ± 0.05[ab]	0.55 ± 0.21[b]	0.2 ± 0.09[a]
22:5 (n-3)	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.04	0.03 ± 0.02
24:0	0.2 ± 0.07[a]	0.21 ± 0.09[a]	0.17 ± 0.07[a]	0.06 ± 0.03[b]	0.31 ± 0.07[c]
22:6(n-3)	4.83 ± 1.8[a]	4.26 ± 1.26[a]	3.46 ± 1.09[a]	0.5 ± 0.28[b]	1.66 ± 0.8[c]
24:1(n-9)	0.03 ± 0.03[a]	0.03 ± 0.03[ab]	0.03 ± 0.03[ab]	0.02 ± 0.04[b]	0.01 ± 0.01[b]
Total SFA	30.33 ± 3.95	31.11 ± 2.61	31.76 ± 2.95	31.14 ± 3.4	28.88 ± 3.46
Total MUFA	43.82 ± 3.97[b]	43.88 ± 4.29[b]	45.95 ± 3.86[ab]	48.54 ± 3.2[a]	31.87 ± 3.47[c]
Total (n-6) FA	19.48 ± 4.36[a]	19.16 ± 3.31[a]	17.43 ± 4.77[a]	18.61 ± 4.73[a]	34.22 ± 5.64[b]
Total (n-3) FA	5.68 ± 2.06[a]	5.14 ± 1.38[a]	4.22 ± 1.29[a]	0.98 ± 0.34[b]	4.11 ± 0.74[a]
Total PUFA	25.16 ± 6.22[a]	24.3 ± 4.56[a]	21.65 ± 5.97[a]	19.58 ± 5.02[a]	38.32 ± 6.04[b]

B: Liver PL fraction fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
12:0	0.02 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
14:0	0.11 ± 0.04[a]	0.13 ± 0.04[a]	0.14 ± 0.04[a]	0.14 ± 0.05[a]	0.2 ± 0.06[b]
14:1(n-5)	0.04 ± 0.04	0.03 ± 0.01	0.09 ± 0.15	0.04 ± 0.04	0.07 ± 0.11
16:0	15.25 ± 1.69[a]	16.28 ± 1.15[ab]	17.24 ± 0.92[b]	15.81 ± 1.2[a]	16.28 ± 0.75[ab]
16:1(n-7)	0.58 ± 0.18[a]	0.68 ± 0.17[ab]	0.78 ± 0.14[b]	0.74 ± 0.16[ab]	0.75 ± 0.12[ab]
18:0	23.81 ± 2.11[a]	22.13 ± 1.69[ab]	21.03 ± 1.04[bc]	22.39 ± 1.47[ab]	19.89 ± 1.22[c]
18:1(n-9)	5.34 ± 0.69[a]	5.3 ± 0.48[a]	5.66 ± 0.65[ab]	5.98 ± 0.48[b]	3.42 ± 0.36[c]
18:1(n-7)	1.74 ± 0.25[a]	2.03 ± 0.34[a]	2.07 ± 0.25[a]	2.12 ± 0.37[a]	3.33 ± 0.56[b]
18:2(n-6)	8.46 ± 1.23[a]	8.62 ± 0.76[a]	8.28 ± 0.87[a]	7.66 ± 0.92[a]	11.65 ± 1.79[b]
18:3(n-6)	0.03 ± 0.01[a]	0.03 ± 0.02[a]	0.03 ± 0.02[a]	0.03 ± 0.01[a]	0.06 ± 0.02[b]
18:3(n-3)	0.01 ± 0.02[a]	0.03 ± 0.03[a]	0.02 ± 0.02[a]	0.02 ± 0.02[a]	0.11 ± 0.04[b]

(*Continued*)

Table 2. (Continued)

B: Liver PL fraction fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
20:0	0.06 ± 0.03	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01
20:1(n-9)	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.12 ± 0.03
20:2(n-6)	0.38 ± 0.07[a]	0.39 ± 0.05[a]	0.39 ± 0.06[a]	0.53 ± 0.13[b]	0.46 ± 0.05[b]
20:3(n-6)	0.8 ± 0.1[a]	0.87 ± 0.13[a]	0.87 ± 0.14[a]	0.66 ± 0.16[b]	0.83 ± 0.1[a]
20:4(n-6)	27.98 ± 2.48[a]	28.11 ± 1.02[a]	27.61 ± 1.57[a]	33.98 ± 1.12[b]	31.51 ± 0.91[c]
20:3(n-3)	0.08 ± 0.03	0.08 ± 0.05	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.02
20:5(n-3)	0.25 ± 0.14[ac]	0.27 ± 0.1[a]	0.21 ± 0.07[a]	0.03 ± 0.06[b]	0.13 ± 0.03[c]
22:0	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[ab]	0.02 ± 0.01[a]	0.01 ± 0.01[b]
22:1(n-9)	0.01 ± 0.01	0.03 ± 0.07	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
22:2(n-6)	0.04 ± 0.01[a]	0.04 ± 0.01[a]	0.04 ± 0.01[a]	0.07 ± 0.02[b]	0.02 ± 0.01[c]
22:4(n-6)	0.23 ± 0.04[a]	0.23 ± 0.02[a]	0.23 ± 0.03[a]	0.58 ± 0.15[b]	0.32 ± 0.04[c]
22:5(n-6)	0.33 ± 0.13[a]	0.33 ± 0.08[a]	0.34 ± 0.08[a]	3.09 ± 0.99[b]	0.45 ± 0.23[a]
22:5 (n-3)	0.04 ± 0.01[a]	0.05 ± 0.02[a]	0.05 ± 0.01[a]	0.1 ± 0.02[b]	0.1 ± 0.05[b]
24:0	0.27 ± 0.08[a]	0.33 ± 0.12[a]	0.32 ± 0.08[a]	0.29 ± 0.08[a]	0.69 ± 0.13[b]
22:6(n-3)	13.58 ± 2.04[a]	13.33 ± 1.02[a]	13.94 ± 0.81[a]	5.26 ± 0.47[b]	8.92 ± 1.88[c]
24:1(n-9)	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.01[b]	0.01 ± 0.01[b]
Total SFA	39.52 ± 1.15[a]	38.94 ± 0.84[a]	38.80 ± 0.9[a]	38.70 ± 0.89[a]	37.12 ± 0.84[b]
Total MUFA	7.81 ± 1.04[ab]	8.18 ± 0.57[abc]	8.70 ± 0.91[bc]	8.99 ± 0.69[c]	7.68 ± 0.67[a]
Total(n-6) FA	38 ± 2.4[a]	38.36 ± 1.27[a]	37.52 ± 1.28[a]	45.97 ± 0.98[b]	44.95 ± 2.08[b]
Total(n-3) FA	14.17 ± 1.98[a]	13.95 ± 1[a]	14.50 ± 0.78[a]	6.04 ± 0.43[b]	9.62 ± 1.86[c]
Total PUFA	52.16 ± 1.17[a]	52.31 ± 0.73[a]	52.01 ± 1.25[a]	52 ± 0.92[a]	54.56 ± 0.72[b]

C: Brain fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
12:0	0.06 ± 0.04[a]	0.05 ± 0.03[a]	0.05 ± 0.04[a]	0.05 ± 0.03[a]	0.12 ± 0.03[b]
14:0	0.24 ± 0.04	0.25 ± 0.04	0.27 ± 0.09	0.28 ± 0.07	0.27 ± 0.06
16:0	17.35 ± 1.49	17.87 ± 1.71	17.74 ± 1.55	17.41 ± 1.07	18.65 ± 0.88
16:1(n-7)	0.3 ± 0.04	0.32 ± 0.05	0.31 ± 0.04	0.3 ± 0.03	0.32 ± 0.03
18:0	17.73 ± 0.65	17.63 ± 0.75	17.75 ± 0.88	17.63 ± 1.73	18.48 ± 0.63
18:1(n-9)	15.87 ± 1.31[ab]	15.73 ± 1.39[ab]	15.55 ± 1.37[ab]	15.96 ± 1.1[a]	14.49 ± 0.75[b]
18:1(n-7)	3.34 ± 0.29[ab]	3.3 ± 0.37[ab]	3.33 ± 0.34[ab]	3.44 ± 0.25[a]	3.04 ± 0.19[b]
18:2(n-6)	0.55 ± 0.06[a]	0.55 ± 0.05[a]	0.57 ± 0.06[a]	0.53 ± 0.07[a]	0.74 ± 0.09[b]
18:3(n-6)	0.04 ± 0.03[ab]	0.06 ± 0.01[a]	0.07 ± 0.01[a]	0.07 ± 0.01[a]	0.02 ± 0.01[b]
18:3(n-3)	0.03 ± 0.02[ab]	0.02 ± 0.01[ab]	0.01 ± 0.01[a]	0.02 ± 0.01[ab]	0.02 ± 0.01[b]
20:0	0.7 ± 0.24	0.56 ± 0.24	0.63 ± 0.19	0.69 ± 0.13	0.54 ± 0.12
20:1(n-9)	1.14 ± 0.62[a]	1.6 ± 0.81[ab]	1.66 ± 0.62[ab]	1.88 ± 0.43[b]	0.39 ± 0.1[c]
20:2(n-6)	0.27 ± 0.06	0.25 ± 0.07	0.27 ± 0.06	0.29 ± 0.05	0.24 ± 0.05
20:3(n-6)	0.42 ± 0.05	0.41 ± 0.06	0.42 ± 0.05	0.41 ± 0.05	0.43 ± 0.05
20:4(n-6)	9.99 ± 0.98	10.17 ± 1.2	9.76 ± 0.89	10.06 ± 0.8	10.86 ± 0.6
22:0	0.72 ± 0.24	0.55 ± 0.21	0.63 ± 0.18	0.69 ± 0.16	0.55 ± 0.12
22:1(n-9)	0.25 ± 0.06[a]	0.25 ± 0.1[ab]	0.27 ± 0.09[a]	0.28 ± 0.04[a]	0.18 ± 0.04[b]
22:2(n-6)	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.01
22:4(n-6)	2.86 ± 0.15	2.91 ± 0.14	2.78 ± 0.19	2.97 ± 0.18	2.83 ± 0.15
22:5(n-6)	1.11 ± 0.2[a]	1.2 ± 0.27[ab]	1.2 ± 0.16[a]	1.46 ± 0.22[b]	0.73 ± 0.34[c]
24:0	1.21 ± 0.37	0.96 ± 0.36	1.09 ± 0.3	1.15 ± 0.21	0.97 ± 0.16
22:6(n-3)	14.17 ± 1.54[ab]	14.53 ± 1.65[ab]	14.52 ± 1.45[ab]	13.1 ± 1.07[a]	15.8 ± 1.04[b]

(*Continued*)

Table 2. (Continued)

C: Brain fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
24:1(n-9)	1.92 ± 0.66	1.36 ± 0.69	1.66 ± 0.59	1.75 ± 0.33	1.36 ± 0.32
Total SFA	37.99 ± 1.21[a]	37.84 ± 1.6[ab]	38.14 ± 1.81[ab]	37.86 ± 2.36[a]	39.57 ± 0.98[b]
Total MUFA	22.79 ± 2.36[a]	22.53 ± 3.14[ab]	22.75 ± 2.73[a]	23.58 ± 2.01[a]	19.76 ± 1.3[b]
Total (n-6) FA	12.38 ± 1.04	12.64 ± 1.27	12.27 ± 0.92	12.81 ± 0.82	13.01 ± 0.7
Total (n-3) FA	17.04 ± 1.55[ab]	17.45 ± 1.69[ab]	17.3 ± 1.48[ab]	16.08 ± 1.14[a]	18.64 ± 1.01[b]
Total PUFA	29.42 ± 2.49[ab]	30.09 ± 2.9[ab]	29.57 ± 2.3[ab]	28.88 ± 1.78[a]	31.65 ± 1.33[b]

D: Eye fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
12:0	0.14 ± 0.04	0.18 ± 0.04	0.21 ± 0.08	0.17 ± 0.06	0.17 ± 0.06
14:0	1.25 ± 0.17	1.26 ± 0.11	1.29 ± 0.15	1.18 ± 0.14	1.21 ± 0.1
14:1(n-5)	0.06 ± 0.05[a]	0.08 ± 0.05[ab]	0.13 ± 0.04[b]	0.07 ± 0.04[a]	0.11 ± 0.04[ab]
16:0	20.84 ± 1.1	20.67 ± 0.58	21.11 ± 0.63	20.97 ± 0.52	20.86 ± 0.62
16:1(n-7)	2.47 ± 0.97	2.62 ± 1.19	3.08 ± 0.67	2.43 ± 0.63	2.6 ± 0.6
18:0	16.38 ± 2.5	14.75 ± 1.62	15.33 ± 1.46	15.66 ± 1.47	16.6 ± 1.62
18:1(n-9)	15.21 ± 2.66[ab]	17.2 ± 2.34[a]	18 ± 3.09[a]	17.15 ± 2.26[a]	13.21 ± 1.62[b]
18:1(n-7)	3.16 ± 0.76	3.39 ± 0.86	3.28 ± 0.26	3.06 ± 0.11	3.14 ± 0.22
18:2(n-6)	3.55 ± 0.87[a]	4.25 ± 1.06[ab]	3.72 ± 1.1[a]	3.84 ± 0.88[a]	5.75 ± 1.86[b]
18:3(n-6)	0.11 ± 0.02[abc]	0.1 ± 0.02[a]	0.1 ± 0.01[ab]	0.11 ± 0.01[bc]	0.12 ± 0.01[c]
18:3(n-3)	0.03 ± 0.02[a]	0.05 ± 0.03[a]	0.03 ± 0.03[a]	0.04 ± 0.03[a]	0.24 ± 0.12[b]
20:0	0.33 ± 0.06	0.28 ± 0.05	0.32 ± 0.03	0.32 ± 0.03	0.33 ± 0.06
20:1(n-9)	0.24 ± 0.05[a]	0.59 ± 1.11[a]	0.26 ± 0.04[a]	0.26 ± 0.05[a]	0.18 ± 0.01[b]
20:2(n-6)	0.35 ± 0.04[a]	0.35 ± 0.03[a]	0.36 ± 0.03[ab]	0.38 ± 0.05[b]	0.37 ± 0.03[ab]
20:3(n-6)	0.29 ± 0.03[a]	0.29 ± 0.04[a]	0.28 ± 0.04[a]	0.28 ± 0.05[a]	0.33 ± 0.04[b]
20:4(n-6)	11.6 ± 0.96[ab]	11.26 ± 0.84[ab]	10.6 ± 0.87[a]	11.85 ± 1.16[b]	11.5 ± 0.57[ab]
20:3(n-3)	0.11 ± 0.21	0.14 ± 0.28	0.03 ± 0.04	0.01 ± 0.02	0.02 ± 0.02
20:5(n-3)	0.04 ± 0.02[ab]	0.06 ± 0.03[ac]	0.05 ± 0.02[a]	0.02 ± 0.01[b]	0.1 ± 0.1[c]
22:0	0.29 ± 0.03	0.26 ± 0.04	0.3 ± 0.03	0.3 ± 0.04	0.31 ± 0.09
22:1(n-9)	0.16 ± 0.04[a]	0.16 ± 0.07[a]	0.12 ± 0.04[a]	0.14 ± 0.05[a]	0.1 ± 0.04[b]
22:2(n-6)	0.07 ± 0.03[ac]	0.08 ± 0.03[a]	0.08 ± 0.02[a]	0.11 ± 0.02[b]	0.04 ± 0.02[c]
22:4(n-6)	1.62 ± 0.2[ab]	1.59 ± 0.14[ab]	1.37 ± 0.44[a]	1.8 ± 0.15[b]	1.64 ± 0.12[ab]
22:5(n-6)	1.12 ± 0.24[a]	1.05 ± 0.13[a]	1.15 ± 0.22[a]	1.72 ± 0.34[b]	0.54 ± 0.1[c]
24:0	0.61 ± 0.13[a]	0.55 ± 0.16[a]	0.63 ± 0.13[a]	0.57 ± 0.16[a]	1.01 ± 0.11[b]
22:6(n-3)	19.21 ± 2.26	18.05 ± 2.45	17.28 ± 2.76	16.63 ± 1.92	18.71 ± 2.55
24:1(n-9)	0.44 ± 0.07[ab]	0.4 ± 0.09[ab]	0.49 ± 0.06[a]	0.47 ± 0.07[ab]	0.42 ± 0.03[b]
Total SFA	39.81 ± 3.59[ab]	37.93 ± 1.81[a]	39.17 ± 1.35[ab]	39.14 ± 1.41[ab]	40.45 ± 1.88[b]
Total MUFA	21.72 ± 4.05[ab]	24.41 ± 3.37[a]	25.33 ± 3.62[a]	23.56 ± 2.7[a]	19.73 ± 2.25[b]
Total (n-6) FA	17.06 ± 0.95[ab]	17.35 ± 1.17[ab]	16.26 ± 0.68[a]	18.27 ± 1.14[b]	18.63 ± 1.88[b]
Total (n-3) FA	20.99 ± 2.29	19.86 ± 2.32	18.74 ± 2.82	18.49 ± 2.01	20.7 ± 2.46
Total PUFA	38.05 ± 2.61[ab]	37.2 ± 2.27[abc]	34.99 ± 2.53[c]	36.76 ± 1.97[bc]	39.32 ± 1.39[a]

E: Testicle fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
12:0	0.29 ± 0.13[a]	0.2 ± 0.08[ab]	0.28 ± 0.31[ab]	0.27 ± 0.25[ab]	0.16 ± 0.04[b]

(*Continued*)

Table 2. (Continued)

E: Testicle fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
14:0	0.82 ± 0.21	0.79 ± 0.15	0.95 ± 0.22	1.03 ± 0.67	0.88 ± 0.23
14:1(n-5)	0.12 ± 0.06[a b]	0.19 ± 0.14[a b]	0.2 ± 0.08[a]	0.2 ± 0.07[a]	0.12 ± 0.04[b]
16:0	30.67 ± 2.03	30.88 ± 1.89	31.1 ± 1.49	31.29 ± 1.42	32.31 ± 1.89
16:1(n-7)	2.23 ± 0.72	2.07 ± 0.63	2.6 ± 0.77	2.05 ± 0.68	1.96 ± 0.71
18:0	6.76 ± 1.99	6.48 ± 1.64	6.18 ± 0.73	7.15 ± 1.86	6.42 ± 0.48
18:1(n-9)	21.21 ± 4.87[a]	20.21 ± 5.2[a]	20.24 ± 3.86[a]	19.47 ± 4.36[a]	12.48 ± 2.08[b]
18:1(n-7)	2.44 ± 0.21	2.54 ± 0.27	2.57 ± 0.24	2.38 ± 0.31	2.4 ± 0.19
18:2(n-6)	5.89 ± 0.95[a]	5.72 ± 0.95[a]	5.64 ± 0.88[a]	5.2 ± 0.85[a]	9.96 ± 3.08[b]
18:3(n-6)	0.02 ± 0.03[a]	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.01[a]	0.13 ± 0.12[b]
18:3(n-3)	0.19 ± 0.1[a]	0.11 ± 0.05[a]	0.14 ± 0.07[a]	0.12 ± 0.04[a]	0.62 ± 0.32[b]
20:0	0.18 ± 0.06[a]	0.18 ± 0.1[a]	0.16 ± 0.03[a]	0.22 ± 0.15[a]	0.13 ± 0.03[b]
20:1(n-9)	0.24 ± 0.06[a]	0.24 ± 0.08[a]	0.22 ± 0.05[a]	0.21 ± 0.06[a]	0.11 ± 0.02[b]
20:2(n-6)	0.27 ± 0.03	0.28 ± 0.04	0.27 ± 0.02	0.27 ± 0.04	0.26 ± 0.04
20:3(n-6)	0.82 ± 0.13[a]	0.87 ± 0.13[a]	0.84 ± 0.09[a]	0.75 ± 0.1[a]	1.04 ± 0.12[b]
20:4(n-6)	13.05 ± 2.13	13.73 ± 1.92	13.52 ± 1.65	14.26 ± 1.74	15.24 ± 2.35
20:3(n-3)	0.1 ± 0.03	0.09 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.11 ± 0.02
20:5(n-3)	0.03 ± 0.02[a b]	0.04 ± 0.02[a]	0.03 ± 0.01[a]	0.02 ± 0.01[b]	0.04 ± 0.02[a]
22:0	0.15 ± 0.02[a]	0.17 ± 0.1[a]	0.13 ± 0.02[a b]	0.2 ± 0.14[a b]	0.24 ± 0.45[b]
22:1(n-9)	0.86 ± 0.18[a]	0.69 ± 0.13[b]	0.7 ± 0.09[b]	0.74 ± 0.12[a b]	0.7 ± 0.14[b]
22:2(n-6)	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.06 ± 0.02[a]	0.06 ± 0.02[a]	0.05 ± 0.06[b]
22:4(n-6)	1.45 ± 0.26[a]	1.57 ± 0.21[a b]	1.5 ± 0.22[a b]	1.55 ± 0.14[a b]	1.71 ± 0.23[b]
22:5(n-6)	10.79 ± 1.78	11.39 ± 1.64	11.19 ± 1.38	11.39 ± 1.12	11.55 ± 1.76
24:0	0.11 ± 0.02[a]	0.12 ± 0.02[a]	0.1 ± 0.02[a]	0.11 ± 0.03[a]	0.08 ± 0.02[b]
22:6(n-3)	0.91 ± 0.15[a]	0.99 ± 0.13[a]	0.94 ± 0.11[a]	0.6 ± 0.08[b]	0.94 ± 0.21[a]
24:1(n-9)	0.05 ± 0.02[a b]	0.07 ± 0.02[a]	0.06 ± 0.02[a b]	0.06 ± 0.02[a b]	0.05 ± 0.01[b]
Total SFA	38.94 ± 2.97	38.79 ± 3.32	38.87 ± 1.97	40.25 ± 3.85	40.18 ± 1.84
Total MUFA	27.14 ± 5.65[a]	25.98 ± 5.92[a]	26.56 ± 4.6[a]	25.08 ± 5.25[a]	17.78 ± 2.81[b]
Total (n-6) FA	30.86 ± 3.09[a]	32.03 ± 2.68[a]	31.5 ± 2.61[a]	31.91 ± 2.03[a]	38.2 ± 1.08[b]
Total (n-3) FA	2.66 ± 0.46[a b]	2.78 ± 0.3[b]	2.68 ± 0.31[a b]	2.37 ± 0.19[b]	3.39 ± 0.16[c]
Total PUFA	33.51 ± 3.52[a]	34.81 ± 2.92[a]	34.18 ± 2.88[a]	34.28 ± 2.16[a]	41.59 ± 1.14[b]

F: Visceral fat fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
12:0	0.24 ± 0.02	0.26 ± 0.03	0.25 ± 0.02	0.25 ± 0.03	0.26 ± 0.02
14:0	1.61 ± 0.16[a]	1.65 ± 0.19[a]	1.7 ± 0.18[a b]	1.63 ± 0.14[a]	1.84 ± 0.11[b]
14:1(n-5)	0.16 ± 0.04[a]	0.15 ± 0.03[a]	0.17 ± 0.04[a]	0.15 ± 0.03[a]	0.22 ± 0.04[b]
16:0	20.64 ± 1.64	21.02 ± 0.67	20.57 ± 0.95	21.03 ± 1.31	20.6 ± 1.43
16:1(n-7)	4.64 ± 0.66[a]	4.88 ± 0.86[a b]	5.06 ± 0.67[a b]	4.83 ± 0.9[a b]	5.93 ± 0.81[b]
18:0	2.24 ± 1.04	2.05 ± 0.9	1.88 ± 0.56	1.88 ± 0.51	2.3 ± 0.49
18:1(n-9)	54.32 ± 2.18[a]	53.74 ± 0.85[a]	53.82 ± 2.01[a]	54.53 ± 1.21[a]	31.21 ± 1.17[b]
18:1(n-7)	2.51 ± 0.52[a]	2.78 ± 0.19[a b]	2.76 ± 0.5[a b]	2.43 ± 0.34[a]	3.29 ± 0.46[b]
18:2(n-6)	11.31 ± 0.77[a]	11.26 ± 0.82[a]	11.5 ± 1.9[a]	11.2 ± 1.28[a]	30.16 ± 2.48[b]
18:3(n-6)	0.06 ± 0.01[a]	0.06 ± 0.01[a]	0.06 ± 0.01[a]	0.06 ± 0.01[a]	0.1 ± 0.01[b]
18:3(n-3)	0.18 ± 0.02[a]	0.2 ± 0.03[a]	0.19 ± 0.05[a]	0.18 ± 0.04[a]	2.52 ± 0.19[b]
20:0	0.3 ± 0.06[a]	0.26 ± 0.03[a]	0.31 ± 0.06[a]	0.32 ± 0.04[a]	0.12 ± 0.02[b]
20:1(n-9)	0.56 ± 0.07[a]	0.54 ± 0.04[a]	0.58 ± 0.05[a]	0.56 ± 0.05[a]	0.2 ± 0.01[b]

(*Continued*)

Table 2. (Continued)

F: Visceral fat fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
20:2(n-6)	0.1 ± 0.02[a]	0.11 ± 0.02[a]	0.11 ± 0.02[a]	0.11 ± 0.03[a]	0.19 ± 0.02[b]
20:3(n-6)	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.07 ± 0.01[b]
20:4(n-6)	0.28 ± 0.15[ab]	0.2 ± 0.06[a]	0.18 ± 0.03[a]	0.17 ± 0.03[a]	0.39 ± 0.07[b]
20:3(n-3)	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.03 ± 0.01[b]
20:5(n-3)	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[b]	0.02 ± 0.0[c]
22:0	0.18 ± 0.03[a]	0.15 ± 0.02[a]	0.18 ± 0.04[a]	0.18 ± 0.03[a]	0.05 ± 0.01[b]
22:1(n-9)	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.01[b]
22:2(n-6)	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[b]
22:4(n-6)	0.02 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.06 ± 0.01[b]
22:5(n-6)	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
24:0	0.09 ± 0.02[ab]	0.07 ± 0.01[a]	0.1 ± 0.03[ab]	0.1 ± 0.02[b]	0.01 ± 0.01[c]
22:6(n-3)	0.19 ± 0.06[a]	0.19 ± 0.05[a]	0.18 ± 0.04[a]	0.01 ± 0.01[b]	0.09 ± 0.02[c]
24:1(n-9)	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.02 ± 0.01[b]
Total SFA	25.4 ± 2.1	25.6 ± 1.1	25.1 ± 1.3	25.5 ± 1.7	25.3 ± 1.9
Total MUFA	62.3 ± 1.9[a]	62.2 ± 1.5[a]	62.5 ± 2.0[a]	62.6 ± 1.3[a]	41.0 ± 2.2[b]
Total (n-6) FA	11.9 ± 0.8[a]	11.8 ± 0.9[a]	12.0 ± 1.9[a]	11.7 ± 1.4[a]	31.0 ± 2.5[b]
Total (n-3) FA	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.3 ± 0.1[b]	2.8 ± 0.3[c]
Total PUFA	12.3 ± 0.8[a]	12.2 ± 1.0[a]	12.4 ± 2.0[a]	11.9 ± 1.4[a]	33.7 ± 2.6[b]

Table G: Kidney fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
12:0	0.12 ± 0.05	0.12 ± 0.05	0.15 ± 0.06	0.14 ± 0.06	0.11 ± 0.04
14:0	0.9 ± 0.32	0.92 ± 0.33	1.11 ± 0.35	0.9 ± 0.35	0.82 ± 0.2
14:1(n-5)	0.09 ± 0.07	0.06 ± 0.02	0.1 ± 0.07	0.08 ± 0.03	0.07 ± 0.02
16:0	20.23 ± 1.19[ab]	21.04 ± 1.14[ab]	21.46 ± 1.1[a]	20.69 ± 1.57[ab]	19.94 ± 0.82[b]
16:1(n-7)	2.2 ± 0.85	2.41 ± 0.87	3.1 ± 1.08	2.39 ± 1.22	2.21 ± 0.7
18:0	12.65 ± 2.23[ab]	12.67 ± 2.26[ab]	11.25 ± 2.37[b]	13.13 ± 2.78[ab]	14.57 ± 1.42[a]
18:1(n-9)	23.73 ± 7.46[a]	21.88 ± 7.04[a]	26.19 ± 8.4[a]	22.41 ± 8.04[a]	12.09 ± 2.76[b]
18:1(n-7)	2.32 ± 0.29	2.43 ± 0.21	2.57 ± 0.23	2.39 ± 0.19	2.39 ± 0.24
18:2(n-6)	8.39 ± 0.57[a]	8.3 ± 0.63[a]	7.96 ± 0.89[ab]	7.14 ± 0.5[b]	12.44 ± 1.83[c]
18:3(n-6)	0.4 ± 0.2	0.55 ± 0.54	0.37 ± 0.27	0.53 ± 0.46	0.4 ± 0.31
18:3(n-3)	0.07 ± 0.03[a]	0.07 ± 0.03[a]	0.08 ± 0.02[a]	0.06 ± 0.01[a]	0.55 ± 0.23[b]
20:0	0.22 ± 0.07[a]	0.19 ± 0.05[a]	0.23 ± 0.07[a]	0.2 ± 0.05[a]	0.15 ± 0.01[b]
20:1(n-9)	0.31 ± 0.1[a]	0.28 ± 0.08[a]	0.32 ± 0.09[a]	0.27 ± 0.07[a]	0.12 ± 0.02[b]
20:2(n-6)	0.24 ± 0.05[abc]	0.23 ± 0.03[bc]	0.22 ± 0.05[c]	0.28 ± 0.07[ab]	0.29 ± 0.03[a]
20:3(n-6)	0.83 ± 0.3[ab]	0.86 ± 0.17[ab]	0.76 ± 0.27[b]	0.86 ± 0.25[ab]	1.09 ± 0.2[a]
20:4(n-6)	21.57 ± 5.7[ab]	22.06 ± 5.19[ab]	19.05 ± 6.89[a]	24.38 ± 6.89[ab]	27.29 ± 3.23[b]
20:3(n-3)	0.07 ± 0.03	0.07 ± 0.02	0.06 ± 0.03	0.08 ± 0.03	0.07 ± 0.02
20:5(n-3)	0.36 ± 0.13[ac]	0.43 ± 0.12[a]	0.26 ± 0.13[ac]	0.03 ± 0.02[b]	0.19 ± 0.05[c]
22:0	0.07 ± 0.04[abc]	0.05 ± 0.02[ac]	0.08 ± 0.04[ab]	0.08 ± 0.03[b]	0.04 ± 0.01[c]
22:1(n-9)	0.15 ± 0.12[a]	0.15 ± 0.15[ab]	0.18 ± 0.1[ab]	0.18 ± 0.13[ab]	0.29 ± 0.07[b]
22:2(n-6)	0.04 ± 0.02[ac]	0.05 ± 0.04[a]	0.03 ± 0.02[ac]	0.06 ± 0.02[b]	0.02 ± 0.01[c]
22:4(n-6)	0.34 ± 0.08[a]	0.33 ± 0.07[a]	0.33 ± 0.16[ab]	0.52 ± 0.12[bc]	0.51 ± 0.05[c]
22:5(n-6)	0.15 ± 0.05[a]	0.14 ± 0.03[a]	0.18 ± 0.16[a]	0.56 ± 0.19[b]	0.17 ± 0.06[a]
22:5(n-3)	0.13 ± 0.05[a]	0.14 ± 0.04[a]	0.11 ± 0.05[ab]	0.07 ± 0.02[b]	0.24 ± 0.04[c]
24:0	0.09 ± 0.04[a]	0.07 ± 0.03[ab]	0.08 ± 0.03[a]	0.08 ± 0.03[a]	0.05 ± 0.02[b]

(*Continued*)

Table 2. (Continued)

Table G: Kidney fatty acid composition.

Fatty acids	Groups[a)]				
	sn-1 DHA	*sn*-2 DHA	*sn*-3 DHA	Tristearin	Normal feed
22:6(n-3)	3.04 ± 0.74[ac]	3.09 ± 0.58[a]	2.45 ± 0.63[ac]	1.1 ± 0.38[b]	2.23 ± 0.41[c]
Total SFA	34.25 ± 1.7	35.04 ± 1.53	34.33 ± 1.39	35.2 ± 0.94	35.64 ± 1.3
Total MUFA	28.78 ± 8.27[a]	27.18 ± 7.94[a]	32.42 ± 9.41[ab]	27.7 ± 9.18[ab]	17.13 ± 3.55[b]
Total (n-6) FA	31.59 ± 5.87[a]	32.16 ± 5.8[a]	28.55 ± 7.41[a]	33.78 ± 7.63[ab]	41.67 ± 2.55[b]
Total (n-3) FA	3.98 ± 0.93[a]	4.1 ± 0.76[a]	3.27 ± 0.83[a]	1.84 ± 0.53[b]	3.77 ± 0.33[a]
Total PUFA	35.57 ± 6.7[a]	36.26 ± 6.54[a]	31.81 ± 7.86[a]	35.61 ± 8.14[a]	45.43 ± 2.78[b]

[a)] During the 5-day intervention period, three groups received structured triacylglycerols (360 mg per day for each rat) with docosahexaenoic acid (DHA) either at *sn*-1, *sn*-2, or *sn*-3 positions and two stearic acid residues in the remaining positions (*sn*-1 DHA, *sn*-2 DHA, *sn*-3 DHA groups), and the tristearin group received tristearin (360 mg per day for each rat), in addition to the ad libitum omega-3 FA deficient diet. The normal feed group received soybean oil based standard feed from the beginning until the end of the animal trial. Values are mean ± SD. $n = 12$ in each group for liver TAG, liver PL, brain, visceral fat, and eye. For rat testicle: $n = 11$ in *sn*-1 DHA group. For rat kidney: $n = 10$ in *sn*-1 DHA group, $n = 11$ in *sn*-2 DHA group, $n = 11$ in *sn*-3 DHA group and $n = 10$ in tristearin group. Values with different superscript letters in the same row indicate that the means are significantly different ($p < 0.05$). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TAG: triacyglycerol, PL: phospholipid.

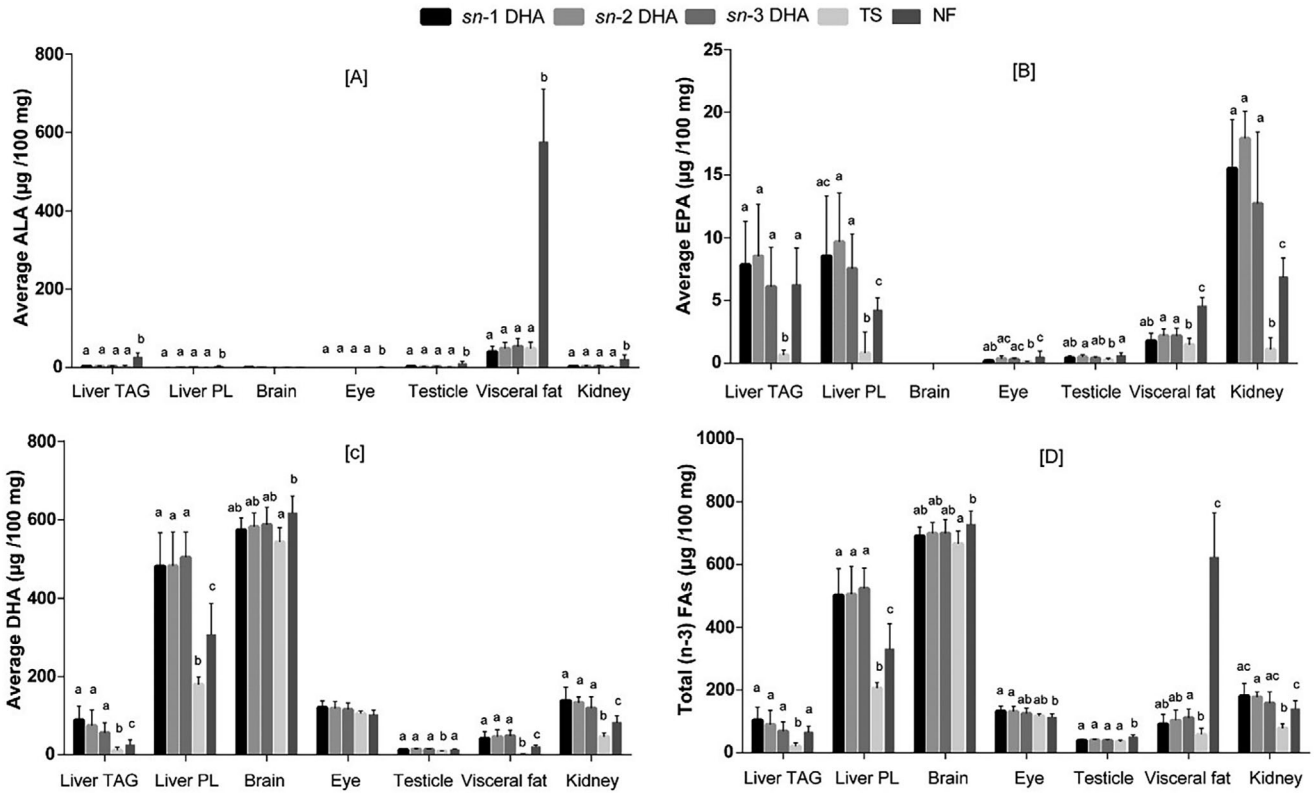

Figure 1. Average absolute content as µg per 100 mg (±SD) of extractable (A) ALA, (B) EPA, (C) DHA, and (D) total (n-3) FA in *sn*-1 DHA, *sn*-2 DHA, *sn*-3 DHA, tristearin, and normal feed groups of rat organs [$n = 12$ for liver TAG, liver PL, brain, visceral fat, and eye; for testicle: $n = 11$ in *sn*-1 DHA; for kidney: $n = 10$ in *sn*-1 DHA group, $n = 11$ in *sn*-2 DHA group, $n = 11$ in *sn*-3 DHA group and $n = 10$ in tristearin group] after 5-day intervention phase. The *sn*-1 DHA, *sn*-2 DHA, *sn*-3 DHA groups received structured triacylglycerols with DHA in the indicated *sn*-position and two stearic acid residues in the other positions (360 mg per day for each rat), and the tristearin group received tristearin (360 mg per day for each rat) for the 5-day intervention period, in addition to the ad libitum omega-3 fatty acid deficient diet. During the 4-week induction period and the 5-day intervention period, the normal feed group received soybean oil based normal feed. Bars with different letters differ from one another within each organ for the fatty acid. ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid, TAG: triacyglycerol, PL: phospholipid.

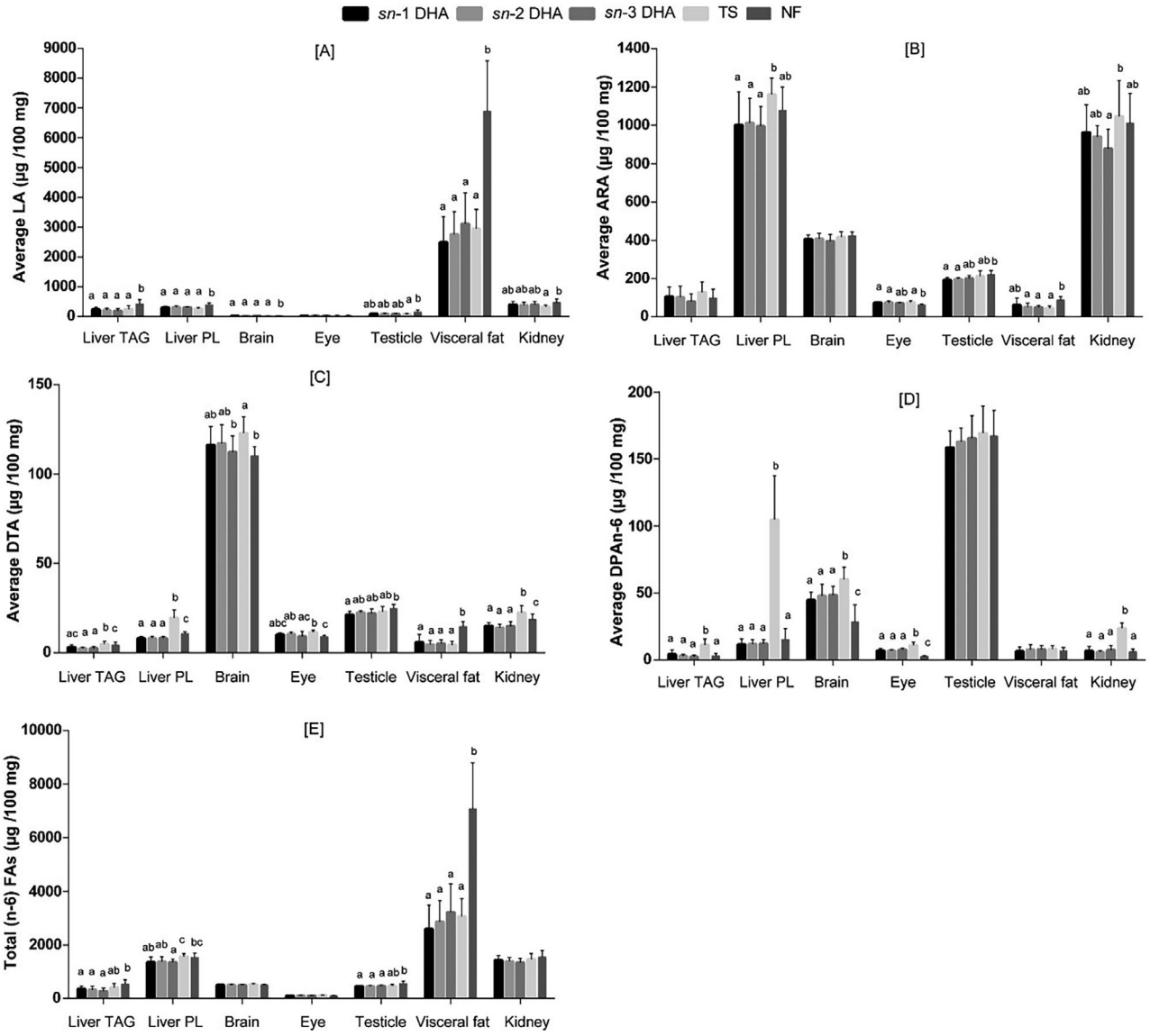

Figure 2. Average absolute content as µg per 100 mg (±SD) of extractable A) LA, B) ARA, C) DTA, D) DPAn-6, and E) total (n-6) FA in *sn*-1 DHA, *sn*-2 DHA, *sn*-3 DHA, tristearin, and normal feed groups of rat organs [$n = 12$ for liver TAG, liver PL, brain, visceral fat, and eye; for testicle: $n = 11$ in *sn*-1 DHA; for kidney: $n = 10$ in *sn*-1 DHA group, $n = 11$ in *sn*-2 DHA group, $n = 11$ in *sn*-3 DHA group and $n = 10$ in tristearin group] after five-day intervention phase. The *sn*-1 DHA, *sn*-2 DHA, *sn*-3 DHA groups received structured triacylglycerols with DHA in the indicated *sn*-position and two stearic acid residues in the other positions (360 mg per day for each rat), and the tristearin group received tristearin (360 mg per day for each rat) for the 5-day intervention period, in addition to the ad libitum omega-3 fatty acid deficient diet. During the 4-week induction period and the 5-day intervention period, the normal feed group received soybean oil based normal feed. Bars with different letters differ from one another within each organ for the fatty acid. LA, linoleic acid; ARA, arachidonic acid; DTA, docosatetraenoic acid; DPAn-6, (n-6) docosapentaenoic acid, TAG: triacylglycerol, PL: phospholipid.

LA, ARA, DTA, DPAn-6, and total (n-6) FA of brain and eye is presented in Figures 1 and 2 respectively. In the rat brain and eye FA composition, a different pattern was observed for DHA accumulation among all the intervention groups than in the liver (Tables 2A,B). The DHA percentage in the brain and eye of the DHA-fed groups was equal to those in the NF group. Neither were differences found in the DHA level among DHA-fed groups ($p > 0.05$). The TS group had a significantly lower DHA level compared to the NF group in the rat brain ($p < 0.001$), whereas the difference in DHA level in the eye between the TS group and other groups did not reach statistical significance (Table 2C and D). The average content of DHA in the brain and eye ranged from (542–617 µg per 100 mg) and (102–120 µg per 100 mg), respectively. The statistical comparison of the tissue content of DHA in the rat brain and eye showed similar differences (Figure 1C) as found in the comparison of the relative percentage of DHA in these tissues (Table 2C and D).

The FA composition of basic feed was reflected in the fatty acid composition of the brain and eyes of rats in different groups. The percentage of LA was found to be higher in the NF group compared to other groups ($p < 0.05$ for the brain; significantly different from *sn*-1 DHA, *sn*-3 DHA, and TS groups in the eye). In

contrast, there were no differences found in the eye LA among the experimental groups when the average absolute content of LA (22–32 µg per 100 mg) was compared (Figure 2A). The relative percentage of ARA in the eye was found to be higher in the TS group compared to the *sn*-3 DHA group ($p < 0.05$) (Table 2D) whereas, the brain ARA percentage of the NF group was almost equal to the DHA-fed groups ($p > 0.05$) (Table 2C). There were no differences in the average absolute content of brain ARA (395–423 µg per 100 mg) among all experimental groups, and the absolute content of ARA in the TS group (75 µg per 100 mg) of the eye was significantly higher compared to the NF group (63 µg per 100 mg) (Figure 2B). The relative percentage of DPAn-6 in the TS group in the brain and eye was found to be the highest among the groups, followed by DHA-fed groups, and the lowest in the NF group (TS group significantly different from *sn*-1 DHA, *sn*-3 DHA, and NF groups in the brain, $p < 0.05$ in the eye). Whereas, the absolute extractable content of DPAn-6 of the TS group in the brain was significantly higher compared to all experimental groups (Figure 2D). The total (n-6) content in the brain was found to be unaffected by the composition of fatty acids in the feed (Table 2C, Figure 2E). The NF group and the TS group had a higher total (n-6) FA percentage in the eye compared to other intervention groups ($p < 0.05$; only significantly different from the *sn*-3 DHA group) (Table 2D). In contrast, there was no difference found among all intervention groups when the absolute extractable amount of total (n-6) FA of the eye was compared (Figure 2E).

In the eye, the ALA percentage and average absolute content were significantly higher in the NF group than in other intervention groups ($p < 0.05$, Table 2D and Figure 1A). The relative percentage and average absolute EPA level in the eye was the highest in the NF group, and the lowest in the TS group ($p < 0.05$) (Table 2D, Figure 1B).

Furthermore, the relative percentage and average absolute total (n-3) PUFA (Table 2C, Figure 1D) and the relative percentage of total PUFA content (Table 2C) in the brain was significantly higher in the NF group than in the TS group ($p < 0.05$), but the levels did not differ among the groups fed with a diet based on peanut oil (TS group and all the DHA-fed groups). In the eye, the relative percentage of the total (n-3) content was found to be unaffected by the feed composition, whereas the total level of PUFAs in the NF group was higher than in the *sn*-3 DHA and the TS groups ($p < 0.05$) (Table 2D). The average tissue content of total (n-3) FA levels of *sn*-1 DHA and *sn*-2 DHA groups in the eye were higher compared to the NF group ($p < 0.05$) (Figure 1D).

3.4. Fatty Acid Composition of Testicle, Visceral Fat, and Kidney

The relative abundance of the fatty acids in the testicle, visceral fat, and kidney are shown in Table 2E–G. The average tissue content (µg per 100 mg) of extractable ALA, EPA, DHA, and total (n-3) FA, and LA, ARA, DTA, DPAn-6, and total (n-6) FA of testicle, visceral fat, and kidney is presented in Figures 1 and 2 respectively. In general, likely due to the lipid extraction method applied in the experiment, the individual fatty acid content of the visceral fat is lower than the typically expected. TS group had a significantly lower relative percentage and average tissue DHA content as compared to the NF ($p < 0.001$) as well as to the DHA-fed groups ($p < 0.05$) in the testicle, visceral fat, and kidney (Table 2E–G, Figure 1C). The percentage of DHA in visceral fat of DHA-fed groups was significantly higher compared to that in the NF group ($p < 0.001$), whereas the DHA level in the testicle and the kidney did not differ between the NF group and DHA-fed groups (Table 2E–G). The DHA content in the DHA-fed groups was significantly higher than in the NF group of visceral fat and kidney, whereas the absolute DHA content of the DHA-fed groups and the NF group was equal in the testicle (Figure 1C).

The high content of ALA in soybean oil was reflected in the higher content of this (n-3) PUFA in the organs of the NF group compared to the other groups (Table 2E–G, Figure 1A). The relative percentages of EPA in the testicle, visceral fat, and kidney in the TS group were also lower than the levels in all other groups The EPA level of the kidney in the *sn*-2 DHA group was the highest among all intervention groups, being 2-fold higher as compared to the NF group ($p < 0.05$) (Table 2G, Figure 1B). There were no statistical differences in the level of DHA, EPA, or other (n-3) PUFAs in the testicle, visceral fat, or kidney between the DHA-fed groups ($p > 0.05$) (Table 2E–G, Figure 1A–C).

LA was found to be higher in the testicle, visceral fat, and kidney of the NF group as compared to the other groups ($p < 0.05$), again reflecting the difference between the FA composition of the soybean oil in the feed of the NF group and the peanut oil used in the (n-3) deficient feed for the other groups (Table 2E–G). The absolute LA content in the visceral fat of the NF group was higher as compared to the other groups, and almost equal absolute LA levels were found in the testicle and kidney of the DHA-fed groups and the NF group (Figure 2A). ARA is an abundant (n-6) PUFA in the kidney (19–27%) and the testicle (13–15%). The NF group had a higher percentage of ARA in the kidney as compared to other groups (Table 2G). The average absolute ARA content in the testicle of the NF group was significantly higher as compared to the *sn*-1 DHA and *sn*-2 DHA groups.

The testicle showed the highest DPAn-6 content (around 11% of total FA) among all organs in all the groups (Table 2E, Figure 2D) without significant differences among the groups. In contrast, the level of DPAn-6 is very low in the kidney, although a statistically significant difference was found when comparing the level in the kidney of the TS group with those of the other groups ($p < 0.05$) (Table 2E, Figure 2D). The NF group had more DTA in the visceral fat and kidney ($p < 0.05$) compared to DHA-fed groups whereas, the DTA level in the NF group of the testicle differed statistically only from the *sn*-1 DHA group (Table 2E–G, Figure 2C). On the contrary, the absolute ARA content in the kidney of the TS group was higher as compared to the NF group ($p < 0.05$).

The three DHA-fed groups did not differ statistically in the total (n-3), total (n-6), or total PUFA levels in the testicle, visceral fat, or kidney.

4. Discussion

Studies on enzyme specificities have previously shown differences in the efficiency of lingual, gastric, and pancreatic enzymes towards both primary positions and the type of fatty acids in TAG molecules during the digestion of lipids.[9,10,12,31,32] Better lymphatic absorption of EPA and DHA from the *sn*-2 position was observed in salmon oil as compared to seal oil, the latter

having DHA mostly at the *sn*-1/*sn*-3 positions of TAGs.[33] Differences in the intramolecular distribution of EPA and DHA in fish and seal oil are suggested to have a different effect on lipid metabolism.[34,35] These pieces of evidence suggest that the lipase specificities could lead to differences in the bioavailability of DHA located in different positions of TAGs. However, the current knowledge on the differences between the accumulation of DHA or other (n-3) PUFAs from *sn*-1, *sn*-2, and *sn*-3 positions is limited.

The present study was conducted in mildly (n-3) deficient SD rats. Before the intervention feeding with the DHA in structured TAGs, the rats were kept on a (n-3) deficient diet for four weeks, which reduced the levels of DHA in various organs and tissues, with the DHA level of the visceral fat being the most affected and the brain DHA the least affected.[36] In the 5-day dietary intervention feeding, the mildly (n-3) deficient rats were fed with *sn*-22:6(n-3)-18:0-18:0, *sn*-18:0-18:0-22:6(n-3), or *sn*-18:0-22:6(n-3)-18:0. The dose of intervention for the present study was comparable to the dosage used in previous studies.[37,38] We measured the DHA accumulation based on the relative proportion and absolute content of this fatty acid in different tissues. In addition to the DHA levels, this study systematically investigated the accumulation of different fatty acids, especially other PUFAs of (n-3) and (n-6) families.

Overall, the intervention feeding with DHA increased the DHA levels in the liver, testicle, visceral fat, and kidney compared to feeding with an equal dosage of tristearin. In previous research, efficient incorporation of DHA into the liver and brain[15] and also in brown adipose tissue[16] of hamsters was observed after a 12-week intervention with structured TAG containing DHA at the *sn*-2 position. Seven days of feeding with a daily dose of 50 mg of pure DHA increased tissue DHA levels in the brain, adipose, skeletal muscle, and liver and EPA levels in the liver in rats.[38] Williams et al. showed that even only 3 days of DHA intervention affected the LC (n-3) PUFA composition of the liver in (n-3) deficient rats.[39] The current study for the first time showed that a short-term feeding was sufficient to replenish the DHA pool of a wide range of organs in mildly (n-3) deficient rats.

The liver is the central organ in lipid metabolism, and the fatty acid composition of the liver is more influenced by dietary fatty acids than that of the brain.[40,41] In our study, the supplementation of DHA resulted in a significant increase in the level of DHA and (n-3) PUFA in the liver. One important aim of the present study was to compare the efficiency of DHA accumulation from different regio- and stereo-specific positions of dietary TAGs. Previous research has shown contradictory results on the influence of the positional distribution of DHA on its bioavailability and accumulation in tissues and organs. While some studies indicated higher tissue accumulation of DHA from the *sn*-2 position of TAG,[23–25] Christensen & Høy reported no significant differences in the liver DHA level in newborn rats after a 3-week intervention with 3.8% DHA of the total fatty acids either as a randomized TAG with DHA equally distributed at *sn*-1, 2, and 3 positions or as a structured TAG with DHA at *sn*-2 position.[18] In our study, we did not find a statistically significant difference in DHA level in the PL fraction of the liver among the *sn*-1 DHA, *sn*-2 DHA, and *sn*-3 DHA groups, which is in line with the findings of Christensen ans Høy considering the dominance of PLs in the lipid fraction of the liver.

Interestingly, the DHA level in liver TAG of the *sn*-1 DHA group (4.8% of total FA) was higher compared to the level of the *sn*-3 DHA group (3.5% of total FA), although the difference was not statistically significant in the multivariate analysis. A similar difference was found in a recent study, where a 4-week feeding of structured TAG containing DHA at the *sn*-1 position resulted in higher DHA content in liver TAG compared to feeding with *sn*-3 DHA.[21] Therefore, these two studies, despite the different periods of intervention, consistently indicated different efficiency between dietary *sn*-1 and *sn*-3 DHA in the accumulation in liver TAG.

The *sn*-position of DHA in the TAG molecule did not influence the accumulation of DHA in the brain or the eyes of the rats. Previous studies have shown an increased level of DHA in the brain, erythrocytes, and liver[15] and the brown adipose tissue[16] from a structured TAG with DHA at the *sn*-2 position as compared to linseed oil, fish oil, fish oil ethyl esters in 10-week old hamsters. The research of Fujimoto et al. showed more efficient absorption of DHA in lymph from the *sn*-2 position than from the *sn*-3 position.[17] Despite the previous evidence of higher bioavailability of *sn*-2 DHA compared to *sn*-1/3 DHA, our results did not confirm the higher tissue accumulation of DHA from the *sn*-2 position as compared to the *sn*-1 or *sn*-3 positions. A lower fecal loss of DHA was shown from the *sn*-2 position compared to the primary positions (*sn*-1 or *sn*-3) of TAGs.[22] However, this was not reflected in the DHA levels in the organ tissues in the current study. The absorption rate of DHA from all three *sn*-positions of TAG was high based on the low fecal content of DHA,[22] which likely explains the equal accumulation of DHA in the organs among the three DHA groups regardless of the *sn*-position of DHA in the TAG molecules. We have previously measured rat plasma TAG and PL levels among DHA-fed groups and control groups.[22] The differences in the DHA levels of the plasma TAG fraction among the intervention groups were reflected in the DHA level of the liver TAG, liver PL fractions, and visceral fat in the present study, but not in the brain, eye, kidney, or testicle. This was in line with the previous finding of a strong correlation between the levels of fatty acids, particularly LC (n-3) PUFAs, of plasma and erythrocytes PLs with the fatty acid profiles of the liver, kidney, heart, and quadriceps tissue, but not with that of the brain, after dietary intervention with different concentrations of ALA.[42]

Although the brain DHA level in the DHA-fed groups was slightly higher compared to the level in the TS group, the difference was not statistically significant. This is in agreement with the previous findings that the brain DHA level is well-buffered against the variation in (n-3) PUFA levels in the diet.[43,44] The DHA level was about 1%, in the testis of the SD rats of the DHA-fed groups and the NF group. While the DHA level in testicles of the group fed with n-3 deficient diet was similar to the level reported in earlier,[45] the levels in the groups fed with n-3 adequate feed and DHA in structured TAGs were lower than the levels reported for SD rats supplemented with fish oil and ALA-rich oil.[45] The difference could have been attributed to the difference in length of intervention feeding and lipid extraction method applied.

The brain is largely dependent on the constant circulation of DHA to replace metabolized DHA of the brain. In addition to plasma pools, DHA is thought to be provided by the DHA reserve in adipose tissue.[46] In our study, the DHA levels in visceral

fat are slightly higher in groups fed with DHA compared to tristearin and normal feed groups. This indicates that the dose and duration of DHA intervention were sufficient to maintain brain DHA levels as well as to increase the DHA levels in the visceral fat. Previously, we reported a higher DHA level in visceral fat after 4 weeks of feeding with structured TAG containing *sn*-3 DHA compared to feeding with TAGs containing *sn*-1 or *sn*-2 DHA. However, in the current study the three DHA groups did not differ in the DHA levels of visceral fat. The difference in the length of intervention feeding may have caused the different findings in these two studies.

The DHA level in the liver PL fraction was increased in DHA-fed groups compared to the TS group and the NF group, but this was accompanied by a decrease in the ARA and DPAn-6 levels. This observation supports the previous findings that LC (n-3) PUFAs have an inhibitory effect on Δ6- and Δ5 desaturases, which are rate-limiting enzymes in the biosynthesis of ARA from LA.[47,48] On the other hand, the higher ARA level in liver PL of the TS group compared to the levels in the DHA-fed group and the NF group was likely a compensative mechanism to maintain the fluidity of the cell membrane.[49] A similar trend was seen in the liver TAGs, brain, eye, testicle, and kidney, even though statistically significant differences were not reached. It should be noted that the highest amount of DPA n-6 was found in the rat testicle, indicating the possible local metabolism of LA in this organ. Similar observations were found by Lin and Salem while studying the uptake and metabolism of deuterated ALA and LA in rats.[50]

DHA levels in organs such as the brain and retina are well buffered against dietary deficiency in (n-3) PUFAs. It takes at least 2-generation of (n-3) deficient feeding to induce significant depletion of DHA levels in these organs and tissues. It has been reported that the full recovery of the DHA levels in the brain of F2 generation (n-3) deficient rats to the normal level took up to 8 weeks after initiation of the (n-3) PUFA repletion diet.[51] In our current study, the 4-week (n-3) deficient feeding induced very mild (n-3) deficiency. Therefore, it is indeed difficult to determine a specific recovery phase clearly differentiating from the saturation state, although the 5-day DHA feeding is a much shorter period compared to the 8-week repletion feeding required to restore the brain DHA levels in F2 generation (n-3) deficient rats. Our findings showed that the dose and the short feeding period of DHA used in this study were sufficient to restore the DHA content in the organs of the (n-3) deficient rats to the same or even higher levels as found in the NF group. In visceral fat, the DHA storage level was increased by 2-fold in DHA-fed groups compared to the NF group. In this regard, the findings obtained with mildly (n-3) deficient rats in this study may to a large extent reflect the impact of DHA feeding on the fatty acid composition and accumulation in tissues of rats in (n-3) normal state.

This study, together with our previous study reporting the absorption and fecal excretion of DHA from stereospecifically structured TAGs,[22] and results of a longer DHA feeding trial with enantio- and regio-specifically structured TAGs in rats without (n-3) deficiency,[21] provides a comprehensive and systematic view of the absorption, excretion, as well as accumulation of DHA in different organs from regio- and stereo-specifically structured TAGs. These findings formed new insights on the influence of the positional distribution of DHA in dietary TAGs on the bioavailability of this important (n-3) PUFA, which significantly strengthens the current understanding of lipid biochemistry and structure-function relationships of dietary lipids.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors acknowledge Leena Vuori, Minttu Matturi, Kang Chen, and Yihe Wang for technical assistance in the lipid analysis. The research was funded by the Research Council of Finland in the project "Chiral lipids in chiral nature: a novel strategy for regio- and stereospecific research of human milk and omega-3 lipids" (Decision No. 310982) and the project "Structures and functions of chiral lipids: A stereospecific & multi-omics approach (Decision No. 356891)", the Graduate School of the University of Turku, the Raisio plc Research Foundation, Finland, the Finnish Food Research Foundation, Orion Research Foundation, Turku University Foundation, the Finland–China Food and Health Network, and theNational Natural Science Foundation of China (Decision No. 81602845).

Conflict of Interest

All authors declare that they have no conflict of interest.

Author Contributions

Conceptualization, B.Y., K.M.L., G.G.H., Y.Z.; methodology, B.Y., A.K., K.M.L., Y.Z., A.Z., G.G.H.; formal analysis A.K.; investigation, A.K., B.Y., K.M.L.; resources, B.Y., K.M.L., Y.Z.,G.G,H.; writing—original draft preparation, BY, AK; writing—review and editing, B.Y., A.K., K.M.L., G.G.H., Y.Z., A.Z.; visualization A.K., B.Y., K.M.L.; supervision, B.Y., K.M.L., Y.Z., G.G.H.; funding acquisition B.Y., A.K., K.M.L., A.Z., G.G.H. All authors have read and approved the submitted manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

absorption, docosahexaenoic acid, (n-3) PUFAs, enantiospecificity, regiospecificity, structured TAG

Received: September 6, 2023
Revised: January 16, 2024
Published online:

[1] J. T. Brenna, N. Salem, A. J. Sinclair, S. C. Cunnane, International Society for the Study of Fatty Acids and Lipids, ISSFAL, *Prostaglandins Leukot. Essent. Fatty Acids* **2009**, *80*, 85.
[2] G. C. Burdge, A. E. Jones, S. A. Wootton, *Br. J. Nutr.* **2002**, *88*, 355.
[3] A. M. Stinson, R. D. Wiegand, R. E. Anderson, *J. Lipid Res.* **1991**, *32*, 2009.

[4] M. J. Weiser, C. M. Butt, M. H. Mohajeri, *Nutrients* **2016**, *8*, 99.
[5] N. G. Bazan, *J. Lipid Res.* **2003**, *44*, 2221.
[6] B. L. Scott, N. G. Bazan, *Proc. Natl. Acad. Sci.* **1989**, *86*, 2903.
[7] D. Li, M. L. Wahlqvist, A. J. Sinclair, *Asia Pac. J. Clin. Nutr.* **2019**, *28*, 1.
[8] F. Shahidi, P. Ambigaipalan, *Annu. Rev. Food Sci. Technol.* **2018**, *9*, 345.
[9] F. Paltauf, F. Esfandi, A. Holasek, *FEBS Lett.* **1974**, *40*, 119.
[10] S. J. Iverson, J. Sampugna, O. T. Oftedal, *Lipids* **1992**, *27*, 870.
[11] R. G. Jensen, F. A. Dejong, R. M. Clark, L. G. Palmgren, T. H. Liao, M. Hamosh, *Lipids* **1982**, *17*, 570.
[12] F. Carrière, E. Rogalska, C. Cudrey, F. Ferrato, R. Laugier, R. Verger, *Bioorg. Med. Chem.* **1997**, *5*, 429.
[13] L. Amate, M. Ramírez, A. Gil, *Lipids* **1999**, *34*, 865.
[14] K. C. Hayes, *Curr. Opin. Lipidol.* **2001**, *12*, 55.
[15] N. M. Bandarra, P. A. Lopes, S. V. Martins, J. Ferreira, C. M. Alfaia, E. A. Rolo, J. J. Correia, R. M. A. Pinto, R. P. Ramos-Bueno, I. Batista, J. A. M. Prates, J. L. Guil-Guerrero, *Nutr. Res.* **2016**, *36*, 452.
[16] P. A. Lopes, N. M. Bandarra, S. V. Martins, M. S. Madeira, J. Ferreira, J. L. Guil-Guerrero, J. A. M. Prates, *Int. J. Food Sci. Nutr.* **2017**, 458.
[17] K. Fujimoto, K. Ito, Y. Endo, F. Hirahara, C. Shiina, S. Nakayama, *Food Sci. Technol. Int. Tokyo* **1998**, *4*, 140.
[18] M. M. Christensen, C. E. Høy, *Lipids* **1997**, *32*, 185.
[19] H. G. Gudmundsson, K. M. Linderborg, H. Kallio, B. Yang, G. G. Haraldsson, *Tetrahedron* **2020**, *76*, 130813.
[20] M. Kalpio, J. D. Magnússon, H. G. Gudmundsson, K. M. Linderborg, H. Kallio, G. G. Haraldsson, B. Yang, *Chem. Phys. Lipids* **2020**, *231*, 104937.
[21] Y. Zhang, M. Kalpio, L. Tao, G. G. Haraldsson, H. G. Guðmundsson, X. Fang, K. M. Linderborg, Y. Zhang, B. Yang, *Food Res. Int.* **2023**, *174*, 113626.
[22] K. M. Linderborg, A. Kulkarni, A. Zhao, J. Zhang, H. Kallio, J. D. Magnusson, G. G. Haraldsson, Y. Zhang, B. Yang, *Food Chem.* **2019**, *283*, 381.
[23] A. Halldorsson, C. D. Magnusson, G. G. Haraldsson, *Tetrahedron* **2003**, *59*, 9101.
[24] G. G. Haraldsson, A. Halldorsson, E. Kulås, *J. Amer. Oil Chem. Soc.* **2000**, *77*, 1139.
[25] J. D. Magnusson, University of Iceland, Reykjavik, **2018**.
[26] C. D. Magnusson, G. G. Haraldsson, *Tetrahedron* **2010**, *66*, 2728.
[27] W. W. Christie, in *Gas Chromatography and Lipids* (W. W. Christie, Ed.), The Oily Press Ltd, Glasgow, **1989**, 11.
[28] J. Folch, M. Lees, G. H. Sloane Stanley, *J. Biol. Chem.* **1957**, *226*, 497.
[29] W. W. Christie, *Lipid Analysis. Isolation, Separation, Identification and Structural Analysis of Lipids*, The Oily Press, PJ Barnes And Associates, Bridgwater **2003**.
[30] W. W. Christie, *J. Lipid Res.* **1982**, *23*, 1072.
[31] R. Duan, in *Fat Digestion and Absorption*, AOAC Press, Rockville, Maryland **2000**.
[32] M. Hamosh, in *Lipases* (Eds: B. Borgström, H. L. Brockman), Elsevier, Amsterdam, Netherlands **1984**, 49.
[33] M. S. Christensen, C. E. Høy, T. G. Redgrave, *Biochim. Biophys. Acta* **1994**, *1215*, 198.
[34] H. Yoshida, J. Kumamaru, M. Mawatari, I. Ikeda, K. Imaizumi, H. Tsuji, A. Seto, *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1293.
[35] H. Yoshida, M. Mawatari, I. Ikeda, K. Imaizumi, A. Seto, H. Tsuji, *J. Nutr. Sci. Vitaminol.* **1999**, *45*, 411.
[36] A. Kulkarni, A. Zhao, B. Yang, Y. Zhang, K. M. Linderborg, *Foods* **2022**, *11*, 208.
[37] S. Ghasemifard, G. M. Turchini, A. J. Sinclair, *Prog Lipid Res* **2014**, *56*, 92.
[38] G. Kaur, D. P. Begg, D. Barr, M. Garg, D. Cameron-Smith, A. J. Sinclair, *Br. J. Nutr.* **2010**, *103*, 32.
[39] M. A. Williams, J. Tinoco, Y. T. Yang, M. I. Bird, I. Hincenbergs, *Lipids* **1989**, *24*, 753.
[40] J. M. Bourre, M. Bonneil, O. Dumont, M. Piciotti, R. Calaf, H. Portugal, G. Nalbone, H. Lafont, *Biochim. Biophys. Acta* **1990**, *1043*, 149.
[41] X. Tang, Z.-J. Li, J. Xu, Y. Xue, J.-Z. Li, J.-F. Wang, T. Yanagita, C.-H. Xue, Y.-M. Wang, *Lipids Health Dis.* **2012**, *11*, 70.
[42] W. C. Tu, B. S. Mühlhäusler, L. N. Yelland, R. A. Gibson, *Prostaglandins Leukot Essent. Fatty Acids* **2013**, *88*, 53.
[43] L. Abedin, E. L. Lien, A. J. Vingrys, A. J. Sinclair, *Lipids* **1999**, *34*, 475.
[44] S. I. Rapoport, M. Igarashi, *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2009**, *81*, 119.
[45] P. S. Chanmugam, M. D. Boudreau, D. H. Hwang, *J. Nutr.* **1991**, *121*, 1173.
[46] R. P. Bazinet, S. Layé, *Nat. Rev. Neurosci.* **2014**, *15*, 771.
[47] H. P. Cho, M. Nakamura, S. D. Clarke, *J. Biol. Chem.* **1999**, *274*, 37335.
[48] M. L. Garg, E. Sebokova, A. B. Thomson, M. T. Clandinin, *Biochem. J.* **1988**, *249*, 351.
[49] H. Tallima, R. El Ridi, *J. Adv. Res.* **2017**, *11*, 33.
[50] Y. H. Lin, N. Salem, *J. Lipid Res.* **2007**, *48*, 2709.
[51] T. Moriguchi, J. Loewke, M. Garrison, J. N. Catalan, N. Salem, *J. Lipid Res.* **2001**, *42*, 419.