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Alternative splicing generates novel *Fads3* transcript in mice

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Abstract

Fads3 is the third member of the fatty acid desaturase gene cluster; with at least eight evolutionarily conserved alternative transcripts (AT), having no clearly established function as are known for *FADS2* and *FADS1*. Here we present identification of a novel *Fads3* transcript in mice (*Fads3AT9*), characterize *Fads3AT9* expression in mouse tissues and evaluate correlations with metabolite profiles. Total RNA obtained from mouse tissues is reverse-transcribed into cDNA and used as template for PCR reactions. Tissue fatty acids were extracted and quantified by gas chromatography. Sequencing analysis revealed complete absence of exon 2 resulting in an open reading frame of 1239 bp, encoding a putative protein of 412 aa with loss of 37 aa compared to classical *Fads3* (*Fads3CS*). *FADS3AT9* retains all the conserved regions characteristic of front end desaturase (cytochrome b5 domain and three histidine repeats). Both *Fads3CS* and *Fads3AT9* are ubiquitously expressed in 11 mouse tissues. *Fads3AT9* abundance was greater than *Fads3CS* in pancreas, liver, spleen, brown adipose tissue and thymus. *Fads3CS* expression is low in pancreas while *Fads3AT9* is over ten-fold greater abundance. The eicosanoid precursor fatty acid 20:4n - 6, the immediate desaturation product of the *Fads1* coded 5-desaturase, was highest in pancreas where *Fads3CS* is low. Changes in expression patterns and fatty acid profiles suggest that *Fads3AT9* may play a role in the regulation and/or biosynthesis of long chain polyunsaturated fatty acids from precursors.

Keywords

Fatty acid desaturase; Alternative transcript; Alternative splicing; Long chain polyunsaturated fatty acids

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Introduction

Polyunsaturated fatty acids (PUFA) are ubiquitous in mammalian tissue. Long chain PUFA (LCPUFA) are bioactive components of membrane phospholipids and serve as substrates for signaling molecules [1]. They can be obtained from the diet or are endogenously synthesized in the liver from dietary PUFA precursors, alpha-linolenic acid (ALA, 18:3n - 3) and linoleic acid (LA, 18:2n - 6) by alternating series of position-specific desaturation and carbon chain-elongation reactions [2]. The nonheme, iron-containing, oxygen-dependent fatty acid desaturases catalyze introduction of double bonds at specific positions within a fatty acid chain. *FADS1* (OMIM#606148), *FADS2* (OMIM#606149) and *FADS3* (OMIM#606150) are located as a cluster within 100 kb region on the long arm of HSA11q12–13.1 locus, while a similar organization is found on mouse chromosome 19 [3, 4]. All three fatty acid desaturase (FADS) genes have evolved by gene duplication events, share 12 exons and 11 introns, and contain a well conserved cytochrome b5 domain and three histidine repeats [4].

The *FADS1* and *FADS2* genes encode classical transcripts (CS) for the 5-desaturase and 4-/ 6-/ 8-desaturase, respectively [2, 5]. Direct evidence for the biochemical function of *FADS3* gene product is enigmatic. The first *Fads3* null mouse is fertile despite its high expression at the implantation site in the mouse uterus [6] and has no overt phenotype, though the biochemical phenotype suggests a role in docosahexaenoic acid (DHA, 22:6n - 3) synthesis [7]. An early in vitro report suggests a unique role as a back end desaturase for *trans*-11 vaccenic acid [8]. Genetic studies have shown associations with familial combined hyperlipidemia in a Mexican population [9] and a minor allele of *FADS3* SNP (rs174455) was found to be negatively associated with DHA in red blood cell phospholipids in the Alspac cohort [10].

We established that primate *FADS3* gives rise to at least eight alternative transcripts that are highly expressed in a tissue-specific manner and are phylogenetically conserved [11]. Others have reported detection of FADS3 proteins [12]. We have shown with mouse embryonic fibroblast (MEF) cells and ribosome foot-printing technology the first positive-sequence-specific-proof of FADS3 translation [5]. Additionally, both *FADS1* and *FADS2* are also alternatively spliced, and we have established a function for a *FADS1 AT* in *FADS2* mediated desaturation [5, 13]. In this study, we report a transcript variant of *Fads3* in adult mouse liver generated by alternative splicing, evidence of its wide expression in 11 mouse tissues. We also report fatty acid profiles emphasizing the FADS target metabolites from 11 different tissues from n = 4 mice.

Materials and methods

Animals

Studies on mice were approved by Cornell University Institutional Animal Care and Use Committee (IACUC, protocol # 2011-0007). C57BL/6 mice were maintained on rodent laboratory diet (7012 Teklad LM-485, Harlan Laboratories, WI) and were fed ad libitum. High quality adult mouse tissues, treated with RNAlater, and maintained at -80 °C, were used to isolate total RNA. We used four mice (2 males and 2 females) average 1 year old at the time of sacrifice.

RNA isolation and cDNA synthesis

Total RNA was isolated from adult mouse tissues using the E.Z.N.A. Total RNA kit (Omega Bio-Tek, GA). The quality and quantity of RNA was analyzed by 260/280 nm ratios using a low volume spectrophotometer (NanoDrop, Thermo Scientific, DE). Total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, NY) according to the manufacturer's instructions.

RT-PCR conditions and identification of alternative transcript

We designed primers to amplify the protein coding region of mouse *Fads3* (GenBank Accession# NM_021890). *Fads3* Forward: ATGGGCGGTGTCGGGGAGCCCGGA and *Fads3* Reverse: TCATTGATGGAGGTATGCATCCAGCCA. RT-PCR was performed using these primers with adult female mouse liver cDNA as template and iProof high-fidelity DNA polymerase (BIO_RAD, CA) in a 20 μ l reaction. Cycling conditions were: initial denaturation at 98 °C for 30 s followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 68 °C for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. PCR products were run on 2 % agarose gels containing ethidium bromide and visualized under UV. Two prominent bands were obtained when separated by electrophoresis on a 2 % agarose gel. The lower band was gel purified, cloned into p GEM T-Easy vector (Promega, WI) and sequenced using T7 forward and SP6 reverse universal primers at the Cornell University life sciences core laboratories.

Expression of *Fads3CS* and *AT9* in mouse tissues

To amplify *Fads3CS*, a forward primer was designed within the exon 1 and reverse primer was designed within in the exon 2 which is missing in the alternative transcript. To amplify *Fads3AT9*, the forward primer bridged the deleted parts of the exon 2. Details of the primers and PCR conditions are provided in Supplementary Table 1. Briefly, cDNA was prepared from 11 different mouse tissues using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, NY) to measure mRNA expression levels of *Fads3CS* and *Fads3AT9*. PCR amplification reactions were performed using EmeraldAmp GT PCR Master Mix (Clontech, CA) in a volume of 20 microliters. PCR products were resolved using 2 % agarose gels and bands visualized under UV light. *18S* was used as control. mRNA expression of *Fads3CS* and *AT9* were quantified using ImageJ software (National Institutes of Health, USA) and the expression levels were normalized to reference gene *18S*. mRNA expression of *Fads3CS* was taken as “1” and the changes between *CS* and *AT9* were measured by using ratio of *AT9/CS*.

Fatty acid analysis

Adult mouse tissues (11 different tissues from 4 mice) were used for fatty acid extraction and analysis. Fatty acid methyl esters (FAME) were prepared using modified one-step method of Garces and Mancha [14] modified for use with soft tissue. Methylated fatty acids were quantified by gas chromatography-flame ionization detection (GC-FID) using an equal weight mixture for response factor calibration and peak structures were identified by GC-covalent adduct chemical ionization tandem mass spectrometry (GC-CACI-MS/MS) [2].

Three injections were performed for each FAME and quantity of each fatty acid species was calculated as percentage by weight in total fatty acids (% , w/w).

Statistical analysis

Data are expressed as mean \pm SD. Statistical significance of fatty acids among eleven tissues was analyzed using one-way ANOVA and Tukey's post hoc test (SPSS software, version 16.0). Different alphabets shown in Table 1 represent statistical significance ($P < 0.05$) between groups. Paired Student's *t* Test was used to analyze the changes in gene expression levels of *Fads3AT9* and *Fads3CS* within each tissue ($*P < 0.05$).

Results and discussion

The open reading frame sequence of house mouse (*Mus musculus*) *Fads3* (GenBank Accession# NM_021890) consists of 1350 bp, encoding a protein of 449 aa and a stop codon. To clone *Fads3* ORF into expression vectors for functional studies we designed primers to amplify 1350 bp of *Fads3*, however, in addition to the expected product size we observed another prominent band using adult mouse liver cDNA (Fig. 1, Top). We carefully gel extracted the prominent band, cloned and sequenced the product resulting in the identification of a novel splice variant of *Fads3* (*Fads3AT9*; GenBank Accession# KM975938). Sequencing analysis revealed complete absence of exon 2 (Fig. 1, Bottom). The putative 1239 bp ORF of *Fads3AT9* identified using ORF finder <<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>>, encodes a protein of 412 aa, resulting from an in-frame loss of 37 aa compared to classical *FADS3*. *FADS3AT9* retains all the conserved regions characteristic of front end desaturase (cytochrome b5 domain and three histidine repeats) (Supplement Fig. 1).

We studied the expression of *Fads3AT9* along with *Fads3CS* using 11 mouse tissues. *AT9* along with *CS* is ubiquitously expressed in all 11 tissues tested; *AT9* expression is higher than *CS* in pancreas, liver, spleen, brown adipose tissue and thymus (Fig. 2, supplementary Fig. 2). *CS* expression is low in pancreas while *AT9* is at much greater abundance (Fig. 2, supplementary Fig. 2). These observations suggest that *AT9* has an important role in pancreatic LCPUFA synthesis.

We used Cell-PLoc 2.0 <<http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/>> [15] to predict subcellular localization of *Fads3CS* and *Fads3AT9*. The protein sequences of *Fads3CS* and *Fads3AT9* in Fasta format were uploaded and the software predicted putative subcellular localization. Both *Fads3CS* and *Fads3AT9* are predicted to be localized to endoplasmic reticulum (ER) with high confidence. *Fads* proteins are known to localize to the ER though recently organelle-specific staining and Western blotting show that primate *FADS1* and *FADS2* localize to both ER and mitochondria [2, 5]. By using the protein molecular weight calculator <<http://www.sciencegateway.org/tools/proteinmw.htm>>, we predict protein size of 51 kDa for *FADS3CS* and 47 kDa for *FADS3AT9*. Earlier, Pedrono et al. [12] also reported existence of several *FADS3* protein isoforms and Shoji et al. reported two *FADS2* isoforms with sizes of 52 and 46 kDa, respectively [16].

We also checked the relevant substrate-product metabolites via fatty acid profiles of the 11 tissues (Table 1). Table 1 is arranged based on highest to lowest total n - 6 PUFA levels. Total n - 6 PUFA levels were highest in the pancreas, followed by liver and spleen and total n - 3 PUFA was highest in cortex and hippocampus, followed by cerebellum. Among these tissues, docosahexaenoic acid (22:6n - 3) was highest in the cortex and hippocampus. We found that the twenty carbon eicosanoid precursor arachidonic acid (20:4n - 6), the immediate product of the *FADS1* 5-desaturase, was highest in pancreas and spleen (Table 1). Earlier, it has been shown that 20:4n - 6 is significantly higher in the pancreatic phospholipid fraction compared to liver and adipose [17]. *AT9* expression was high but *Fads3CS* low in pancreas, suggesting that AT9 may be playing a key role in LCPUFA biosynthesis in this tissue via direct catalysis by an AT9 protein, or some modification of the *Fads1CS* catalytic function. Alternative splicing regulates enzymatic activities of the proteins, their interactions with ligands and substrate specificity [18]. We previously reported that a *FADS1* splice variant (*FADS1AT1*) potentiates *FADS2* mediated production of 18:3n - 6 [5]. Widespread expression patterns and fatty acid profiles in mouse tissues suggest that *Fads3AT9* may play an important role in the regulation and/or biosynthesis of LCPUFA from precursors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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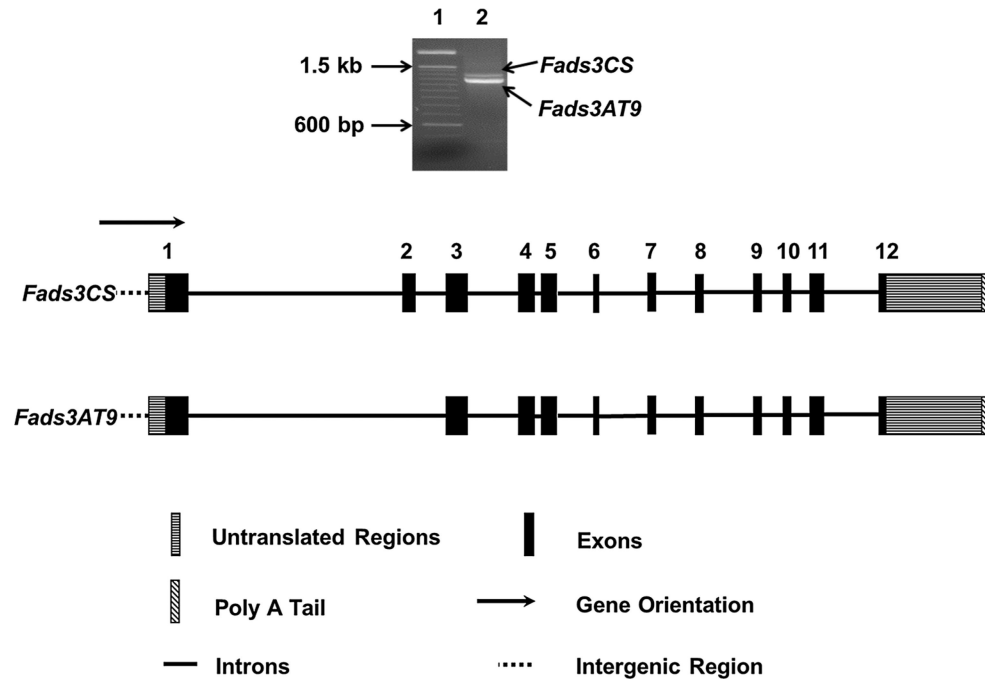


Fig. 1. Amplicons of *Fads3CS* and *Fads3AT9* and cartoon image of *Fads3CS* and *Fads3AT9* gene structure Top: *Fads3CS*–Classical splicing, *Fads3AT9*–alternative transcript. The products were separated on 2 % agarose gel and visualized under UV using ethidium bromide. *Lane 1*: 100 base pair molecular weight marker and *Lane 2*: PCR products amplified by RT-PCR. Bottom: *Fads3CS*–Classical splicing, *Fads3AT9* with missing exon 2 is shown. Numbers 1–12 are exons

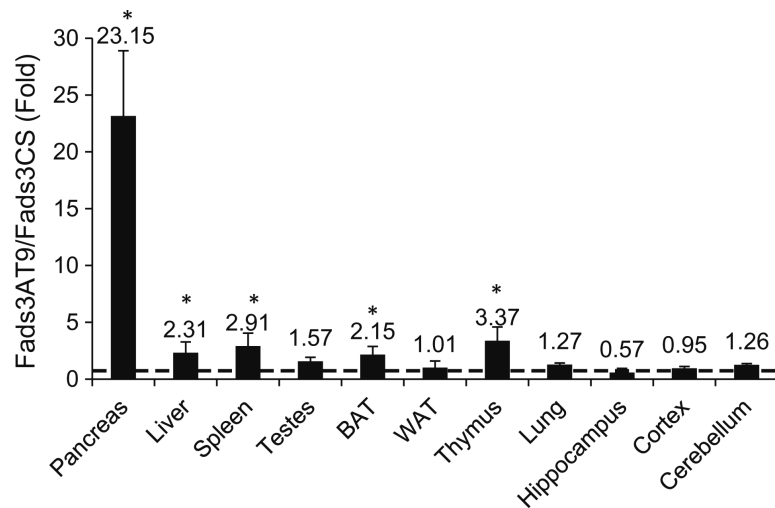


Fig. 2. Determination of mRNA expression of *Fads3AT9* in eleven tissues using Image J software. Relative mRNA levels of *Fads3CS* and *Fads3AT9* were normalized to reference gene *18S*. mRNA expression of *Fads3CS* was regarded as “1” (*dash line*) and the change between *CS* and *AT9* was measured by ratio of *AT9/CS*. Pancreases, liver, spleen, brown adipose tissue and thymus have higher levels of *Fads3AT9* compared to *Fads3CS*. Data is expressed as mean \pm SD (n = 4) and asterisk represents $P < 0.05$

Table 1

Fatty acid profiles of mouse tissues

Name of FA	Pancreas	Liver	Spleen	Testes	BAT	WAT	Thymus	Lung	Hippocampus	Cortex	Cerebellum
12:0	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.04 ± 0.02 ^a	0.21 ± 0.03 ^b	0.18 ± 0.02 ^c	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
14:0	0.32 ± 0.10 ^a	0.34 ± 0.08 ^a	0.52 ± 0.04 ^a	0.57 ± 0.06 ^{ab}	1.87 ± 0.46 ^d	1.11 ± 0.34 ^{bc}	1.47 ± 0.16 ^{cd}	1.16 ± 0.15 ^{bc}	0.14 ± 0.05 ^a	0.20 ± 0.06 ^a	0.13 ± 0.03 ^a
15:0	0.09 ± 0.03 ^{ab}	0.13 ± 0.01 ^{abc}	0.20 ± 0.05 ^c	0.06 ± 0.02 ^{ab}	0.07 ± 0.01 ^{ab}	0.14 ± 0.03 ^{bc}	0.10 ± 0.03 ^b	0.21 ± 0.06 ^c	0.05 ± 0.01 ^{ab}	0.06 ± 0.03 ^{ab}	0.04 ± 0.01 ^a
16:0	23.90 ± 0.83 ^{bc}	24.52 ± 0.79 ^c	24.69 ± 0.63 ^c	30.83 ± 0.69 ^d	19.47 ± 1.84 ^a	19.20 ± 1.37 ^a	20.24 ± 1.04 ^a	37.01 ± 1.20 ^e	20.67 ± 1.21 ^{ab}	25.33 ± 2.28 ^e	22.43 ± 0.27 ^{abc}
18:0	13.41 ± 0.72 ^c	10.81 ± 1.77 ^{cde}	17.31 ± 0.29 ^f	8.45 ± 1.53 ^{bc}	5.82 ± 0.83 ^{ab}	2.89 ± 0.75 ^a	7.89 ± 1.02 ^{bcd}	11.91 ± 0.78 ^{de}	22.00 ± 0.59 ^g	21.19 ± 0.64 ^g	21.65 ± 0.40 ^g
20:0	0.02 ± 0.00 ^a	0.07 ± 0.04 ^{ab}	0.35 ± 0.05 ^{cd}	0.33 ± 0.09 ^{cd}	0.44 ± 0.08 ^d	0.22 ± 0.03 ^{bc}	0.00 ± 0.00 ^a	0.23 ± 0.07 ^c	0.27 ± 0.07 ^c	0.32 ± 0.03 ^{cd}	0.59 ± 0.05 ^e
22:0	0.11 ± 0.01 ^{bc}	0.14 ± 0.03 ^c	0.24 ± 0.06 ^d	0.03 ± 0.01 ^b	0.06 ± 0.00 ^{ab}	0.03 ± 0.00 ^{ab}	0.07 ± 0.01 ^{bc}	0.28 ± 0.04 ^d	0.08 ± 0.01 ^{abc}	0.00 ± 0.00 ^a	0.02 ± 0.01 ^{ab}
23:0	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.14 ± 0.02 ^b	0.09 ± 0.04 ^c	0.10 ± 0.01 ^c
24:0	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.10 ± 0.04 ^b	0.05 ± 0.00 ^a	0.06 ± 0.01 ^b
Total SFA	37.83 ± 1.28 ^c	36.01 ± 1.16 ^c	43.33 ± 0.58 ^{de}	40.30 ± 2.24 ^{cd}	27.95 ± 2.89 ^{ab}	23.76 ± 2.21 ^a	29.76 ± 0.27 ^b	50.81 ± 0.65 ^f	43.45 ± 1.62 ^{de}	47.21 ± 2.76 ^{ef}	45.03 ± 0.59 ^{de}
14:1	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.02 ± 0.00 ^a	0.11 ± 0.03 ^b	0.12 ± 0.03 ^b	0.12 ± 0.04 ^b	0.04 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
16:1n-9	0.08 ± 0.01 ^a	0.38 ± 0.05 ^{cde}	0.29 ± 0.03 ^{abcd}	0.53 ± 0.04 ^{de}	0.54 ± 0.13 ^{de}	0.51 ± 0.03 ^{de}	0.34 ± 0.04 ^{bcd}	2.55 ± 0.23 ^f	0.43 ± 0.04 ^{de}	0.17 ± 0.02 ^{abc}	0.12 ± 0.01 ^{ab}
16:1n-7	0.89 ± 0.24 ^{ab}	1.83 ± 0.51 ^{ab}	0.96 ± 0.20 ^{ab}	1.04 ± 0.43 ^{ab}	3.41 ± 0.40 ^{cd}	5.73 ± 0.64 ^c	4.62 ± 0.72 ^d	2.45 ± 0.26 ^{bc}	0.55 ± 0.04 ^a	0.62 ± 0.06 ^a	0.55 ± 0.06 ^a
18:1n-9 + n-7	12.62 ± 0.97 ^{ab}	15.67 ± 1.05 ^{abc}	9.90 ± 0.45 ^a	15.45 ± 1.42 ^{abc}	33.34 ± 2.95 ^d	35.90 ± 1.53 ^d	31.41 ± 1.18 ^{cd}	11.70 ± 0.78 ^a	18.69 ± 1.46 ^{abc}	18.86 ± 0.33 ^{abc}	26.11 ± 1.65 ^{bcd}
20:1n-9	0.17 ± 0.01 ^a	0.23 ± 0.04 ^a	0.27 ± 0.05 ^{ab}	0.34 ± 0.03 ^{abd}	0.74 ± 0.11 ^{bc}	0.61 ± 0.26 ^{ac}	0.78 ± 0.18 ^{bc}	0.40 ± 0.06 ^{abd}	1.07 ± 0.24 ^c	0.84 ± 0.08 ^{cd}	2.70 ± 0.44 ^e
20:1n-7	0.04 ± 0.02 ^a	0.08 ± 0.04 ^{abd}	0.12 ± 0.03 ^{abd}	0.08 ± 0.04 ^{abd}	0.12 ± 0.02 ^{abd}	0.08 ± 0.03 ^{ab}	0.19 ± 0.02 ^{bc}	0.05 ± 0.03 ^a	0.25 ± 0.04 ^c	0.19 ± 0.05 ^{de}	0.51 ± 0.10 ^f
22:1n-9	0.06 ± 0.00 ^{ac}	0.07 ± 0.00 ^{ac}	0.08 ± 0.01 ^{ac}	0.00 ± 0.00 ^c	0.03 ± 0.01 ^{ce}	0.02 ± 0.00 ^{ce}	0.06 ± 0.02 ^{ac}	0.18 ± 0.04 ^b	0.10 ± 0.04 ^{ae}	0.06 ± 0.01 ^{ac}	0.13 ± 0.03 ^{ab}
24:1	0.07 ± 0.03 ^{ac}	0.07 ± 0.02 ^{ac}	0.00 ± 0.00 ^c	0.04 ± 0.02 ^{ac}	0.03 ± 0.01 ^c	0.07 ± 0.02 ^{ac}	0.03 ± 0.00 ^c	0.19 ± 0.04 ^b	0.11 ± 0.03 ^{ab}	0.17 ± 0.06 ^b	0.92 ± 0.02 ^d
Total MUFA	13.91 ± 1.18 ^a	18.34 ± 1.46 ^a	11.61 ± 0.43 ^a	17.48 ± 1.75 ^a	38.20 ± 2.85 ^{bd}	43.04 ± 1.67 ^b	37.55 ± 0.38 ^d	17.54 ± 1.01 ^{ac}	21.21 ± 1.75 ^a	20.91 ± 0.40 ^a	31.03 ± 2.25 ^d
18:2n-6	19.38 ± 2.46 ^{ab}	21.47 ± 2.43 ^a	9.50 ± 0.26 ^c	5.29 ± 1.16 ^{df}	29.44 ± 2.06 ^c	29.84 ± 3.44 ^c	19.25 ± 1.77 ^b	8.33 ± 0.84 ^{cd}	0.83 ± 0.08 ^g	0.84 ± 0.02 ^g	1.21 ± 0.16 ^g
18:3n-6	0.16 ± 0.05 ^b	0.47 ± 0.14 ^c	0.06 ± 0.01 ^a	0.10 ± 0.01 ^{ab}	0.06 ± 0.01 ^a	0.09 ± 0.02 ^{ab}	0.08 ± 0.02 ^{ab}	0.27 ± 0.05 ^d	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
20:2n-6	0.42 ± 0.09 ^a	0.30 ± 0.08 ^a	1.32 ± 0.24 ^b	0.10 ± 0.01 ^a	0.24 ± 0.03 ^a	0.18 ± 0.03 ^a	0.70 ± 0.01 ^c	0.50 ± 0.05 ^a	0.10 ± 0.03 ^a	0.10 ± 0.01 ^a	0.29 ± 0.02 ^a
20:3n-6	0.78 ± 0.17 ^{bc}	0.63 ± 0.19 ^{ab}	1.00 ± 0.10 ^c	1.30 ± 0.01 ^c	0.21 ± 0.03 ^d	0.18 ± 0.04 ^d	0.53 ± 0.00 ^{bc}	1.03 ± 0.09 ^e	0.32 ± 0.06 ^{cd}	0.29 ± 0.02 ^d	0.40 ± 0.02 ^{ad}
20:4n-6	22.82 ± 0.67 ^a	13.48 ± 2.53 ^b	20.97 ± 0.85 ^a	12.76 ± 0.82 ^b	1.56 ± 0.27 ^c	0.43 ± 0.09 ^c	6.27 ± 1.11 ^{de}	11.60 ± 0.79 ^{bd}	12.70 ± 0.92 ^{bf}	9.66 ± 0.52 ^{def}	7.70 ± 0.83 ^e
22:4n-6	0.53 ± 0.08 ^{ac}	0.43 ± 0.10 ^{ac}	3.57 ± 0.68 ^b	1.58 ± 0.03 ^c	0.31 ± 0.05 ^a	0.33 ± 0.08 ^a	1.47 ± 0.26 ^c	3.49 ± 0.46 ^b	3.37 ± 0.26 ^b	2.13 ± 0.41 ^c	1.52 ± 0.10 ^c
22:5n-6	0.42 ± 0.10 ^{ad}	0.25 ± 0.08 ^{ad}	0.47 ± 0.09 ^a	13.68 ± 0.19 ^b	0.06 ± 0.02 ^d	0.07 ± 0.03 ^d	0.42 ± 0.06 ^a	0.48 ± 0.16 ^a	0.41 ± 0.08 ^{ad}	0.29 ± 0.08 ^{ad}	0.07 ± 0.02 ^d

Name of FA	Pancreas	Liver	Spleen	Testes	BAT	WAT	Thymus	Lung	Hippocampus	Cortex	Cerebellum
Total n – 6 PUFA	44.51 ± 2.03 ^a	37.02 ± 0.33 ^b	36.88 ± 1.07 ^b	34.81 ± 0.51 ^{bc}	31.87 ± 2.02 ^c	31.10 ± 3.57 ^c	28.70 ± 0.36 ^c	25.70 ± 0.97 ^d	17.73 ± 0.94 ^e	13.32 ± 0.92 ^f	11.18 ± 0.87 ^f
18:3n – 3	0.25 ± 0.02 ^a	0.91 ± 0.25 ^{bc}	0.20 ± 0.05 ^{ad}	0.14 ± 0.09 ^{ad}	1.04 ± 0.29 ^b	1.66 ± 0.22 ^c	0.81 ± 0.02 ^e	0.16 ± 0.04 ^{ad}	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d
20:5n – 3	0.49 ± 0.07 ^a	0.28 ± 0.06 ^b	0.13 ± 0.02 ^c	0.07 ± 0.00 ^{de}	0.03 ± 0.01 ^e	0.04 ± 0.02 ^e	0.04 ± 0.01 ^{de}	0.11 ± 0.02 ^{cd}	0.00 ± 0.00 ^e	0.00 ± 0.00 ^e	0.04 ± 0.01 ^e
22:5n – 3	0.43 ± 0.06 ^a	0.42 ± 0.09 ^a	1.41 ± 0.24 ^b	0.26 ± 0.01 ^a	0.09 ± 0.03 ^a	0.08 ± 0.04 ^a	0.58 ± 0.05 ^b	1.12 ± 0.25 ^b	0.11 ± 0.04 ^a	0.10 ± 0.02 ^a	0.19 ± 0.08 ^a
22:6n – 3	2.53 ± 0.10 ^a	6.98 ± 1.04 ^b	6.29 ± 0.03 ^b	6.41 ± 0.10 ^b	0.69 ± 0.07 ^c	0.22 ± 0.03 ^c	2.36 ± 0.47 ^{ad}	4.49 ± 0.66 ^d	17.38 ± 0.39 ^e	18.37 ± 2.25 ^e	12.41 ± 1.07 ^f
Total n – 3 PUFA	3.71 ± 0.07 ^a	8.58 ± 0.94 ^b	8.03 ± 0.28 ^b	6.87 ± 0.01 ^{bc}	1.85 ± 0.37 ^e	2.00 ± 0.29 ^e	3.80 ± 0.52 ^{ac}	5.88 ± 0.48 ^c	17.49 ± 0.36 ^f	18.46 ± 2.26 ^f	12.64 ± 1.12 ^g
20:2n – 9	0.02 ± 0.00 ^a	0.04 ± 0.02 ^{ab}	0.07 ± 0.00 ^a	0.05 ± 0.01 ^{ad}	0.02 ± 0.01 ^a	0.04 ± 0.01 ^{ab}	0.01 ± 0.00 ^a	0.00 ± 0.00 ^a	0.09 ± 0.04 ^{cd}	0.08 ± 0.01 ^{bd}	0.10 ± 0.02 ^c
20:3n – 9	0.07 ± 0.02 ^{ad}	0.06 ± 0.03 ^{abcd}	0.00 ± 0.00 ^b	0.15 ± 0.04 ^c	0.04 ± 0.02 ^{abcd}	0.04 ± 0.01 ^{ab}	0.07 ± 0.00 ^{ab}	0.09 ± 0.02 ^{cd}	0.09 ± 0.02 ^{cd}	0.04 ± 0.01 ^{ab}	0.07 ± 0.01 ^{sd}
Total n – 9 PUFA	0.09 ± 0.03 ^b	0.10 ± 0.03 ^{ab}	0.07 ± 0.00 ^a	0.19 ± 0.05 ^c	0.07 ± 0.03 ^{ab}	0.06 ± 0.01 ^{ab}	0.08 ± 0.00 ^{ab}	0.09 ± 0.02 ^{bc}	0.18 ± 0.05 ^{bc}	0.12 ± 0.03 ^{ab}	0.18 ± 0.02 ^b