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Two weeks of docosahexaenoic acid (DHA) supplementation increases synthesis-secretion kinetics of n-3 polyunsaturated fatty acids compared to 8 weeks of DHA supplementation

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Running Title: Higher n-3 synthesis-secretion kinetics with acute dietary DHA

Abbreviations

ACSL – very long-chain acyl-CoA synthetase

ALA – α-linolenic acid

ARA – arachidonic acid

DHA – docosahexaenoic acid

DGLA – dihomo-γ-linolenic acid

DPAn-3 – n-3 docosapentaenoic acid

DPAn-6 – n-6 docosapentaenoic acid

ELOVL – elongation of very long-chain

EPA – eicosapentaenoic acid

FADS – fatty acid desaturase

F_{PUFA} – turnover

GC-MS – gas chromatography-mass spectrometry

GLA – γ-linolenic acid

$k_{1,PUFA}$ – synthesis-secretion coefficient

LNA – linoleic acid

PC – phoshatidylcholine

PE – phosphatidylethanolamine

PFB – pentafluorylbenzyl

PS – phosphatidylserine

PUFA – polyunsaturated fatty acid

SDA – stearidonic acid

S_{max} – maximum first derivative

$t_{1/2,PUFA}$ – half-life

TAG – triacylglycerol

THA – tetracosahexaenoic acid

TLE – total lipid extract

TPAn-3 – n-3 tetracosapentaenoic acid

UHPLC – ultra-high performance liquid chromatography

Abstract

Docosahexaenoic acid (DHA, 22:6n-3) must be consumed in the diet or synthesized from n-3 polyunsaturated fatty acid (PUFA) precursors. However, the effect of dietary DHA on the metabolic pathway is not fully understood. Presently, 21-day old Long Evans rats were weaned onto one of four dietary protocols: 1) 8 weeks of 2% ALA (ALA), 2) 6 weeks ALA followed by 2 weeks of 2% ALA + 2% DHA (DHA), 3) 4 weeks ALA followed by 4 weeks DHA and 4) 8 weeks of DHA. After the feeding period, $^{2}H_{5}$-ALA and $^{13}C_{20}$-eicosapentaenoic acid (EPA, 20:5n-3) were co-infused and blood was collected over three hours for determination of whole-body synthesis-secretion kinetics. The synthesis-secretion coefficient (ml/min, means ± SEM) for EPA (0.238 ± 0.104 vs. 0.021 ± 0.001) and DPAn-3 (0.194 ± 0.060 vs. 0.020 ± 0.008) synthesis from plasma unesterified ALA, and DPAn-3 from plasma unesterified EPA (2.04 ± 0.89 vs. 0.163 ± 0.025) were higher ($p < 0.05$) after 2 weeks compared to 8 weeks of DHA feeding. The daily synthesis-secretion rate (nmol/d) of DHA from EPA was highest after 4 weeks of DHA feeding (843 ± 409) compared to no DHA (70 ± 22). Liver gene expression of ELOVL2 and FADS2 were lower ($p < 0.05$) after 4 versus 8 weeks of DHA. Higher synthesis-secretion kinetics after 2 and 4 weeks of DHA feeding suggests an increased throughput of the PUFA metabolic pathway. Furthermore, these findings may lead to novel dietary strategies to maximize DHA levels while minimizing dietary requirements.

Keywords

Docosahexaenoic acid; nutrition; metabolism; lipids; liver; n-3 fatty acids; kinetics

1. Introduction

N-3 polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA, 22:6n-3) that are high in the heart [1] and brain [2, 3] must be synthesized from shorter chain n-3 PUFA or consumed directly from the diet. Specifically, α-linolenic acid (ALA, 18:3n-3) is the most consumed n-3 PUFA in the North American diet consumed primarily from cooking oil sources such as vegetable seed oils and soybean oils [4]. DHA synthesis rates from ALA are considered to be very low [5-15], however these studies did not consider the synthesis of DHA from alternative metabolic precursors such as eicosapentaenoic acid (EPA, 20:5n-3). When co-infusing ALA and EPA tracers simultaneously in rodents, our lab has demonstrated that the daily synthesis-secretion rate of DHA from plasma unesterified EPA (172 ± 62 nmol/d) is not statistically different than from ALA (98 ± 28 nmol/d [16]. This results in a total DHA synthesis-secretion rate of 270 nmol/d – nearly 3 times that of ALA alone – and suggests that DHA synthesis rates have been underestimated and may not be as low as previously determined. Furthermore, DHA synthesis rates from plasma unesterified ALA appear to be more than sufficient to meet brain DHA requirements [17], however, synthesis rates in relation to whole-body DHA requirements have not been assessed.

The effect of a wide range of dietary linoleic acid (LNA, 18:2n-6) [18] and ALA [19] compositions over 8 weeks have been determined on DHA synthesis-secretion kinetics. Briefly, increasing dietary ALA from 3% to 10% of total fat in rodents does not affect daily DHA synthesis-secretion rates despite a higher capacity for DHA synthesis following the 3% ALA diet [19]. Increasing dietary linoleic acid (LNA, 18:2n-6) from 10% to 40% of total fat in the diet of rodents does not increase synthesis-secretion rates of DHA, nor is there an effect on capacity for DHA synthesis-secretion [18]. However, in both studies increasing dietary ALA and LNA from 0.1% and 1.5%, respectively, increases daily synthesis-secretion rates and capacity for synthesis-secretion. Although DHA synthesis kinetics appear to be alterable across a range of ALA consumption, human interventions suggest that ALA intakes between 2 and 14 grams per day may do not change plasma phospholipid DHA concentrations [20].

Dietary consumption of DHA itself is the most efficient way to increase plasma DHA levels [20], and it is commonly believed that DHA intake will decrease or even shut down DHA synthesis from metabolic precursors. Humans fed 6.5 grams per day of DHA for 90 days demonstrate significantly lower deuterated n-3 metabolites in plasma, including DHA, compared to a low DHA diet following an oral dose of deuterated ALA [8]. Conversely, EPA + DHA intake of 1.5 grams per day did not alter the conversion of labeled ALA into plasma DHA, but did show increases in labeled plasma phosphatidylcholine (PC) EPA and n-3 docosapentaenoic acid (DPAn-3, 22:5n-3) [5]. Additionally, studies in rats have shown an inhibition of DHA and/or n-3 synthesis in response to increases in dietary DHA [21, 22]. However, the aforementioned human and rodent studies utilize oral tracers to determine the appearance of labeled n-3 PUFA over hours and days, and are thus unable to account for turnover of synthesized DHA, such as that which is metabolized for other functions in body. Using a 3-hour infusion model our lab has attempted to ameliorate these limitations and has demonstrated that dietary DHA (2% of total fat) over 15 weeks shuts down DHA synthesis from ALA [17]. However, the diet also contained very low amounts of dietary ALA (0.25% of total fat), thus potentially limiting substrate availability for DHA synthesis. In the presence of sufficient ALA, the effect of acute dietary DHA consumption on DHA synthesis may be different and has not been previously assessed. Therefore, an assessment of the effect of DHA feeding over time in the presence of similar amounts of dietary ALA is warranted.

In the present study, we aimed to determine the effects of short- and long-term DHA intake on the synthesis-secretion kinetics of n-3 PUFA. To address this, rats were weaned onto one of four diets for a total of 8 weeks providing 0, 2, 4 or 8 weeks of 2% DHA in total fatty acids. Synthesis-secretion kinetics were determined following the co-infusion of $^{2}H_{5}$-ALA and $^{13}C_{20}$-EPA over 3 hours in unrestrained free-living rats. Briefly, we determined that 2 weeks of dietary DHA significantly increased the synthesis-secretion coefficient for EPA and DPAn-3 from plasma unesterified ALA, and for DPAn-3 from plasma unesterified EPA. The daily-synthesis-secretion rate was also higher after 4 weeks of DHA compared to the ALA control diet. Higher synthesis-secretion kinetics after 2 and 4 weeks of dietary DHA suggests an increased throughput of the PUFA metabolic pathway as a potential means for reaching steady-state DHA

levels more rapidly. Furthermore, these findings may lead to the implementation of new dietary strategies to maximize DHA levels while minimizing dietary requirements.

2. Materials and Methods

2.1 Animals

All experimental procedures were performed in agreement with the policies set out by the Canadian Council on Animal Care and were approved by the Animal Ethics Committee at the University of Toronto. Four Long Evans dams with eight 18-day-old male non-littermate Long Evans pups were ordered from Charles River Laboratories (St. Constant, QC, Canada). Following arrival at the University of Toronto, the dam and pups were acclimated for 3 days on the 2% ALA diet and then weaned at 21 days old. At weaning, the 21-day-old pups were placed on a 10% by weight fat diet containing either 2% ALA (% weight) in total fatty acids or 2% DHA + 2% ALA in total fatty acids as the only dietary n-3 fatty acids following one of four dietary protocols: 1) 8 weeks of 2% ALA (0DHA), 2) 6 weeks of 2% ALA then 2 weeks of 2% DHA + 2% ALA (2DHA), 3) 4 weeks of 2% ALA then 4 weeks of 2% DHA + 2% ALA (4DHA) or 4) 8 weeks of 2% DHA + 2% ALA (8DHA) (**Figure 1**). Each dietary protocol lasted eight weeks following weaning until 11 weeks of age. During this time and prior to cannulation, rats were handled frequently and housed in pairs [16].

2.2 Diets

The diets were modified from the AIN-93G custom low n-3 PUFA rodent diet (Dyets, Inc., Bethlehem, PA) [23, 24]. The diets contained 10% lipids by weight, and the fat content of the diet by weight was 32.8% safflower oil, 63.2% hydrogenated coconut oil and 4% added fatty acid ethyl esters. The added ethyl esters were 2% oleate ethyl ester (Nu-Chek Prep, Inc., Elysian, MN) and 2% ALA ethyl ester (gift from BASF Pharma Callanish Ltd., Isle of Lewis, UK) for the 2% ALA diet, and 2% DHA ethyl ester (gift from BASF Pharma Callanish Ltd.) and 2% ALA ethyl ester for the 2% DHA + 2% ALA diet. Added ethyl ester oils were the only n-3 fatty acids present in the diet. Each oil was determined to be >98% pure by GC-FID. Actual composition of the diets was determined at the time of use by GC-FID to be 2.07 ± 0.01 % (mean ± SEM) by weight ALA in total fatty acids in the ALA diet, and 1.72% DHA and 1.91% ALA in the DHA + ALA diet, as reported previously [24].

2.3 Surgery and $^{2}H_5$-ALA, $^{13}C_{20}$-EPA and $^{13}C_{18}$-LNA Infusion

At 8 weeks post-weaning, 11-week-old rats were subjected to surgery to implant a catheter into each of the jugular vein and the carotid artery, as previously described in detail [18]. Baseline plasma unesterified and total fatty acid concentrations were measured in plasma drawn from the carotid artery one day after surgery and two days prior to stable isotope infusion. Modified from the method of Rapoport, Igarashi, and Gao [25] and Domenichiello et al. [17], animals were co-infused with 0.563 μmol/100 g body weight of $^{2}H_5$-ALA (purity >95% confirmed by GC-FID and GC-mass spectrometry(MS); Cambridge Isotope Laboratories, Inc., Tewksbury, MA), 0.281 μmol/100 g body weight of $^{13}C_{20}$-EPA (generously provided by Dr. Joseph Hibbeln, National Institutes of Health) and 2.25 μmol/100 g body weight of $^{13}C_{18}$-LNA (Cambridge Isotopes) into the jugular vein for 180 min. Infusate preparation, 3-hour steady-state infusions and blood collections from the carotid artery (0, 30, 60, 90, 120, 150 and 180 min) were performed as previously described in detail [18]. All blood samples were centrifuged for 15 min at 500g (PC-100 microcentrifuge; Diamed, ON, Canada) and the plasma was collected and stored at -80°C. Total blood plasma volumes were determined using the method of Schreihofer, Hair and Stepp and modified by our lab [17, 26].

2.4 Lipid Extractions from Baseline Serum

Total lipid extracts (TLE) for total fatty acid and for unesterified fatty acid measurements were obtained from 10 μL and 40 μL of serum, respectively, by a method modified from Folch, Lees, and Sloane Stanley [27] and described in detail previously [28]. Briefly, lipids were extracted with 2:1:0.75 chloroform:methanol:0.88% potassium chloride (v:v:v) a known amount of heptadecanoic acid (17:0, NuChek Prep Inc., Elysian, MN, USA) as internal standard. The mixtures were vortexed, centrifuged at 500 *g* for 10 min, and the lower, chloroform lipid-containing layer was pipetted into a new test tube. TLEs for total fatty acid determinations were stored for hydrolysis as described later. TLEs for unesterified fatty acid determinations were subject to thin-layer chromatography (TLC), the unesterified

fatty acids were collected, isolated from silica gel and stored for derivatization to pentafluorylbenzyl (PFB) esters as described in detail previously [28, 29].

2.5 Lipid Extractions from Stable Isotope Infused Plasma

TLE were obtained from 50 μL of stable isotope infused plasma by the Folch method as described earlier containing a known amount of heptadecanoic acid (NuChek Prep Inc.) as internal standard. Four-fifths of the TLE was stored for hydrolysis and stable isotope enrichment of total fatty acids, and the remaining one-fifth of the TLE was used for isolation of isotopically labeled unesterified fatty acid by the TLC method described earlier. The isolated labeled unesterified fatty acids were stored for later derivatization to PFB esters.

2.6 Hydrolysis of Total Endogenous and Isotopically Labeled Total Fatty Acid Pools

The TLE from baseline serum and infused plasma used to determine endogenous and labeled fatty acid concentrations, respectively, was evaporated under nitrogen and the lipids were hydrolyzed in 2 mL of 10% potassium hydroxide in methanol (w:v), as previously described [16, 29, 30]. Hydrolyzed fatty acids from the total lipid pool were collected and stored at -80 °C for later derivatization to PFB esters.

2.7 PFB Esterification

The plasma unesterified fatty acids and hydrolyzed fatty acids from total lipid pools were dried under nitrogen and derivatized to PFB esters, as previously described [29]. Briefly, 100 μL of acetonitrile:diisoproplyamine:pentafluorylbenzyl bromide (1000:10:1, v:v:v) was added and heated at 60 °C for 15 minutes. The reagents were then evaporated under nitrogen, re-aliquoted in 40 μL of hexane and analyzed for endogenous and labeled fatty acids by gas chromatography-mass spectrometry (GC-MS).

2.8 Endogenous and Labeled Fatty Acid Determination by GC-MS

Fatty acid PFB esters were analyzed on an Agilent 5977A quadrupole mass spectrometer coupled to an Agilent 7890B gas chromatograph (Agilent Technologies, Mississauga, ON) in negative chemical ionization mode, as described previously [29] and recently modified by our lab [30]. The fatty acid PFB esters were injected via an Agilent 7693 autosampler into a DB-FFAP 30 m x 0.25 mm i.d. x 25 μm film thickness capillary column (J&W Scientific from Agilent Technologies, Mississauga, ON) interfaced directly into the ion source with helium as the carrier gas. Fatty acids were analyzed in selected ion monitoring mode using [M-H]- for parent ion identification with ion dwell times of 500 μs. The [M-H]- parent ions for endogenous and labeled fatty acids are presented in **Supplemental Table 1**.

2.9 Whole Blood Complex Acyl-Lipid Species Determination by UPLC-MS/MS

Baseline whole blood samples were collected one day post-surgery and two days prior to infusions and frozen at -80°C for later analysis of complex acyl-lipid species [31]. Total lipids were extracted by a modified Folch method from 50 μL of whole blood by the method described earlier containing a mix of deuterated internal standards (Splash Lipidomix, Avanti Polar Lipids, Alabaster, AL, USA). Lipid extracts were dried under a stream of N_2 gas and reconstituted in 100 μL 65:35:5 isopropanol:acetonitrile:water + 0.1% formic acid, and were stored at 4ºC until analysis by a Waters ACQUITY UPLCI-Class system coupled to a Waters Synapt G2Si Quadrupole-Time-of-Flight mass spectrometer (Waters Inc., Milford, MA, USA). A reversed phase, binary multi-step ultra-high performance liquid chromatography (UHPLC) protocol was used with a Waters ACQUITY CSH C18 column, with dimensions 15 cm x 2.1 mm x 1.7 μm. The mobile phase consisted of A: 60:40 acetonitrile:water + 10mM ammonium formate + 0.1% formic acid, and B: 90:10 isopropanol:acetonitrile + 10mM ammonium formate + 0.1% formic acid. The gradient protocol used was as follows: 32% B from 0 – 1.5 min, 45% B from 1.5 – 4 min, 50% B from 4 – 8 min, 55% B from 8 – 18 min, 60% B from 18 – 20 min, 70% B from 20 – 35 min, 95% B from 35 – 40 min, 95% B from 40 – 45 min, decreased to 32%

B from 45 – 47 min, and allowed to equilibrate until the 48 minute mark. The flow was set at 260 μL/min, column temperature at 45°C and tray temperature at 4°C. The mass spectrometer was operated in High Resolution negative ion mode, scan range 100 to 1200 *m/z*, and tandem mass spectrometry data was acquired under data-independent acquisition conditions. Compound identifications and extracted ion peak areas were generated using MassLynx Software (Waters Inc., Milford, MA, USA). Raw peak areas were normalized within complex lipid classes using the appropriate internal standards from the Splash Lipidomix mix, such that all native phosphatidylcholine (PC) species were normalized using the PC internal standard peak, phosphatidylethanolamine (PE) species were normalized using the PE standard peak, etc.

2.10 Fatty Acyl-CoA Analysis

After 180 min of infusion, animals continuing to be infused were anaesthetized with 5% isoflurane and the median lobe of the liver was freeze-clamped, collected, immediately placed in liquid nitrogen and stored at -80°C for acyl-CoA analysis. Liver samples were pulverized under liquid nitrogen and acyl-CoAs extracted as performed previously [32]. Briefly, 10 mg of liver was homogenized for 150s in 500 μL of 150 mM aqueous ammonium bicarbonate, then mixed with 500 μL of 3:1:1 acetonitrile:isopropanol:methanol and sonicated for 3 min at 4°C. The homogenate was centrifuged at 16 000 *g* for 10 min and the supernatant collected. The remaining pellet was extracted again and the supernatants were combined into one vial and dried under nitrogen. The solid residue was then re-suspended in 500 μL of 15 mM sodium hydroxide in 1:1 water:methanol, centrifuged at 14 000 *g* for 5 min and the supernatant collected for LC-MS/MS analysis.

Fatty acyl-CoA liver extracts were detected using a Waters ACQUITY UPLCI-Class system (Waters Inc.) and a5500 QTRAP mass spectrometer (Applied Biosystems, Fosters City, CA) equipped with a Waters ACQUITY UPLC BEH C8 column (2.1 x 100 mm, 1.7μm). The initial HPLC conditions of elution were set at 300 μl/min gradient system consisting of 95% A (15 mM ammonium hydroxide)

and 5% B (acetonitrile with 15 mM ammonium hydroxide). The initial gradient was maintained for 1.5 minutes, and eluent B was linearly increased to 37.8% over 8.5 min, then to 100% over 1 min and finally rinsed for 2 min with 100% B and allowed to equilibrate to the starting conditions for 5 minutes. The MS was operated in positive electrospray ionization mode with a source temperature of 500 °C and the ion spray voltage setting of 5500 eV. Data was acquired by multiple reaction monitoring (MRM) mode with mass transitions as follows: 1028.4 to 521.4 (m/z) for ALA-CoA, 1052.3 to 545.3 for EPA-CoA, 1078.3 to 571.3 for DHA-CoA and 1020.6 to 513.5 for the internal standard 10Z heptadecenoyl Coenzyme A. Sample concentrations were calculated by plotting peak area ratios (analyte/internal standard) against calibration curves generated from extracted standard mixes.

2.11 Equations

To determine the synthesis rates of n-3 PUFA from ALA and EPA, the appearance of $^{2}H_5$-PUFA and $^{13}C_{20}$-PUFA, respectively, in the plasma-esterified pool were measured and fit to a Boltzmann sigmoidal curve ([^{2}H/^{13}C-PUFA] x plasma volume vs. time) using nonlinear regression [17] (Graphpad Prism Version 4.0, La Jolla, CA, USA). At any point on the curves, the slope (*S*) is determined by the ability of the whole body to synthesize $^{2}H_5$-PUFA from $^{2}H_5$-ALA or $^{13}C_{20}$-PUFA from $^{13}C_{20}$-EPA and the ability of the periphery to uptake $^{2}H_5$-PUFA (equation 1) or $^{13}C_{20}$-PUFA (equation 2), respectively.

$$S = k_{1,\mathrm{PUFA}}[^{2}H_5\text{-ALA}]_{\mathrm{unesterified}} - k_{-1,\mathrm{PUFA}}[^{2}H_5\text{-PUFA}]_{\mathrm{esterified}} \quad (Eq.\ 1)$$

$$S = k_{1,\mathrm{PUFA}}[^{13}C_{20}\text{-EPA}]_{\mathrm{unesterified}} - k_{-1,\mathrm{PUFA}}[^{13}C_{20}\text{-PUFA}]_{\mathrm{esterified}} \quad (Eq.\ 2)$$

Where $k_{1,\mathrm{PUFA}}$ is the steady-state synthesis-secretion coefficient for any PUFA, representing a measure for the proportion of infused label that is converted to downstream products, $[^{2}H_5\text{-ALA}]_{\mathrm{unesterified}}$, $[^{13}C_{20}\text{-EPA}]_{\mathrm{unesterified}}$ are the plasma concentrations of the infusate, $k_{-1,\mathrm{PUFA}}$ is the disappearance coefficient for any individual PUFA, and $[^{2}H_5/^{13}C_{20}\text{-PUFA}]_{\mathrm{esterified}}$ is the concentration of an individual PUFA in the plasma

that has been synthesized from the respective infusate, packaged into lipoprotein and secreted into the plasma.

The maximum first derivative (S_{max}) of the curves are assumed to be the time point when the uptake of esterified labeled PUFA from the periphery is negligible, i.e., 0 (equation 3 and 4).

$$S_{max} = k_{1,PUFA}[^{2}H_5\text{-ALA}]_{unesterified} - 0 \qquad (Eq.\ 3)$$

$$S_{max} = k_{1,PUFA}[^{13}C_{20}\text{-EPA}]_{unesterified} - 0 \qquad (Eq.\ 4)$$

Therefore, the derivative at this point is equal to the rate of $^{2}H_5$-PUFA or $^{13}C_{20}$-PUFA synthesis-secretion. By correcting the S_{max} by the substrate tracee:tracer ratio, the rates of actual PUFA synthesis are determined, $J_{syn,PUFA}$ (nmol/min) [17].

$$J_{syn,PUFA} = S_{max}\,([ALA]_{unesterified} / [^{2}H_5\text{-ALA}]_{unesterified}) = k_{1,DHA}[ALA]_{unesterified} \qquad (Eq.\ 5)$$

$$J_{syn,PUFA} = S_{max}\,([EPA]_{unesterified} / [^{13}C_{20}\text{-EPA}]_{unesterified}) = k_{1,DHA}[EPA]_{unesterified} \qquad (Eq.\ 6)$$

The diet consumed for this study contained no n-3 PUFA other than ALA and/or DHA, therefore it can be assumed that the plasma total n-3 PUFA were constant during the infusion period. Therefore, the turnover rate (F_{PUFA}) and half-life ($t_{1/2,PUFA}$) of total n-3 PUFA in the plasma can be determined by Eq. 7 and 8, respectively.

$$F_{PUFA} = J_{syn,PUFA} / (V_{plasma} * [PUFA]_{esterified}) \qquad (Eq.\ 7)$$

$$t_{1/2,PUFA} = 0.693 / F_{PUFA} \qquad (Eq.\ 8)$$

The same equations were used for determination of synthesis-secretion kinetic parameters of n-6 PUFA from unesterified LNA ($^{13}C_{18}$-LNA infusate) with results reported in **Supplemental Table 2**.

2.12 Gene Expression

mRNA expression was determined by reverse transcriptase qualitative polymerase chain reaction (RT-qPCR). RNA extraction was performed using Trizol reagent (Invitrogen Co., Frederick, MD) and RNA was isolated and assessed as described previously [33]. Briefly, RNA was separated from other cellular components using chloroform, the RNA was centrifuged to a pellet, washed in ethanol and dissolved in deionized water. RNA was measured for purity using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE) and subsequently reverse transcribed to cDNA with reverse transcriptase for higher stability with a MJ mini personal thermal cycler (Biorad Laboratories, Mississauga, ON). Aliquots of cDNA were loaded onto a 96-well PCR plate along with forward and reverse primers for the genes of interest and Fast SYBR Green master mix fluorescent dye (Applied Biosystems, Streetsville, ON) to bind to the double stranded DNA allowing detection of the amplified qPCR products. The samples were incubated at 95°C for 10 min, followed by 40 cycles of 95°C and 60°C in order to amplify the target sequence using a Biorad CFX Real-Time PCR detection system (Biorad). The threshold cycle number (ΔCt) for target genes FADS1 (F – 5' CCTCTTGTAAAGCACGAGCC, R – 5' CAAGGGGTCACACTGTTCCT), FADS2 (F – 5' TCAAAACCAACCACCTGTTCTTC, R – 5' ACCAGGCGATGCTTTCCA), ELOVL2 (F – 5' TGCTTGCCCGTGAGAGCCAC, R – 5' TGCCACAGGAAGGCGACGAC), ELOVL5 (F – 5' CTCTCGGGTGGCTGTACTTC, R – 5' AGAGGCCCCTTTCTTGTTGT), ACSL3 (F – 5' GGCTACTCTTCACCACAGACA, R – 5' CGATCCATGATTTCCGGCAC) and ACSL6 (F – 5'TGAATGCACAGCTGGGTGTA, R – 5' ATGTGGTTGCAGGGCAGAG) were expressed relative to the ΔCt of the 18S housekeeping gene (Sigma-Aldrich; F – 5' GATCCATTGGAGGGCAAGTCT, R – 5' AACTGCAGCAACTTTAATATACGCTATT). The expression of target genes were then determined using the $2^{-\Delta\Delta Ct}$ method [34], and the values were expressed relative to the 0DHA dietary protocol.

2.13 Statistics

Differences between dietary protocols for all dependent measures were assessed by one-way ANOVA followed by Tukey's HSD post-hoc test. Normality was assessed by the Shapiro-Wilk test for normality, and in the case of non-normally distributed groups, the data was log transformed prior to statistical analysis. All data is presented as means ± SEM.

3. Results

3.1 Fatty Acid Concentrations

Plasma unesterified fatty acid concentrations were determined two days prior to infusions and plasma unesterified infusate concentrations were determined as an average throughout the 180 min infusion period. Specific activity ([unesterified fatty acid infusate]/[endogenous unesterified fatty acid]) is also reported (**Figure 2**). No differences ($p > 0.05$) were determined for plasma unesterified ALA across the four dietary conditions (6.7 ± 1.5 to 14.5 ± 2.7 nmol/mL), however the 3-hour average plasma unesterified $^{2}H_{5}$-ALA was higher ($p > 0.05$) in the 8DHA group (1.0 ± 0.1 nmol/mL) compared to all other dietary groups (0.5 ± 0.1 to 0.7 ± 0.1 nmol/mL). Specific activity was not different ($p > 0.05$) between the four dietary conditions. Plasma unesterified EPA was higher ($p < 0.05$) in the 4DHA (2.2 ± 1.2 nmol/mL) and the 8DHA groups (2.1 ± 0.5) compared to the 0DHA (0.33 ± 0.03) and 2DHA groups (0.7 ± 0.3) with no differences ($p > 0.05$) in the concentration of the plasma $^{13}C_{20}$-EPA infusate. As a result of the high endogenous plasma EPA concentrations in the 4DHA and 8DHA groups, the measured specific activities of 0.09 ± 0.02 for both are significantly lower ($p < 0.05$) than determined following the 0DHA dietary intervention (0.48 ± 0.12).

Plasma esterified n-3 PUFA concentrations were used to determine turnover of newly synthesized n-3 PUFA for each dietary conditions (**Table 1**). Only EPA and DHA total plasma concentrations were higher ($p < 0.05$) after 4 and 8 weeks of dietary DHA compared to the 0DHA condition. Plasma EPA was 1.6 and 1.4-fold higher and plasma DHA was 2.3 and 1.6-fold higher in the 4DHA and 8DHA groups, respectively, compared to the 0DHA control group. Interestingly, 18:4n-3 and 20:3n-3 were 3- and 0.9-fold lower ($p < 0.05$), respectively, in the 2DHA group compared to 0DHA and this was maintained in the 8DHA group. Only 20:4n-3, n-3 tetracosapentaenoic acid (TPAn-3, 24:5n-3) and tetracosahexaenoic acid (THA, 24:6n-3) showed no differences ($p > 0.05$) across all dietary conditions. For plasma n-6 PUFA, 18:3n-6, 20:2n-6, 22:4n-6, 22:5n-6, 24:4n-6 and 24:5n-6 were all lower ($p < 0.05$) in the 2DHA group compared to the 0DHA group, and this was maintained in the 4DHA and 8DHA group for all n-6 PUFA except 18:3n-6 and 20:2n-6.

3.2 Lipid and Acyl-CoA Species

Concentrations of DHA and EPA-containing phospholipid species and one DHA-containing triacylglycerol (TAG) were measured in whole blood collected two days before infusions and presented in **Figure 3**. The PC species 16:0/DHA-PC (50 ± 5 and 46 ± 4 nmol/mL) and 18:0/DHA-PC (87 ± 13 and 65 ± 7) were higher ($p < 0.05$) following the 4DHA and 8DHA dietary protocols, respectively, compared to 0DHA (26 ± 4 and 38 ± 7, respectively). In addition, 16:0/DHA-phosphatidylethanolamine (PE) was higher ($p < 0.05$) in the 2DHA (9.2 ± 1.5) and 8DHA group (7.2 ± 0.5) compared to 0DHA (3.1 ± 0.4) and 18:0/DHA-phosphatidylserine (PS) was higher in the 4DHA group (18 ± 1.3) and higher still ($p < 0.05$) in the 8DHA group (34 ± 2.3) compared to both 0DHA (5.1 ± 1.7) and 2DHA groups (9.2 ± 1.0). Finally, 16:0/EPA-PC was higher ($p < 0.05$) in the 8DHA group (10.1 ± 0.8) compared to the 0DHA control group (6.6 ± 0.8). No differences ($p > 0.05$) between dietary conditions were determined for 16:1/DHA-PC, 18:1/DHA-PC, 18:0/EPA-PC or 16:0/18:1/DHA-TAG.

Liver fatty acyl-CoA concentrations (ng/mg) were also determined and presented for ALA, EPA and DHA-CoA (**Figure 4**). No differences ($p > 0.05$) were detected across dietary groups for ALA-CoA or EPA-CoA. DHA-CoA was 67 – 94% higher ($p < 0.05$) in all dietary DHA groups compared to the 0DHA group (0.12 ± 0.02 ng/mg).

3.3 PUFA synthesis-secretion coefficients, rates, turnover rates and half-lives

Synthesis-secretion parameters for EPA, DPAn-3 and DHA were determined from plasma unesterified ALA as well as for DPAn-3 and DHA from plasma unesterified EPA for maximum first derivative (S_{max}, nmol/min), synthesis-secretion coefficient ($k_{1,n}$, nmol/min), daily synthesis-secretion rates (nmol/day), turnover (%/day) and halflife (days) (**Table 2**). The synthesis-secretion coefficients for EPA synthesis from ALA were 4.4, 4.4 and 10.3-fold higher ($p < 0.05$) after 2 weeks of dietary DHA (2DHA group) compared to 0, 4 and 8 weeks of DHA feeding, respectively. Similarly, the synthesis-secretion coefficient for DPAn-3 synthesis from ALA was 8.7-fold higher ($p < 0.05$) in the 2DHA group compared to the 8DHA group. Furthermore, a faster turnover ($p < 0.05$) and shorter half-life was

determined for newly synthesized DPAn-3 from the plasma unesterified ALA pool in the 2DHA group compared to 0DHA. An 11.5-fold higher ($p < 0.05$) synthesis-secretion coefficient for DPAn-3 from plasma unesterified EPA was also identified in the 2DHA group compared to 8DHA, and a 14.4-fold faster turnover and 3.6-fold shorter half-life was determined in the 2DHA versus 0DHA group. Synthesis-secretion kinetic parameters for DHA from plasma unesterified ALA showed no differences ($p > 0.05$) for any measure. Strikingly, the daily synthesis-secretion rate for DHA from plasma unesterified EPA was 11-fold higher ($p < 0.05$) in the 4DHA group compared to the 0DHA group. As a summary, daily synthesis-secretion rates (nmol/d, means ± SEM) for the sum of EPA, DPAn-3 and DHA were 957 ± 412, 6939 ± 4044, 1996 ± 636 and 1037 ± 312 from ALA ($p = 0.093$), and 313 ± 85, 2380 ± 1093, 3393 ± 1905 and 771 ± 217 from EPA ($p = 0.065$) for the 0DHA, 2DHA, 4DHA and 8DHA groups, respectively (data not shown).

A similar trend for PUFA synthesis-secretion kinetics were determined for other n-3 PUFA from plasma unesterified ALA (**Supplemental Table 3**) and EPA (**Supplemental Table 4**). For example, the synthesis-secretion coefficients for stearidonic acid (SDA, 18:4n-3), 20:3n-3 and THA were 6.5, 5.5 and 10.4-fold higher ($p < 0.05$) in the 2DHA group compared to the 8DHA group. Furthermore, turnover is faster and half-lives shorter for ALA in the 2DHA and 4DHA groups compared to 0DHA, for SDA in the 2DHA group compared to the 0DHA and 8DHA groups, and for 20:3n-3 in the 2DHA group compared to the 0DHA group. In addition, the TPAn-3 synthesis-secretion coefficient from EPA is 1.6-fold higher ($p < 0.05$) in the 4DHA group compared to 8DHA. Finally, similar effects of 2 weeks DHA feeding on n-6 PUFA synthesis-secretion kinetic measures from plasma unesterified LNA were determined (**Supplemental Table 4**).

3.4 Gene Expression

Expression of mRNA targets were determined in liver samples collected immediately following 180 min infusions and are reported in **Figure 5**. Expression of fatty acid desaturase 2 (FADS2) gene encoding for Δ6-desaturase was two times lower ($p < 0.05$) in the 4DHA group compared to the 8DHA

group with no other differences shown between dietary conditions. Similarly, ELOVL2 encoding for the elongase-2 enzyme was 74% lower ($p < 0.05$) in the 4DHA group compared to 8DHA, but did not obtain statistical significance compared to the 0DHA group ($p = 0.055$). No significant differences ($p > 0.05$) were determined between dietary conditions for FADS1, ELOVL5, ACSL3 or ACSL6.

4. Discussion

In the present study, we demonstrate higher synthesis-secretion coefficients after 2 weeks of DHA feeding compared to 8 weeks of DHA feeding for the synthesis of DPAn-3 from plasma unesterified ALA and EPA, and for the synthesis of EPA from plasma unesterified ALA. Furthermore, synthesis-secretion coefficient for EPA synthesis from ALA is more than 5 times higher in the 2DHA group compared to 0DHA (Table 2). Most strikingly, we demonstrate an 11-fold higher daily synthesis-secretion rate for DHA from plasma unesterified EPA in the 4DHA group compared to the 0DHA group. Similarly, high synthesis-secretion coefficients for 18:4n-3, 20:3n-3 and THA from plasma unesterified ALA (Supplemental Table 1), 24:5n-3 from EPA (Supplemental Table 2) and 20:2n-6 from LNA (Supplemental Table 3) were determined for the 2DHA and/or 4DHA groups. In addition, higher daily synthesis-secretion rates were determined for 18:4n-3 from ALA in the 2DHA group compared to 8DHA (Supplemental Table 1), and for 20:2n-6 and 20:3n-6 from LNA in the 2DHA group compared to 0DHA (Supplemental Table 3). Furthermore, 8 weeks of DHA feeding (8DHA) yielded no significant differences compared to no DHA (0DHA) for all kinetic measures except for the half-life of newly synthesized DPAn-3 from plasma unesterified EPA that was 4.3-fold lower in the 8DHA group. Our kinetic determinations for the ALA-only diet (0DHA) are in reasonable agreement with a recently published article assessing all n-3 and n-6 PUFA on the same diet during the same 8-week feeding period [28].

To the best of our knowledge, this is the first study to demonstrate that a chronic 8-week DHA feeding protocol compared to an ALA-only control does not affect synthesis rates or kinetics of any downstream n-3 PUFA, including DHA. In fact, the synthesis-secretion kinetics of DHA from plasma unesterified ALA were remarkably consistent across all diets, despite significantly different synthesis-secretion kinetics of both DPAn-3 and THA. This may provide evidence to suggest that the peroxisomal β-oxidation of THA to DHA is a regulatory step in the DHA synthesis pathway in response to DHA feeding. Our results were surprising as previous studies using oral doses of labeled ALA in adult rats following a 1.3% DHA diet over 8 weeks [21] and in artificially reared rat pups over 20 days of a 2%

DHA diet [22] determined lower conversion of the labeled precursor into DHA. Similarly, humans fed 6.5 g/d DHA over 90 days lowered the appearance of labeled DHA in plasma [8], and humans consuming 1.5 g/d EPA + DHA over 60 days lowered the appearance of labeled EPA and DHA but not DHA [5]. Diets from both rodent and human studies contained sufficient ALA, and although similarities exist between these studies and our own, one key difference exists: the appearance of labeled DHA in plasma from an oral dose of labeled ALA is determined over many days and cannot account for metabolism/oxidation or storage of both the precursor and the product during this period. Labeled DHA measures following an oral dose of ALA may then be more accurately described as an accumulation of labeled DHA that is highly influenced by turnover. Our 3-hour infusion methodology allows for real-time kinetic measure determinations in plasma from steady-state levels and prior to DHA uptake into peripheral tissues, thereby limiting the contributions from metabolism and storage. This would suggest that faster turnover of DHA – and not DHA synthesis – may be responsible for the lower accretion of labeled DHA in orally dosed rodents and humans. Interestingly, we have shown previously that synthesis-secretion rates of all n-3 PUFA appear to be primarily determined by turnover, and not synthesis when fed an ALA-only diet [28]. Although, we were unable to demonstrate faster turnover of newly synthesized DHA in the 8DHA group, future studies infusing labeled DHA may provide a more direct measure of DHA turnover in response to changes in dietary n-3 PUFA intake.

The finding of a generally higher throughput of both plasma unesterified ALA and EPA in the PUFA biosynthesis pathway following acute DHA feeding (2 and 4 weeks) compared to more chronic DHA feeding (8 weeks) was unexpected. This finding could not be explained by a concomitantly higher mRNA expression of the genes involved for the encoding of the biosynthesis pathway enzymes – FADS1 (Δ5-desaturase), FADS2 (Δ6-desaturase), ELOVL2 (elongase-2) or ELOVL 5 (elongase-5) (Figure 5). In fact, ELOVL2 and FADS 2 expression were lower in the 4DHA group compared to the 8DHA group. However, this does not exclude the possibility of higher enzyme activity, higher protein content or both. In fact, an increase in Δ5-desaturase and Δ6-desaturase enzyme activity has recently been demonstrated

despite lower mRNA expression of FADS1 and FADS2 in nutritional models [35, 36]. The higher synthesis-secretion coefficient after 2 weeks of DHA feeding for the synthesis of 18:4n-3 from plasma ALA and DPAn-3 from EPA identified in our study is indicative of a higher enzyme activity of Δ6-desaturase and elongase-2 and/or elongase-5. Interestingly, gene expression for elongase-2 (ELOVL2) and Δ6-desaturase (FADS2) are significantly lower after 4 weeks of DHA feeding compared to 8 weeks of feeding. This suggests a compensatory reduction in mRNA expression following the higher enzyme activity suggested *via* the synthesis-secretion coefficients after 2 weeks of DHA feeding. Conversely, higher mRNA expression following 8 weeks of DHA feeding may be a compensatory response for lower enzyme activity. We must also consider the possibility that synthesis-secretion kinetics and/or mRNA expression could be highest following even shorter DHA feeding periods (i.e. one week), and future studies should attempt to answer this question. The disagreement between mRNA expression and synthesis-secretion rates is interesting in the context of human populations. A meta-analysis determined a role for both dietary fatty acids and single nucleotide polymorphisms (SNPs) in the FADS gene cluster on blood DHA and EPA levels [37]. However, other studies have shown no effect of FADS SNPs on blood DHA levels [38, 39]. Variations in the relationship between diet, gene expression and enzyme activity may help to understand some of these conflicting findings as they relate to SNPs and blood PUFA levels.

The metabolic purpose for the increased PUFA biosynthetic throughput is not clear, however, the introduction of DHA in the diet may signal an upregulation of the pathway as a means to reach steady-state blood or tissue DHA levels more rapidly. Interestingly, very-high ALA feeding (up to 64% in total fatty acids) has been shown to achieve similar DHA levels in numerous tissues [40, 41] as compared to the present study in plasma, and may further indicate the ability to increase throughput of the pathway in response to dietary n-3 changes. However, our 3-hour steady-state infusion model may not solely represent changes in the PUFA biosynthesis pathway. By measuring the conversion of a labeled precursor (i.e. $^{2}H_{5}$-ALA or $^{13}C_{20}$-EPA) into labeled PUFA products (i.e. $^{2}H_{5}$-DHA or $^{13}C_{20}$-DHA) in the plasma, we are measuring the summed rate of liver uptake, elongation/desaturation, incorporation into

complex lipids/lipoproteins and export back into the plasma. Although the potential role of each of these steps cannot be ignored, we have demonstrated that gene expression for ACSL3 and ACSL6 encoding for DHA-specific CoA synthetases and the incorporation of DHA into phospholipids and triacylglycerols [42, 43] are not different across all dietary groups (Figure 5). In addition, no differences in the levels of liver DHA-CoA between the 2DHA, 4DHA or 8DHA were shown which further supports that differences in DHA-CoA synthesis was not responsible for the higher synthesis-secretion kinetics determined between the 2DHA or 4DHA and 8DHA group, and in turn suggests that changes in the incorporation of DHA into complex lipids does not explain the increase in DHA synthesis-secretion from plasma unesterified EPA determined after 4 weeks of DHA feeding. However, the general upregulation of our kinetic model makes it difficult to exclude the role that additional alternate pathways may play. Future studies assessing these dietary strategies in knockout or enzyme inhibitor models may help to more precisely elucidate the mechanisms involved. Furthermore, 5-minute tracer infusions can be designed so that newly synthesized downstream tracer products can be isolated in the liver prior to export into the plasma [11, 22, 44], thereby eliminating or reducing the potential contribution from lipoprotein repackaging and export into circulation. Regardless of the mechanism, we demonstrate a clear upregulation for the mobilization of the n-3 PUFA into the plasma pool following an acute 2-week DHA feeding period that appears to involve an increase in the synthesis of n-3 PUFA downstream to both ALA and EPA.

Rodent models of pregnancy have also been shown to increase synthesis and accretion of DHA [45] and protein levels of Δ6-desaturase late in pregnancy [33]. This increase in synthesis is associated with increases in specific DHA-containing lipid species in both plasma and liver, most significantly 16:0/DHA-PC [33]. Furthermore, n-3 blood biomarkers may be able to detect adherence and/or non-adherence to n-3 dietary advice [46], suggesting that specific EPA or DHA-containing complex acyl-lipid species may respond differently to acute or chronic DHA feeding. It is possible that in a similar manner specific lipid species are more responsive to increases in newly synthesized DHA versus exogenously derived DHA. For this reason, we identified the major DHA and EPA-containing lipid species in whole blood across the four dietary protocols. Although whole blood 16:0/DHA-PC was not different between

the three DHA fed groups, differences may be present when comparing 8 weeks of DHA feeding to even more acute DHA intake periods of shorter than two weeks. Of the nine additional DHA and EPA-containing lipid species reported, only 18:0/22:6-PS was higher after 8 weeks of feeding compared to after 2 and 4 weeks feeding. This finding identifies 18:0/DHA-PS as a potential blood biomarker for chronic dietary DHA intake and may prove to be an important tool for assessing dietary adherence to n-3 advice, and studies designed more towards answering such questions need to be developed.

The diets used in our study contained ALA and/or DHA as the only n-3 fatty acids present in the diet. Mechanistically, this allows us to identify the introduction of DHA into the diet acutely as the primary influence on the higher n-3 synthesis-secretion kinetics compared to chronic DHA. However, unless taken as a supplement DHA is rarely if ever consumed independently from EPA as both are found in high amounts in seafood, fish and fish oil supplements [4], with EPA normally present in higher amounts and often as high as twice that of DHA. The inclusion of EPA in the diet is expected to significantly impact the synthesis-secretion rates of DHA [16], as plasma unesterified EPA should increase with increased dietary EPA [47]. In our current study, DHA feeding did not increase plasma unesterified EPA levels until four weeks of feeding, and this increase is the primary reason for the higher DHA synthesis-secretion rates. Therefore, we hypothesize that acute fish oil feeding providing high dietary EPA along with DHA would push this higher rate of DHA synthesis-secretion to occur earlier in the feeding period and with greater magnitude. In order to fully realize the effects of acute DHA feeding on synthesis-secretion rates, future studies should be aimed at investigating the effects of acute DHA + EPA or fish oil feeding on the synthesis-secretion kinetics of n-3 PUFA from plasma unesterified EPA and ALA.

In summary, we determined that an acute 2-week DHA feeding period results in higher n-3 PUFA synthesis-secretion kinetics from plasma unesterified ALA and EPA precursors compared to chronic DHA intake, and daily synthesis-secretion rates of DHA from EPA were higher after 4 weeks of DHA feeding compared to an ALA-only control. This upregulation of n-3 synthesis-secretion kinetics with acute DHA feeding may occur as a means to reach steady-state DHA levels more rapidly.

Furthermore, 8 weeks of DHA feeding did not result in any differences in DHA synthesis-secretion kinetics or daily synthesis-secretion rates compared to an ALA-only control, suggesting that turnover of DHA may be higher with DHA feeding. The higher synthesis-secretion kinetics with acute DHA feeding was an unexpected yet exciting finding, and suggests that novel dietary strategies such as a repeated acute DHA feeding protocol may serve to attain similar tissue and blood DHA levels as chronic DHA intake while minimizing dietary requirements.

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Conflicts of Interest

RPB holds a Canada Research Chair in Brain Lipid Metabolism. RPB has received research grants from Bunge Ltd, Arctic Nutrition, the Dairy Farmers of Canada and Nestle Inc, as well as travel support from Mead Johnson and mass spectrometry equipment and support from Sciex. In addition, RPB is on the executive of the International Society for the Study of Fatty Acids and Lipids and held a meeting on behalf of Fatty Acids and Cell Signaling, both of which rely on corporate sponsorship. RPB has given expert testimony in relation to supplements and the brain. RPB also provides complimentary fatty acid analysis for farmers, food producers and others involved in the food industry, some of whom provide free food samples. There are no other conflicts of interest.

References

[1] Berger MM, Delodder F, Liaudet L, Tozzi P, Schlaepfer J, Chiolero RL, et al. Three short perioperative infusions of n-3 PUFAs reduce systemic inflammation induced by cardiopulmonary bypass surgery: a randomized controlled trial. Am J Clin Nutr. 2013;97:246-54.

[2] Igarashi M, Ma K, Gao F, Kim HW, Greenstein D, Rapoport SI, et al. Brain lipid concentrations in bipolar disorder. J Psychiatr Res. 2010;44:177-82.

[3] Lacombe RJS, Chouinard-Watkins R, Bazinet RP. Brain docosahexaenoic acid uptake and metabolism. Mol Aspects Med. 2018.

[4] Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. Am J Clin Nutr. 2011;93:950-62.

[5] Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. Br J Nutr. 2003;90:311-21.

[6] Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. Br J Nutr. 2002;88:355-63.

[7] Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr. 2002;88:411-20.

[8] Emken EA, Adlof RO, Duval SM, Nelson GJ. Effect of dietary docosahexaenoic acid on desaturation and uptake in vivo of isotope-labeled oleic, linoleic, and linolenic acids by male subjects. Lipids. 1999;34:785-91.

[9] Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta. 1994;1213:277-88.

[10] Hussein N, Ah-Sing E, Wilkinson P, Leach C, Griffin BA, Millward DJ. Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. J Lipid Res. 2005;46:269-80.

[11] Igarashi M, Ma K, Chang L, Bell JM, Rapoport SI, DeMar JC, Jr. Low liver conversion rate of alpha-linolenic to docosahexaenoic acid in awake rats on a high-docosahexaenoate-containing diet. J Lipid Res. 2006;47:1812-22.

[12] Mayes C, Burdge GC, Bingham A, Murphy JL, Tubman R, Wootton SA. Variation in [U-13C] alpha linolenic acid absorption, beta-oxidation and conversion to docosahexaenoic acid in the pre-term infant fed a DHA-enriched formula. Pediatr Res. 2006;59:271-5.

[13] McCloy U, Ryan MA, Pencharz PB, Ross RJ, Cunnane SC. A comparison of the metabolism of eighteen-carbon 13C-unsaturated fatty acids in healthy women. J Lipid Res. 2004;45:474-85.

[14] Pawlosky RJ, Hibbeln JR, Lin Y, Goodson S, Riggs P, Sebring N, et al. Effects of beef- and fish-based diets on the kinetics of n-3 fatty acid metabolism in human subjects. Am J Clin Nutr. 2003;77:565-72.

[15] Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N, Jr. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. J Lipid Res. 2001;42:1257-65.

[16] Metherel AH, Domenichiello AF, Kitson AP, Hopperton KE, Bazinet RP. Whole-body DHA synthesis-secretion kinetics from plasma eicosapentaenoic acid and alpha-linolenic acid in the free-living rat. Biochim Biophys Acta. 2016;1861:997-1004.

[17] Domenichiello AF, Chen CT, Trepanier MO, Stavro PM, Bazinet RP. Whole body synthesis rates of DHA from alpha-linolenic acid are greater than brain DHA accretion and uptake rates in adult rats. J Lipid Res. 2014;55:62-74.

[18] Domenichiello AF, Kitson AP, Chen CT, Trepanier MO, Stavro PM, Bazinet RP. The effect of linoleic acid on the whole body synthesis rates of polyunsaturated fatty acids from alpha-linolenic acid and linoleic acid in free-living rats. J Nutr Biochem. 2016;30:167-76.

[19] Domenichiello AF, Kitson AP, Metherel AH, Chen CT, Hopperton KE, Stavro PM, et al. Whole-Body Docosahexaenoic Acid Synthesis-Secretion Rates in Rats Are Constant across a Large Range of Dietary alpha-Linolenic Acid Intakes. J Nutr. 2017;147:37-44.

[20] Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr. 2006;83:1467S-76S.

[21] Lin YH, Shah S, Salem N, Jr. Altered essential fatty acid metabolism and composition in rat liver, plasma, heart and brain after microalgal DHA addition to the diet. J Nutr Biochem. 2011;22:758-65.

[22] DeMar JC, Jr., DiMartino C, Baca AW, Lefkowitz W, Salem N, Jr. Effect of dietary docosahexaenoic acid on biosynthesis of docosahexaenoic acid from alpha-linolenic acid in young rats. J Lipid Res. 2008;49:1963-80.

[23] DeMar JC, Jr., Ma K, Bell JM, Rapoport SI. Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids. J Neurochem. 2004;91:1125-37.

[24] Metherel AH, Chouinard-Watkins R, Trepanier MO, Lacombe RJS, Bazinet RP. Retroconversion is a minor contributor to increases in eicosapentaenoic acid following docosahexaenoic acid feeding as determined by compound specific isotope analysis in rat liver. Nutr Metab (Lond). 2017;14:75.

[25] Rapoport SI, Igarashi M, Gao F. Quantitative contributions of diet and liver synthesis to docosahexaenoic acid homeostasis. Prostaglandins Leukot Essent Fatty Acids. 2010;82:273-6.

[26] Schreihofer AM, Hair CD, Stepp DW. Reduced plasma volume and mesenteric vascular reactivity in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol. 2005;288:R253-61.

[27] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226:497-509.

[28] Metherel AH, Lacombe RJS, Chouinard-Watkins R, Hopperton KE, Bazinet RP. Complete assessment of whole-body n-3 and n-6 PUFA synthesis-secretion kinetics and DHA turnover in a rodent model. J Lipid Res. 2018;59:357-67.

[29] Pawlosky RJ, Sprecher HW, Salem N, Jr. High sensitivity negative ion GC-MS method for detection of desaturated and chain-elongated products of deuterated linoleic and linolenic acids. J Lipid Res. 1992;33:1711-7.

[30] Metherel AH, Domenichiello AF, Kitson AP, Lin YH, Bazinet RP. Serum n-3 Tetracosapentaenoic Acid and Tetracosahexaenoic Acid Increase Following Higher Dietary alpha-Linolenic Acid but not Docosahexaenoic Acid. Lipids. 2017;52:167-72.

[31] Aristizabal Henao JJ, Metherel AH, Smith RW, Stark KD. Tailored Extraction Procedure Is Required To Ensure Recovery of the Main Lipid Classes in Whole Blood When Profiling the Lipidome of Dried Blood Spots. Anal Chem. 2016;88:9391-6.

[32] Morin-Rivron D, Christinat N, Masoodi M. Lipidomics analysis of long-chain fatty acyl-coenzyme As in liver, brain, muscle and adipose tissue by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2017;31:344-50.

[33] Chalil A, Kitson AP, Aristizabal Henao JJ, Marks KA, Elzinga JL, Lamontagne-Kam DME, et al. PEMT, Delta6 desaturase, and palmitoyldocosahexaenoyl phosphatidylcholine are increased in rats during pregnancy. J Lipid Res. 2018;59:123-36.

[34] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8.

[35] Rincon-Cervera MA, Valenzuela R, Hernandez-Rodas MC, Marambio M, Espinosa A, Mayer S, et al. Supplementation with antioxidant-rich extra virgin olive oil prevents hepatic oxidative stress and reduction of desaturation capacity in mice fed a high-fat diet: Effects on fatty acid composition in liver and extrahepatic tissues. Nutrition. 2016;32:1254-67.

[36] Valenzuela R, Echeverria F, Ortiz M, Rincon-Cervera MA, Espinosa A, Hernandez-Rodas MC, et al. Hydroxytyrosol prevents reduction in liver activity of Delta-5 and Delta-6 desaturases, oxidative stress, and depletion in long chain polyunsaturated fatty acid content in different tissues of high-fat diet fed mice. Lipids Health Dis. 2017;16:64.

[37] Smith CE, Follis JL, Nettleton JA, Foy M, Wu JH, Ma Y, et al. Dietary fatty acids modulate associations between genetic variants and circulating fatty acids in plasma and erythrocyte membranes: Meta-analysis of nine studies in the CHARGE consortium. Mol Nutr Food Res. 2015;59:1373-83.

[38] Roke K, Walton K, Klingel SL, Harnett A, Subedi S, Haines J, et al. Evaluating Changes in Omega-3 Fatty Acid Intake after Receiving Personal FADS1 Genetic Information: A Randomized Nutrigenetic Intervention. Nutrients. 2017;9.

[39] Tanaka T, Shen J, Abecasis GR, Kisialiou A, Ordovas JM, Guralnik JM, et al. Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study. PLoS Genet. 2009;5:e1000338.

[40] Rincon-Cervera MA, Valenzuela R, Hernandez-Rodas MC, Barrera C, Espinosa A, Marambio M, et al. Vegetable oils rich in alpha linolenic acid increment hepatic n-3 LCPUFA, modulating the fatty acid metabolism and antioxidant response in rats. Prostaglandins Leukot Essent Fatty Acids. 2016;111:25-35.

[41] Valenzuela BR, Barrera RC, Gonzalez-Astorga M, Sanhueza CJ, Valenzuela BA. Alpha linolenic acid (ALA) from Rosa canina, sacha inchi and chia oils may increase ALA accretion and its conversion into n-3 LCPUFA in diverse tissues of the rat. Food Funct. 2014;5:1564-72.

[42] Marszalek JR, Kitidis C, Dirusso CC, Lodish HF. Long-chain acyl-CoA synthetase 6 preferentially promotes DHA metabolism. J Biol Chem. 2005;280:10817-26.

[43] Pelerin H, Jouin M, Lallemand MS, Alessandri JM, Cunnane SC, Langelier B, et al. Gene expression of fatty acid transport and binding proteins in the blood-brain barrier and the cerebral cortex of the rat: differences across development and with different DHA brain status. Prostaglandins Leukot Essent Fatty Acids. 2014;91:213-20.

[44] Igarashi M, Ma K, Chang L, Bell JM, Rapoport SI. Rat heart cannot synthesize docosahexaenoic acid from circulating alpha-linolenic acid because it lacks elongase-2. J Lipid Res. 2008;49:1735-45.

[45] Metherel AH, Kitson AP, Domenichiello AF, Lacombe RJS, Hopperton KE, Trepanier MO, et al. Maternal liver docosahexaenoic acid (DHA) stores are increased via higher serum unesterified DHA uptake in pregnant long Evans rats. J Nutr Biochem. 2017;46:143-50.

[46] Patterson AC, Metherel AH, Hanning RM, Stark KD. The percentage of DHA in erythrocytes can detect non-adherence to advice to increase EPA and DHA intakes. Br J Nutr. 2014;111:270-8.

[47] Browning LM, Walker CG, Mander AP, West AL, Madden J, Gambell JM, et al. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. Am J Clin Nutr. 2012;96:748-58.

Table 1 – Plasma polyunsaturated fatty acid concentrations following 8 weeks of dietary intervention

Total N-3 Fatty Acids (nmol/mL)				
Fatty Acid	0DHA	2DHA	4DHA	8DHA
ALA	62.4 ± 11.7^{a}	25.5 ± 4.3^{b}	42 ± 4.8^{ab}	32 ± 3.4^{b}
SDA	1.08 ± 0.16^{a}	0.27 ± 0.03^{c}	0.61 ± 0.09^{ab}	0.62 ± 0.2^{bc}
20:3n-3	2.62 ± 0.41^{a}	1.4 ± 0.18^{b}	1.85 ± 0.31^{ab}	1.4 ± 0.2^{b}
20:4n-3	1.70 ± 0.27	0.98 ± 0.13	1.55 ± 0.22	1.30 ± 0.21
EPA	24 ± 2.7^{a}	28.6 ± 5.3^{ab}	62.8 ± 14.8^{b}	56.7 ± 13.5^{b}
DPAn-3	28.3 ± 2.8^{ab}	18.8 ± 1.5^{a}	37.9 ± 6.1^{b}	23.4 ± 2.2^{a}
DHA	164 ± 22^{a}	309 ± 29^{ab}	534 ± 67^{c}	425 ± 47^{bc}
TPAn-3	0.47 ± 0.04	0.33 ± 0.04	0.53 ± 0.1	0.36 ± 0.04
THA	3.15 ± 0.33	2.19 ± 0.25	3.37 ± 0.61	2.86 ± 0.40
Total N-6 Fatty Acids (nmol/mL)				
Fatty Acid	0DHA	2DHA	4DHA	8DHA
LNA	1498 ± 88	1228 ± 69	1427 ± 59	1371 ± 64
GLA	22.2 ± 2.2^{a}	11.4 ± 1.1^{b}	17.3 ± 1.9^{ab}	22 ± 6.8^{a}
20:2n-6	24 ± 3^{a}	12.3 ± 1.3^{b}	16.5 ± 3.2^{ab}	11.8 ± 1.9^{b}
DGLA	36.2 ± 4.8	25.3 ± 3.3	41.6 ± 4.9	45.4 ± 8
ARA	1473 ± 112	1541 ± 100	1608 ± 86	1444 ± 87
22:4n-6	53.6 ± 4.7^{a}	30.7 ± 2.8^{b}	35.7 ± 3.3^{b}	17.5 ± 1.8^{c}
DPAn-6	57.8 ± 7^{a}	19.2 ± 1.7^{b}	14.7 ± 1.3^{b}	7.57 ± 0.68^{b}
24:4n-6	0.67 ± 0.03^{a}	0.47 ± 0.04^{b}	0.44 ± 0.04^{bc}	0.32 ± 0.02^{c}
24:5n-6	1.67 ± 0.21^{a}	0.74 ± 0.11^{b}	0.68 ± 0.11^{b}	0.4 ± 0.05^{b}

Different superscript letters represent statistically different values between diets as determined by Tukey's post hoc test following a significant one-way ANOVA, $p < 0.05$. Values are expressed as means ± SEM, n = 7 – 8. ALA, α-linolenic acid; ARA, arachidonic acid; DGLA, di-homo-γ-linolenic acid; DHA, docosahexaenoic acid; DPAn-3, n-3 docosapentaenoic acid; DPAn-6, n-6 docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LNA, linoleic acid; SDA, stearidonic acid; THA, tetracosahexaenoic acid; TPAn-3, n-3 tetracosapentaenoic acid; 0DHA, no DHA feeding; 2DHA, 2weeks of DHA feeding; 4DHA, 4 weeks of DHA feeding; 8DHA, 8 weeks of DHA feeding.

Table 2 - Kinetic parameters for whole-body synthesis-secretion of EPA, DPAn-3 and DHA from plasma unesterified ALA and EPA

Source	Fatty Acid	Diet	Maximum First Derivative (S_{max}, nmol/min)	Synthesis-secretion coefficient ($k_{1,n}$, ml/min)	Daily synthesis-secretion rate (nmol/d)	Turnover (%/day)	Half-life (d)
ALA	EPA	0DHA	0.031 ± 0.013^{ab}	0.044 ± 0.016^{a}	336 ± 81	2.09 ± 0.55	0.56 ± 0.15
		2DHA	0.115 ± 0.05^{a}	0.238 ± 0.104^{b}	4679 ± 3635	38.4 ± 34.3	0.27 ± 0.07
		4DHA	0.02 ± 0.006^{b}	0.044 ± 0.013^{a}	854 ± 330	1.57 ± 0.37	1.17 ± 0.66
		8DHA	0.021 ± 0.002^{ab}	0.021 ± 0.001^{a}	441 ± 98	2.68 ± 1.46	0.77 ± 0.2
	ANOVA		*0.011**	*0.005**	*0.208*	*0.045**	*0.045**
	DPAn-3	0DHA	0.029 ± 0.013	0.040 ± 0.015^{ab}	464 ± 254	2.09 ± 1.00^{a}	1.46 ± 0.6^{a}
		2DHA	0.098 ± 0.034	0.194 ± 0.06^{a}	2117 ± 775	16.0 ± 6.6^{b}	0.38 ± 0.24^{b}
		4DHA	0.033 ± 0.015	0.081 ± 0.044^{ab}	1011 ± 357	2.88 ± 0.87^{ab}	0.4 ± 0.09^{ab}
		8DHA	0.018 ± 0.005	0.020 ± 0.008^{b}	459 ± 202	3.74 ± 1.47^{ab}	0.55 ± 0.15^{ab}
	ANOVA		*0.064*	*0.019**	*0.139*	*0.045**	*0.045**
	DHA	0DHA	0.008 ± 0.005	0.01 ± 0.005	157 ± 120	0.12 ± 0.08	48.2 ± 17.1
		2DHA	0.009 ± 0.003	0.02 ± 0.009	143 ± 57	0.06 ± 0.03	41.3 ± 19
		4DHA	0.004 ± 0.001	0.01 ± 0.004	132 ± 39	0.03 ± 0.01	86.2 ± 61.4
		8DHA	0.005 ± 0.001	0.006 ± 0.002	136 ± 56	0.32 ± 0.26	36.2 ± 11.5
	ANOVA		*0.688*	*0.679*	*0.588*	*0.890*	*0.890*
EPA	DPAn-3	0DHA	0.064 ± 0.027^{ab}	0.501 ± 0.149^{ab}	243 ± 66	1.12 ± 0.29^{a}	1.84 ± 0.87^{a}
		2DHA	0.289 ± 0.13^{a}	2.041 ± 0.888^{a}	2039 ± 994	17.3 ± 10.1^{b}	0.40 ± 0.20^{b}
		4DHA	0.055 ± 0.022^{ab}	0.6 ± 0.196^{ab}	2550 ± 1627	5.95 ± 2.97^{ab}	0.45 ± 0.15^{ab}
		8DHA	0.024 ± 0.003^{b}	0.163 ± 0.025^{b}	501 ± 159	3.03 ± 0.69^{ab}	0.35 ± 0.07^{b}
	ANOVA		*0.023*	*0.030*	*0.113*	*0.047*	*0.047*
	DHA	0DHA	0.015 ± 0.005	0.132 ± 0.036	70 ± 22^{a}	0.06 ± 0.02	63.3 ± 34.5
		2DHA	0.081 ± 0.046	0.569 ± 0.31	341 ± 199^{ab}	0.12 ± 0.06	17.7 ± 5.6
		4DHA	0.021 ± 0.011	0.305 ± 0.17	843 ± 409^{b}	0.16 ± 0.08	19.4 ± 5.5
		8DHA	0.015 ± 0.006	0.102 ± 0.036	270 ± 76^{ab}	0.11 ± 0.03	14.2 ± 5.1
	ANOVA		*0.299*	*0.411*	*0.045*	*0.662*	*0.622*

Different superscript letters represent statistically different kinetic values between diets and within a kinetic measure for each individual n-3 PUFA as determined by Tukey's post hoc test following a significant one-way ANOVA, $p < 0.05$. One-way ANOVA p values are represented in *italics* with a * representing a significance level of $p < 0.05$. Values are expressed as means ± SEM, n = 7 – 8. ALA – α-linolenic acid; DHA, docosahexaenoic acid; DPAn-3 – n-3 docosapentaenoic acid; EPA, eicosapentaenoic acid; 0DHA, no DHA feeding; 2DHA, 2weeks of DHA feeding; 4DHA, 4 weeks of DHA feeding; 8DHA, 8 weeks of DHA feeding.

Figure 1

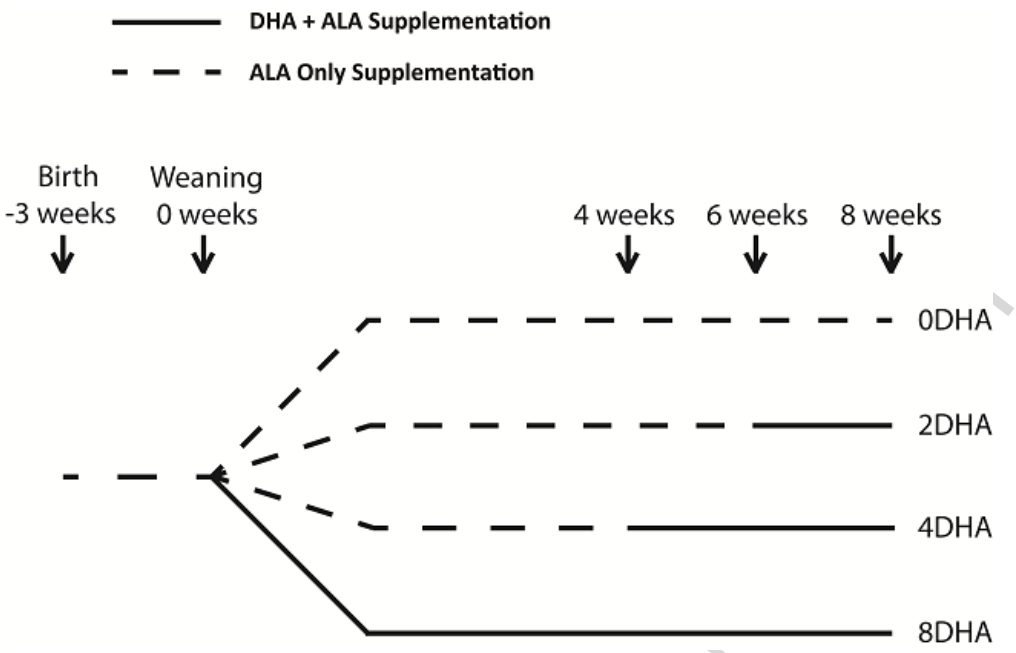

Figure 1 – Study feeding design and timeline implemented for 8 weeks post-weaning. ALA, α-linolenic acid; DHA, docosahexaenoic acid; 0DHA, no DHA feeding; 2DHA, 2weeks of DHA feeding; 4DHA, 4 weeks of DHA feeding; 8DHA, 8 weeks of DHA feeding.

Figure 2

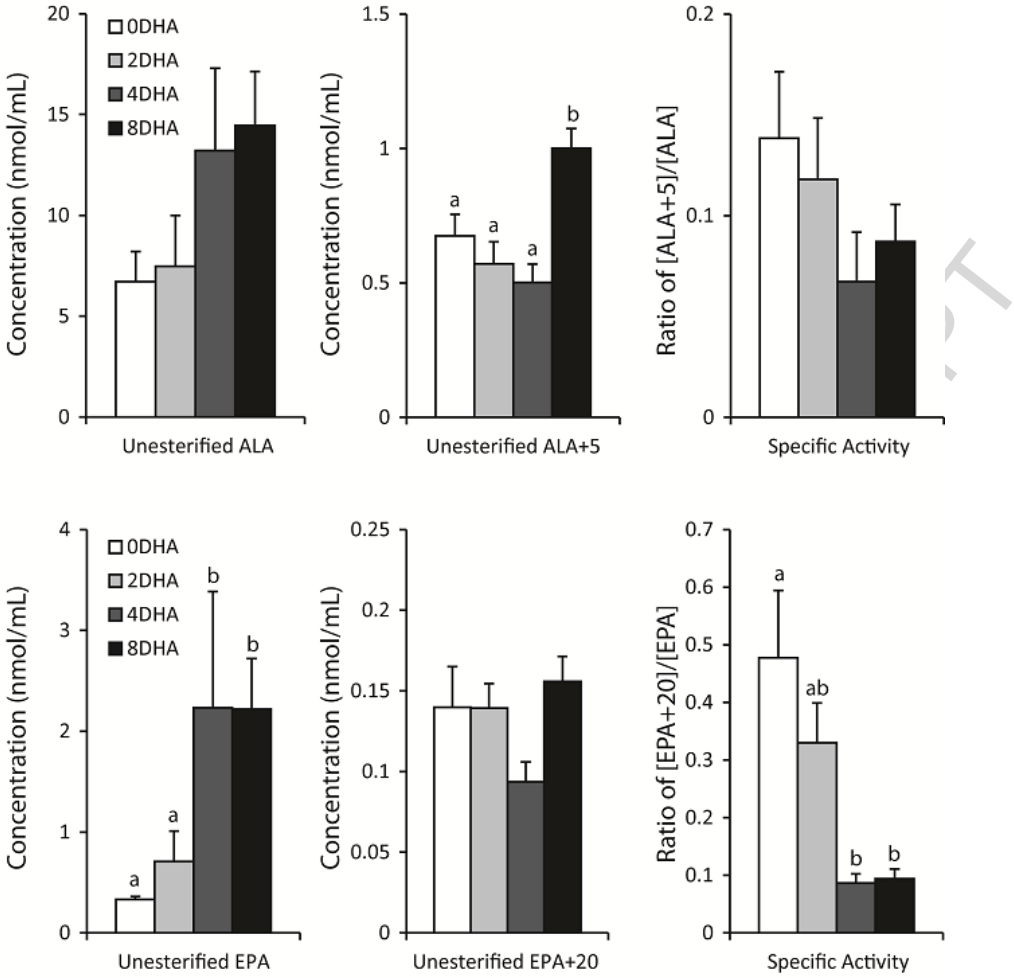

Figure 2 – Plasma unesterified fatty acid, average labeled unesterified fatty acid infusate and specific activity during 3-hour steady-state co-infusion of unesterified $^{2}H_{5}$-ALA and $^{13}C_{20}$-EPA after an 8-week dietary intervention. Different superscript letters represent statistically different values between diets as determined by Tukey's post hoc test following a significant one-way ANOVA, $p < 0.05$. Values are expressed as means ± SEM, n = 7 – 8. DHA, docosahexaenoic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; 0DHA, no DHA feeding; 2DHA, 2weeks of DHA feeding; 4DHA, 4 weeks of DHA feeding; 8DHA, 8 weeks of DHA feeding.

Figure 3

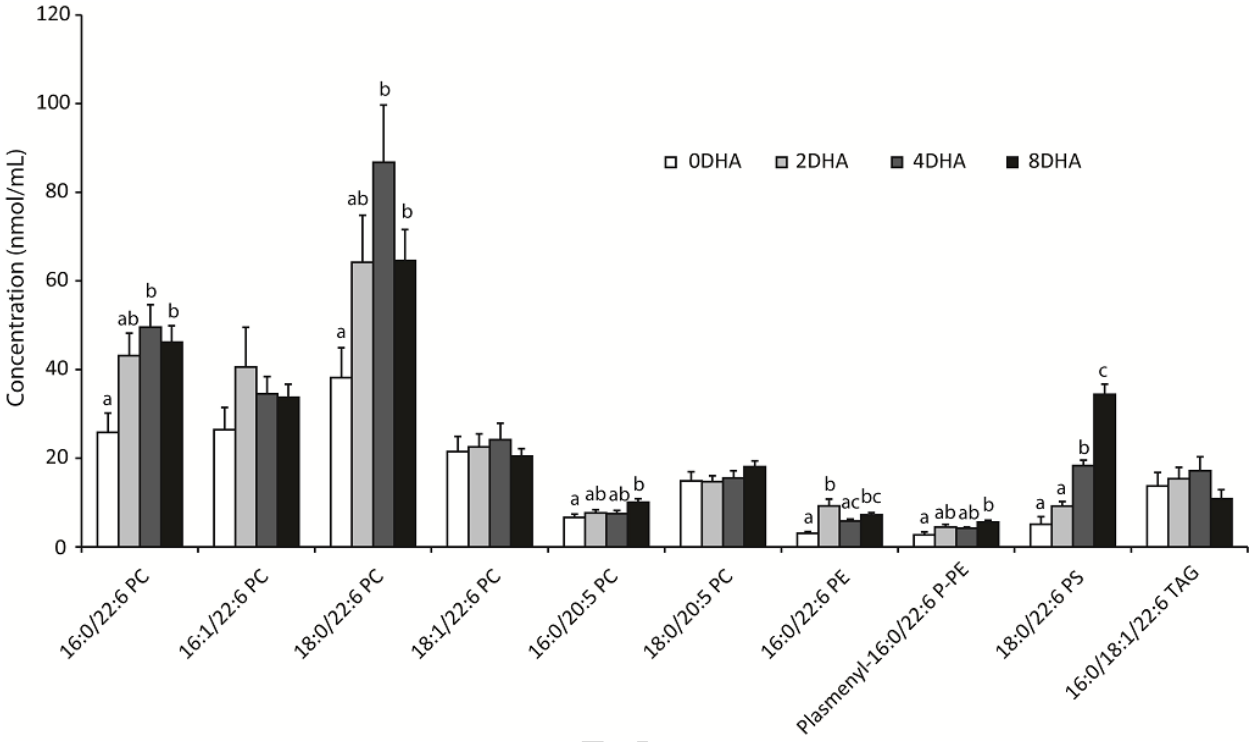

Figure 3 – Concentrations of most abundant EPA and DHA-containing lipid species in whole blood following 8 weeks of dietary intervention. Different superscript letters represent statistically different concentrations between diets and within lipid species as determined by Tukey's post hoc test following a significant one-way ANOVA, $p < 0.05$. Values are expressed as means ± SEM, n = 7 – 8. DHA, docosahexaenoic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; TAG, triacylglycerol; 0DHA, no DHA feeding; 2DHA, 2weeks of DHA feeding; 4DHA, 4 weeks of DHA supple feeding mentation; 8DHA, 8 weeks of DHA feeding.

Figure 4

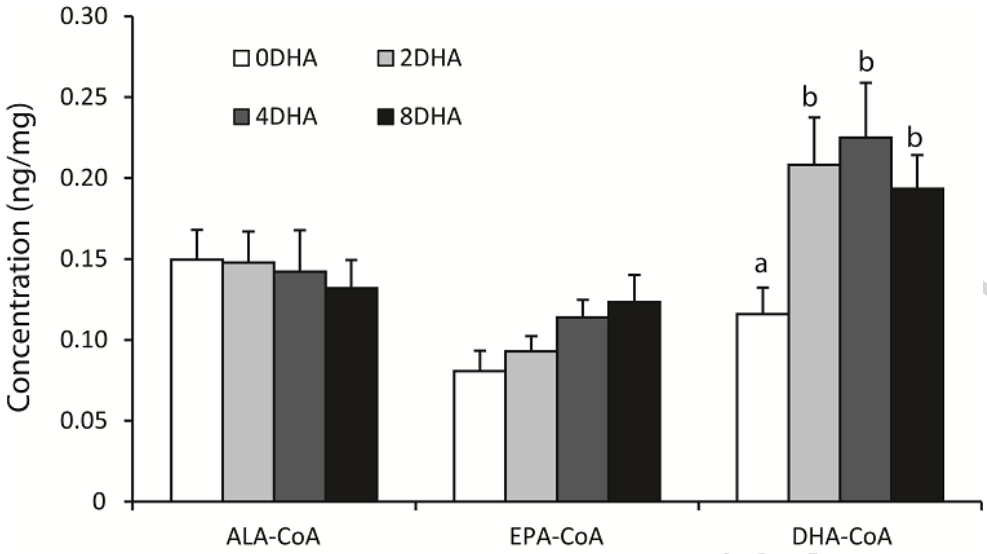

Figure 4 – ALA-CoA, EPA-CoA and DHA-CoA liver concentrations following 8 weeks of dietary intervention. Different superscript letters represent statistically different concentrations between diets and acyl-CoA species as determined by Tukey's post hoc test following a significant one-way ANOVA, $p < 0.05$. Values are expressed as means ± SEM, n = 7 – 8. ALA, α-linolenic acid; CoA, coenzyme A; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; 0DHA, no DHA feeding; 2DHA, 2weeks of DHA feeding; 4DHA, 4 weeks of DHA feeding; 8DHA, 8 weeks of DHA feeding.

Figure 5

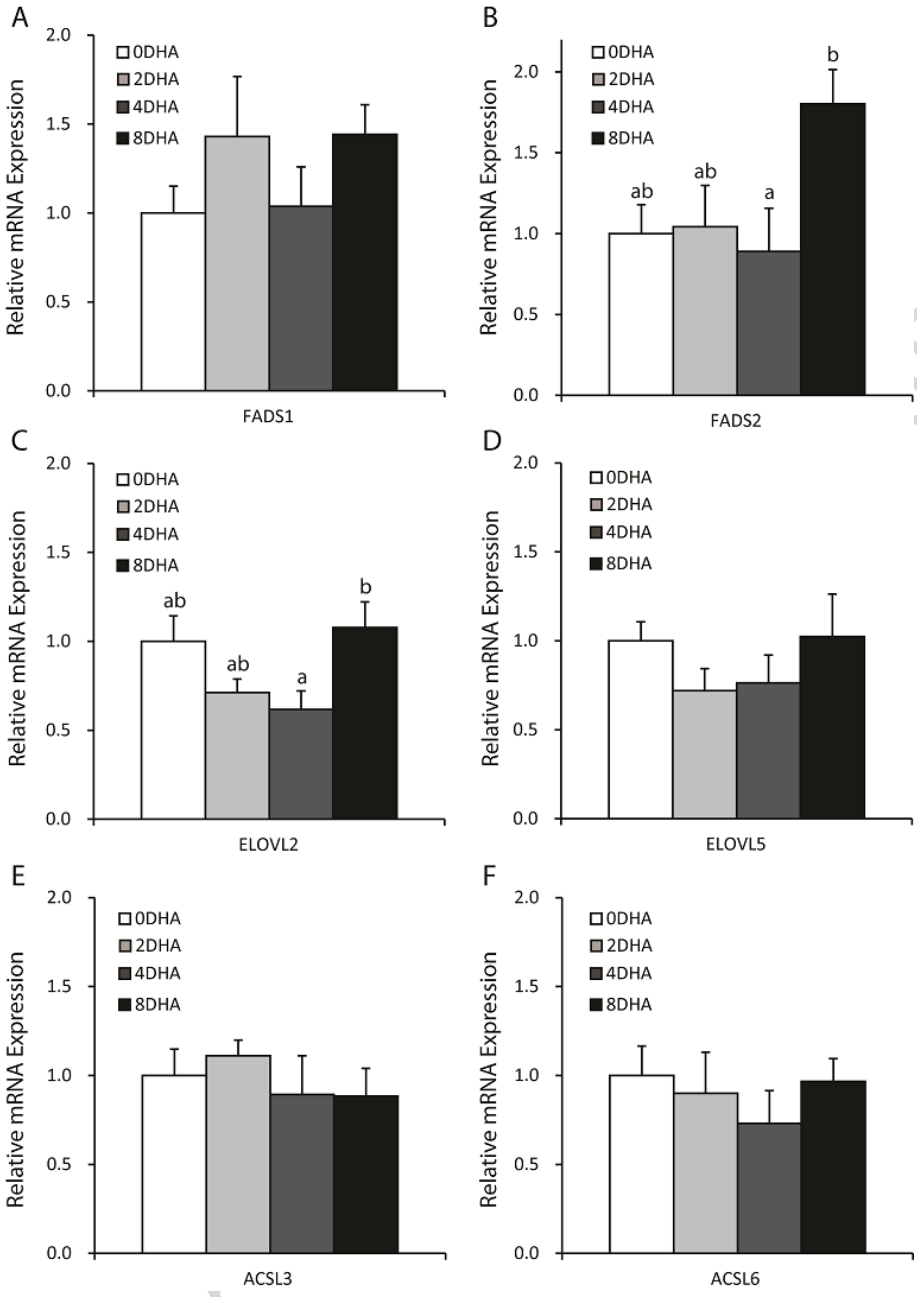

Figure 5 – Liver mRNA expression following 8 weeks of dietary intervention. Different superscript letters represent statistically different concentrations between diets and acyl-CoA species as determined by Tukey's post hoc test following a significant one-way ANOVA, $p < 0.05$. Values are expressed relative to the 0DHA group as means ± SEM, n = 7 – 8. ACSL, long-chain fatty acyl-CoA synthetase; CoA, coenzyme A; DHA, docosahexaenoic acid; ELOVL, elongation of very long-chain; FADS, fatty acid desaturase; 0DHA, no DHA feeding; 2DHA, 2weeks of DHA feeding; 4DHA, 4 weeks of DHA feeding; 8DHA, 8 weeks of DHA feeding.

Highlights

1. Isotopic fatty acid kinetic modelling is determined after dietary DHA feeding
2. Synthesis-secretion coefficients for EPA and DPAn-3are higher after 2 weeks DHA
3. Daily DHA synthesis-secretion rate is higher after 4 weeks DHA
4. DHA kinetics are not different after 8 weeks DHA compared to ALA
5. ELOVL2 and FADS2 expression are lower after 4 weeks compared to 8 weeks DHA