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Original Research Article

Determining the half-life and turnover rate of EPA, n–3 docosapentaenoic acid, and DHA in humans using the natural abundance of carbon-13: a secondary analysis of a randomized clinical trial

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A B S T R A C T

Background: Tools to measure omega-3 (n–3) polyunsaturated fatty acid (PUFA) half-lives and turnover deserve attention, as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play vital roles in the body. Plants and marine life vary in fixed carbon-13 signatures ($\delta^{13}C$), allowing *in vivo* tracing.

Objectives: To determine the half-lives and turnover of n–3 PUFAs using $\delta^{13}C$ in this secondary analysis.

Methods: During a 28-d randomized crossover study (6-wk wash-in phase/washout phase), 12 participants (aged 19–34 y) were supplemented with 4.2 g/d α-linolenic acid (ALA) (flax oil) or 4.3 g/d DHA, 1.0 g/d EPA, and 0.2 g/d docosapentaenoic acid (n–3 DPA) (fish oil). Blood was collected on days 0, 1, 3, 7, 14, and 28. Plasma n–3 PUFA $\delta^{13}C$ signatures were analyzed by gas chromatography–isotope ratio mass spectrometry (GC-IRMS) to calculate half-lives and turnover.

Results: $\delta^{13}C$ signatures of flax oil ALA, fish oil EPA, n–3 DPA, and DHA were -33.2 ± 1.4, -26.3 ± 2.2, -26.2 ± 1.5, and -25.4 ± 1.3 mUr, respectively. Baseline signatures were not statistically different. Over time, $\delta^{13}C$ of EPA, n–3 DPA, and DHA converged toward the isotopic signature of the fish oil supplement ($P < 0.05$). Using $\delta^{13}C$ signatures, half-lives of EPA, n–3 DPA, and DHA (3.4 ± 2.7, 6.4 ± 5.3, and 6.3 ± 8.9 d, mean ± SD, respectively) and turnover rates (61.9 ± 53.1, 6.0 ± 2.9, and 78.0 ± 44.5 nmol/mL/d, respectively) were calculated.

Conclusions: This is the first study to investigate n–3 PUFA half-lives and turnover in humans using GC-IRMS and the natural variance of ^{13}C in commercial fish oils. These results were similar to preclinical model values and other clinically used methods but added the first estimate for n–3 DPA half-life and turnover rate. Compound-specific isotope analysis is a valuable tool in human studies for determining n–3 PUFA half-lives and turnover rate, as well as factors affecting them, including diet, exercise, sex, genetics, and disease.

Keywords: fatty acid metabolism, omega-3 PUFAs, DHA, MS, biomarkers, compound-specific isotope analysis, half-life, turnover rates

Introduction

DHA, an omega-3 (n–3) PUFA, is vital for cell membrane structure and is abundant throughout the nervous system, particularly in the brain and eyes, contributing up to 97% and 93% of the total n–3 PUFAs present, respectively [1,2]. Low DHA concentrations have been associated with impaired brain development in infants, suboptimal vision, and an increased risk of neurological and psychiatric diseases like Alzheimer disease and attention-deficit/hyperactivity disorder [1–3]. Whether these lower concentrations are due to a lack of DHA in the diet or increased metabolic consumption leading to higher DHA turnover rates is not yet agreed upon.

Abbreviations: ALA, α-linolenic acid; BF3, boron trifluoride; ^{13}C, carbon-13; CI, confidence interval; CSIA, compound-specific isotope analysis; DPA, docosapentaenoic acid; FAME, fatty acid methyl ester; FFQ, food frequency questionnaire; GC-FID, gas chromatography-flame ionization detection; GC-IRMS, gas chromatography-isotope ratio mass spectrometry; Q-Q, quantile-quantile; RMSE, root mean square error; USGS, United States Geological Survey.

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DHA and EPA are concentrated in oily fish, shellfish, and algae, and n–3 PUFA supplements formulated from these are all viable sources [4,5]. Docosapentaenoic acid (n–3 DPA) is found in similar foods and supplements but in lower concentrations [6]. Unlike DHA and EPA, the functions of n–3 DPA are lesser known. Aside from being a potential reservoir for DHA, n–3 DPA has been shown to promote inflammation resolution, lower triglycerides, and to be converted to a series of oxylipins whose functions are yet to be fully understood [7].

Dietary assessment methods, such as food frequency questionnaires (FFQs), recalls and records, and plasma concentrations used to assess DHA consumption and metabolism in studies, are useful tools with inherent strengths but substantial limitations [8]. For example, dietary assessment methods like FFQs show recall bias, with inconsistent results and poor validity, whereas DHA plasma concentrations can be affected by sex, genetics, obesity, and chronic disease [8,9]. Additional methods should be explored to better understand n–3 PUFA metabolism with the goal of informing dietary requirements.

Compound-specific isotope analysis (CSIA) is a technique that has been used to trace fatty acids in rodent tissues and human plasma [10–17]. Due to differences in photosynthetic processes, different carbon fixation pathways exist, and as a result, plants and marine life have varying amounts of carbon-13 (^{13}C) in their produced nutrients [18]. This results in plants being categorized into either C3 or C4 plants, where C3 plants (i.e., soy and flax) have a naturally lower or more negative ^{13}C signature or value, typically between −23 and −32 mUr, compared with C4 plants (i.e., corn and sugarcane), which have higher or less negative values, −10 to −16 mUr [10]. Marine sources like fish tend to have an intermediate ^{13}C range (−18 to −30 mUr) [8]. Upon consumption, ^{13}C-containing nutrients are incorporated into tissues, allowing investigators to trace their metabolism via CSIA [10–17]. CSIA measures the natural abundance of the ^{13}C:^{12}C isotope ratio ($\delta^{13}C$) in targeted compounds, allowing for a relatively cost-effective, natural tracer technique. As $\delta^{13}C$ is not modified in metabolism, the investigators could trace the metabolism of dietary food components like DHA in humans with repeated dosing over extended periods. Using these $\delta^{13}C$ signatures, half-lives and turnover rates of these nutrients can be determined, providing important insight into n–3 PUFA metabolism. Turnover rates in particular have been shown to be a primary determinant of tissue and blood n–3 PUFA concentrations and can help to determine the relationship between n–3 PUFA consumption, synthesis, and concentrations [19].

Prior to the current analysis, EPA, n–3 DPA, and DHA half-lives and turnover had not been investigated in adult humans using CSIA. Previously, highly enriched and uniformly labeled fatty acids have been used in studies to determine half-lives in humans [20,21]; however, with this secondary analysis of a clinical trial [22], the investigators validated the use of CSIA at the natural abundance level in human plasma with commercially available supplements. Using CSIA, the investigators determined the half-lives (days) and turnover rates (nmol/mL/d) of EPA and DHA (3–7 d and 60–80 nmol/mL/d), as well as n–3 DPA (6.4 d and 6.0 nmol/mL/d) in human plasma.

Methods

Study design

In a double-blind crossover study, 12 healthy participants aged 19–34 y were supplemented with either 4.2 g/d of α-linolenic acid (ALA) from flax oil or 4.3 g/d DHA from fish oil for 28 d with 6-wk wash-in and washout phases (Figure 1) [22]. As this is an exploratory secondary analysis of a randomized clinical trial, the study design, sample size calculation, intervention details, recruitment period, participant losses, harms, baseline characteristics, etc., are reported in the study by Gabbs et al. [22]. Participants underwent a minimum 6-wk wash-in phase, during which they were instructed to avoid foods high in ALA (≥0.3 g/serving) or EPA + DHA (≥0.1 g/serving) and to maintain these restrictions for the remainder of the study. Participants were randomly assigned to either the flax oil supplement or the fish oil supplement for the first phase and then provided the other for the second phase. This was done according to a statistician-generated randomization code.

At baseline (day 0), participants' height, weight, and blood pressure were measured, and blood samples were collected after an overnight fast. Following baseline, participants provided fasted blood samples on days 1, 3, 7, 14, and 28. The methods for blood collection have been previously described in detail in the study by Gabbs et al. [22]. Briefly, after being extracted by a trained phlebotomist in BD Vacutainer Blood Collection Tubes containing EDTA, blood samples were centrifuged at 1100 rpm at 4°C, and then an antioxidant cocktail was added to the aliquots before samples were aliquoted and frozen and stored in a −80°C freezer until undergoing analysis. Adherence to the study and its required baseline diet, which excludes n–3 PUFA-rich foods, was assessed by the number of supplements returned, plasma

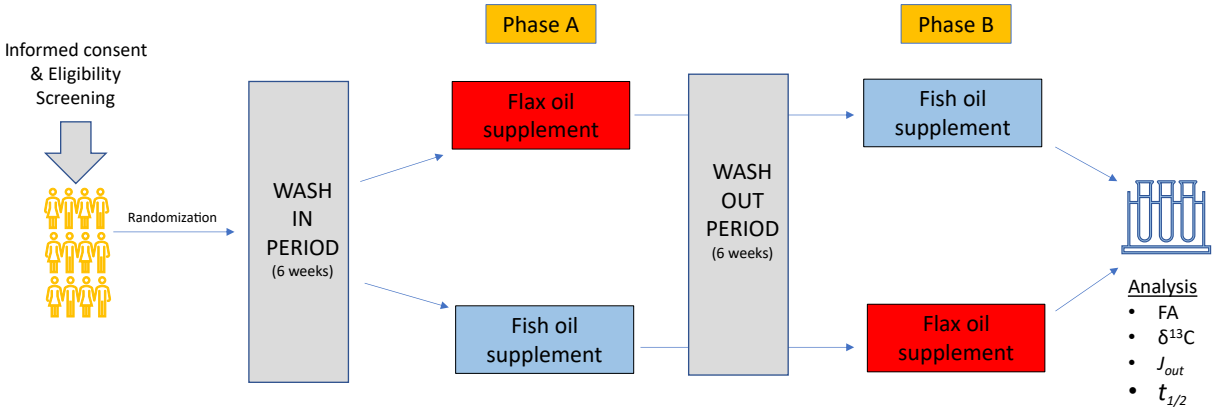


FIGURE 1. Study design of a secondary analysis of a clinical trial. Twelve healthy males and females aged 19–34 y with a BMI between 18 and 28 kg/m^2 were randomized in a double-blind crossover design to receive either 4.2 g/d of ALA from flax oil or 4.3 g/d DHA, 1.0 g/d of EPA, and 0.2 g/d n–3 DPA from fish oil for 28 days with 6-wk wash-in and wash out phases. Plasma samples from baseline, and days 1, 3, 7, 14, and 28 were taken during each phase.

fatty acid composition, and the completion of 3-d food records [22]. The wash-in and washout phases varied in length to ensure female participants began the supplementation phase, 9 ± 2 d after the beginning of menses.

Participants

Participants were recruited from December 2014 to August 2015. The intervention was administered from January 2015 to February 2016, and no follow-up data collection occurred postintervention. As described in the study by Gabbs et al. [22], participants were recruited in Winnipeg, Manitoba, via multiple media outlets. Ethics approval was provided by the University of Manitoba Research Ethics Board and St Boniface Hospital Research Review Committee, and the trial was registered in December 2014 on clinicaltrials.gov: NCT02317588 (https://www.clinicaltrials.gov/study/NCT02317588). After obtaining informed consent, 12 individuals (6 males, 6 nonpregnant, and nonlactating females) aged 19–34 y with a BMI between 18 and 28 kg/m^2 and blood pressure <140/90 mm Hg were enrolled. Participant inclusion and exclusion criteria, as well as detailed subject characteristics, are presented in the study by Gabbs et al. [22]. Participants needed to be healthy and free from disease or infections, nonsmokers, have a stable body weight for the last 6 mo (±3 kg), drink <15 alcoholic beverages per week, and have not consumed any anti-inflammatory drugs or n–3 PUFA supplements in the last 3 mo.

Supplements

Daily doses of either 4.2 g ALA from flax oil (NOW Foods; natural product number: 80002313) or 4.3 g DHA, 1.0 g/d of EPA, and 0.2 g/d n–3 DPA from fish oil (Super DHA Gems; Carlson Laboratories; natural product number: 80012587) were consumed. Capsules were packaged by a pharmacy into opaque packs to maintain blinding [22]. The fatty acid composition and δ^{13}C signatures of the supplements were analyzed by GC-flame ionization detection (FID) and GC-combustion–isotope ratio MS (GC-C-IRMS), respectively. The δ^{13}C signatures of the ALA found in the flax oil supplement and the DHA found in the fish oil supplement were −33.2 and −25.4 mUr, respectively (Table 1).

TABLE 1
Fatty acid composition and δ^{13}C signatures of n–3 PUFA supplements (± SD).

Fatty acid	Relative % of fatty acids	
	Flax oil	Fish oil
SFAs	8.38 ± 0.06	8.90 ± 0.07
MUFAs	15.89 ± 0.03	14.84 ± 0.08
n–6 PUFAs	15.01 ± 0.03	5.31 ± 0.01
18:3n–3	60.02 ± 0.09	0.40 ± 0.02
18:3n–3 δ^{13}C	−33.25 ± 1.40	−27.41 ± 3.65
20:3n–3	0.04 ± 0.01	0.29 ± 0.001
20:5n–3	0.02 ±0.01	12.14 ± 0.03
20:5n–3 δ^{13}C	ND	−26.26 ± 2.24
22:5n–3	ND	2.33 ± 0.03
22:5n–3 δ^{13}C	ND	−26.18 ± 1.53
22:6n–3	ND	49.93 ± 0.69
22:6n–3 δ^{13}C	ND	−25.40 ± 1.29
n–3 PUFAs	60.1 ± 0.04	65.10 ± 0.30

Abbreviations: δ^{13}C, delta carbon-13; ND, not detected.
Supplements were measured in triplicate. Values are means ± SD.

Lipid extraction

Plasma samples were sent from Winnipeg, Canada, to Toronto, Canada, on dry ice. Samples were extracted using the Folch method and converted to fatty acid methyl esters (FAMEs) and checked for quality via GC-FID as previously described [13,23]. One male participant missed the days 14 and 28 visits for the DHA supplementation phase, and so lipid extraction was not done for this participant on these days. With 100 µL of plasma, lipid extraction was performed using a 2:1 chloroform:methanol solution and 50 µL (4 mg/mL concentration) of an internal standard composed of triheptadecanoin, cholesteryl heptadecanoate, and heptadecanoic acid from Nuchek Prep, Inc and 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine from Avanti Research. Samples were vortexed, and then before centrifuging for 5 min at 1460 rpm, 1 mL potassium chloride (0.88 g/100 mL; 0.88%) was added. The lower chloroform phase, which contains dissolved lipids, was removed by pipette and added into glass test tubes. This was then dried under nitrogen, and 300 µL of hexane and 1 mL of 14% boron trifluoride (BF3)–methanol complex was added. All samples were methylated using a single bottle of BF3–methanol complex to prevent differing δ^{13}C-methyl among BF3–methanol lots. This was vortexed and then incubated for 1 h at 100°C, checking every 15 min for evaporation. Once cooled, 1 mL of Milli-Q water and 1 mL of hexane was added, and the samples were vortexed again. Following centrifugation at 1460 rpm for 5 min, the upper hexane layer containing the FAMEs was transferred to small test tubes, and they were dried down under nitrogen. Samples were then reconstituted with 100 µL of heptane, vortexed, and then transferred into 0.25 mL inserts, recapped, and put onto the GC-FID to check for quality and peak identification, and then the GC-IRMS for δ^{13}C signature analysis.

FAME analysis by GC-FID and GC-IRMS

Fatty acid concentrations were previously reported in the study by Gabbs et al. [22] from baseline and days 1, 3, 7, 14, and 28, and here, the investigators present the mean values. Three µL were injected into a 100 m, 0.25 mm i.d., 0.20 µm df Supelco SP-2560 (by Sigma–Aldrich) capillary column in a Thermo Scientific Trace 1310 GC interfaced to an MAT 253 IRMS (Thermo Finnigan MAT) by a GC Iso Link II combustion interface (Thermo Scientific) using a TriPlus RSH autosampler (Thermo Fisher Scientific). The carbon isotope ratio and separation of the n–3 PUFAs of interest were executed by the following column temperature parameters: 60°C then increasing the rate to 15°C/min until 180°C followed by a 1.5°C/min rate increase until 240°C with an 18-min hold, totaling 66 min. A carrier flow rate of 1.2 mL/min was set. Following that, isolated fatty acids were combusted using GC-Isolink II held at 1000°C and interfaced to MAT 253 IRMS (Thermo Fisher Scientific) through a continuous flow of ConFlo IV (Thermo Fisher Scientific). The carbon dioxide was then dried by gas through a Nafion dryer before entering the MAT 253 IRMS ion source for isotope detection. Stop and start slopes of 0.2 mV/s and 1.4 mV/s with distinct corrections were used to integrate peak detection. At 1020°C in the oxidation and 650°C in the reduction reactors of a ConFlo IV continuous-flow interface (Thermo Fisher Scientific), CO_2 gas was introduced to the MAT 253 IRMS [13]. A linearity test of the fatty acid peak range for MAT 253 IRMS was quantified using a 10-point concentration curve (0.00075, 0.003, 0.0075, 0.015, 0.15, 0.45, 0.75, 2, 5, and 10 mg/mL). Linearity test samples of United States Geological Survey 71 (USGS71) were injected, and amplitude 44 as well as δ^{13}C signature were plotted for simple regression analysis. The limits of quantification/detection for fatty acid isotope analysis were determined to be in the peak range of 23 mV (0.75 µg/mL) to 19,000 mV (10 mg/mL). FID runs were used to calculate the

estimated peak on IRMS prior to IRMS separation. Peaks above or below the linearity peak range were adjusted by either reconstitution solvent volume or injection volume to ensure all fatty acids of interest were within the quantification range. Run quality and $\delta^{13}C$ reproducibility were monitored with 3 independent USGS samples every 10 samples. A linearity test was conducted biweekly to ensure the precision of the isotope analysis.

Carbon isotope ratio normalization

Similar to previously described methods [13], all carbon isotope ratios detected by the IRMS were normalized using consensus-validated 20-carbon FAME reference material USGS70, USGS71, and USGS72 (Reston Stable Isotope Laboratory) with isotope ratios of −30.53, −10.50, and −1.54, respectively. Reference materials were created and injected 2 times through each round of samples on the IRMS. Unadjusted linear regression equations were created and used to generate normalizing equations for each round of samples. The R^2 values calculated for all equations were >0.9883.

Statistics and calculations

Sample size

This is a secondary analysis of a clinical trial, and the original sample size was calculated by Gabbs et al. [22] for their primary outcome and was based on pilot data of 5 individuals supplemented with an ALA- or EPA/DHA-rich supplement for 6 wk. Using presupplementation and postsupplementation, means and SDs of oxylipins derived from ALA (9-hydroxy-octadecatrienoic acid) and DHA (17-hydroxy-DHA), $n = 7$ and 3, respectively, provided a power of >0.80 with an $\alpha = 0.05$. An $n = 12$ was used to provide sufficient power for oxylipins present at low concentrations and to account for potential dropouts [22].

Half-lives and turnover rates

Data are reported as means ± SDs. Half-lives and turnover rates were estimated via nonlinear mixed-effects modeling of $\delta^{13}C$ signature time courses, including treatment, period, and sequence effects; missing observations were handled implicitly via maximum likelihood (missing at random assumption). As 1 male participant missed day 14 and 28 visits for the DHA supplementation phase model–based estimation, all observed time points were used, and the missing data were left blank. A sensitivity check was run via complete case analysis, and the results were statistically similar when excluding the participant's data. Due to the missing data, a mixed model repeated-measures 2-way analysis of variance model (supplement × time) was run to determine the difference between supplementation groups for ALA, EPA, n–3 DPA, and DHA $\delta^{13}C$ signatures. As a crossover design was used, the data were also analyzed to include period and sequence in the model with the mixed-effects model, $\delta^{13}C = \beta_0 + \beta_1$ Treatment $+ \beta_2$ Time $+ \beta_3$ (Treatment × Time) $+ \beta_4$ Period $+ \beta_5$ Sequence $+ u + \varepsilon$, with a subject-specific random intercept u and a first-order autoregressive within-subject correlation structure. Type 3 tests with Kenward–Roger degrees of freedom were reported.

For half-life ($t_{1/2}$) calculations, GraphPad Prism 10.6.0 was used, and statistical significance was determined by a P value < 0.05. To justify the sufficiency of the model, root mean square error (RMSE), quantile-quantile (Q-Q) plot and residual plot were used. For time point comparisons, Tukey-adjusted post hoc pairwise contrasts (fish fed compared with flax fed) were performed at each scheduled day. $\delta^{13}C$-DHA signatures were plotted over time and modeled with a 1-phase exponential decay function, generating a curve of best fit:

$$y = (y_0 - plateau)e^{-kx} + plateau$$

where y_0 is the best-fit curve at baseline, plateau is the value of the best-fit curve at an infinite time, and k is the decay constant. At the individual-subject level, the half-lives (in days) of EPA, n–3 DPA, and DHA were determined and summarized (mean ± SD) using the following formula:

$$\frac{1}{n}\sum_{i=1}^{n}\frac{\ln(2)}{k_i}$$

where k_i is the decay constant and n are the number of participants. Using the k values for each subject, the median, mean, and ±SD were calculated. To estimate 95% confidence intervals (CIs), the investigators applied the bootstrap method by resampling subject IDs 500 times; for each bootstrap sample, the mean half-life across subjects was recalculated, and the 2.5th and 97.5th percentiles of the resulting bootstrap distribution were used to construct the CI. The turnover of EPA, n–3 DPA, and DHA in plasma or turnover rate (J_{out}, nmol/mL/d) was calculated using the experimentally derived half-lives and the following formula:

$$J_{\text{out}} = (0.693 \times CFA)/t_{1/2}$$

where C_{FA} is the mean n–3 PUFA plasma concentration of participants calculated using the concentration data in the study by Gabbs et al. [22] over 28 d and $t_{1/2}$ is the experimentally derived half-life. To estimate the net incorporation rate of the n–3 PUFAs of interest, the n–3 PUFA turnover from the n–3 PUFA plasma pool, or the $J_{out,}$ is assumed to be equal when the net incorporation rate of the n–3 PUFAs and the n–3 PUFA turnover rate are in a steady state [24]. J_{out} values in mg/d for EPA, n–3 DPA, and DHA were also calculated based on plasma volume derived from participants' mean weight of 73.4 ± 11.2 kg, where total plasma volume was estimated using 3000 mL per 70 kg body weight [25].

Results

Changes in plasma n–3 PUFA $\delta^{13}C$ signatures

The plasma samples from baseline and days 1, 3, 7, 14, and 28 were analyzed by GC-IRMS to determine $\delta^{13}C$ n–3 PUFA signatures for the 2 supplement groups and were compared (Figure 2). At baseline, the $\delta^{13}C$ signature for the fish oil group was slightly lower for DHA (−26.99 mUr) than the flax oil group (−26.25 mUr). ALA, EPA, and n–3 DPA were slightly higher in the fish oil group (−33.10, −30.99, and −26.41 mUr, respectively) than the flax oil group (−33.21, −31.13, and −27.61 mUr, respectively). The plasma ALA $\delta^{13}C$ signatures at baseline were similar to the $\delta^{13}C$-ALA signatures present in the flax oil supplement, and there were no statistically significant differences within or between the groups after 28 d. However, EPA and n–3 DPA $\delta^{13}C$ signatures between groups were significantly different at day 28 for supplement ($P < 0.01$) and EPA, n–3 DPA, and DHA for time ($P < 0.0001$). The significant differences found for the supplement indicate a move over the course of the study toward the $\delta^{13}C$ signatures of the supplements. Moreover, a statistically significant interaction for time × supplement was seen in the $\delta^{13}C$ DHA signature ($P < 0.05$). Between supplement groups for DHA, there are no differences at baseline, day 1, or day 3; however, differences became statistically significant at days 7, 14, and 28 ($P = 0.016$, $P = 0.034$, and $P = 0.004$, respectively). For DHA, the time effect was dependent on the supplement; however, this significant interaction is primarily driven by the fish oil group. For the flax oil group, there were no significant differences between the signatures over time. Within the fish oil group, there were significant differences between baseline and day 7 ($P < 0.05$), day 1 and days 7, 14, and 28 ($P < 0.005$), as well as between day 3 and days 7, 14, and 28 ($P < 0.01$).

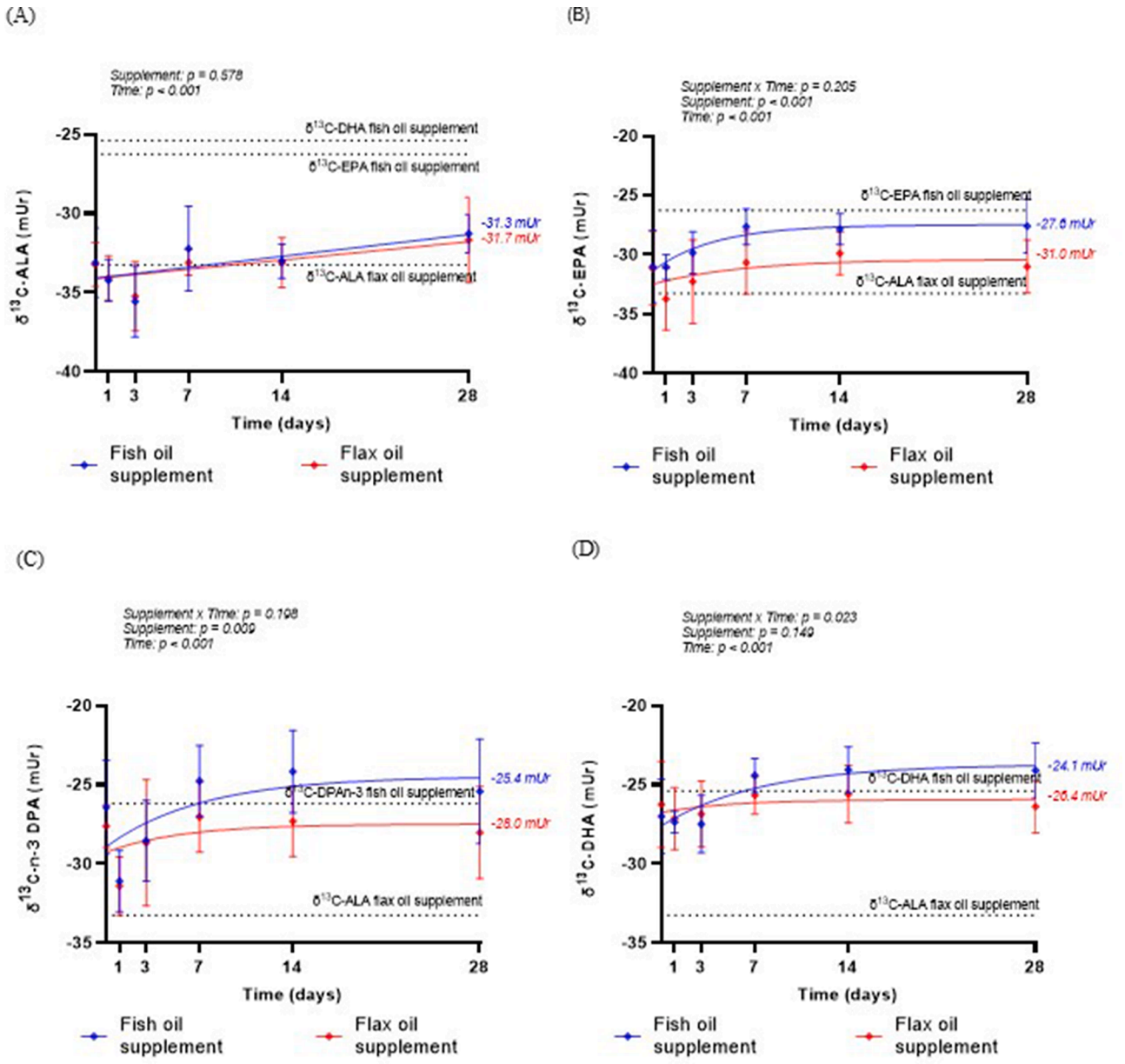


FIGURE 2. Changes in plasma δ^{13}C n–3 PUFA signatures. Change in omega-3 (n–3) PUFAs. (A) δ^{13}C-α-linolenic acid (ALA) vs. time, (B) δ^{13}C-eicosapentaenoic acid (EPA) vs. time, (C) δ^{13}C-n–3 docosapentaenoic acid (n–3 DPA) vs. time, and (D) DHA vs. Time following flax and fish oil supplementation in a crossover study. Dotted lines correspond to the signature of the fish oil supplement and flax oil supplement. Fish oil group is in blue and flax oil group is in red. Data are mean ± SD and modeled with a 1-phase decay function (n = 9–12 per time point). δ^{13}C, carbon-13 signature; mUr, millUrey.

Whether the sequence of the treatment affected results was investigated. For DHA, period/sequence were not significant (P = 0.588 and 0.744). Similarly for EPA, no period/sequence effects (P = 0.422 and 0.195) were seen, nor were they seen for n–3 DPA either. (P = 0.768 and 0.652). Because neither the period effect nor the sequence effect was statistically significant in the crossover mixed model, there is no evidence of residual carryover.

Plasma PUFA half-life and turnover rate

The plasma half-lives of EPA, n–3 DPA, and DHA from fish oil were calculated and mapped out at the individual-subject level (see Supplemental Figure 1: Individual δ^{13}C EPA, n–3 DPA, and DHA from fish oil group in Supplemental Materials) and summarized across subjects (Table 2). They were found to be 3.4 ± 2.7 (median: 2.8; 95% CI: 1.96, 5.06), 6.4 ± 5.3 (median: 4.5; 95% CI: 3.42, 9.55), and 6.3 ± 8.9 (median: 2.5; 95% CI: 1.95, 10.92) days, respectively. The plasma turnover rates of EPA, n–3 DPA, and DHA were calculated at the individual-subject level and summarized across subjects and were found to be 61.9 ± 53.1, 6.0 ± 2.9, and 78.0 ± 44.5 nmol/mL/d, respectively (Table 2). When corrected for mean plasma volume, J_{out} values for EPA, n–3 DPA, and DHA were 58.9 ± 50.5, 6.3 ± 3.1, and 80.6 ± 45.9 mg/d, respectively (Table 2). As the δ^{13}C-ALA signature of the flax oil supplement was similar to that of the participants' diet, plasma half-lives and turnover rates could not be modeled accurately.

TABLE 2
Plasma n–3 PUFA concentrations, half-lives, and turnover (± SD) in subjects supplemented with fish oil.

Fish oil group	EPA	n–3 DPA	DHA
Concentration (nmol/mL)[1]	176.8 ± 37.2	40.2 ± 7.8	312.9 ± 58.1
$t_{1/2}$ (d)	3.4 ± 2.7	6.4 ± 5.3	6.3 ± 8.9
J_{out} (nmol/mL/d)	61.9 ± 53.1	6.0 ± 2.9	78.0 ± 44.5
J_{out} (mg/d)	58.9 ± 50.5	6.3 ± 3.1	80.6 ± 45.9
y^0, mUr	−31.5 ± 2.2	−28.8 ± 2.6	−27.7 ± 1.5
Plateau, mUr	−27.1 ± 2.3	−23.7 ± 2.9	−22.8 ± 4.5
k, 1/d[2]	0.34 ± 0.3	0.16 ± 0.1	0.55 ± 1.0

Abbreviations: $t_{1/2}$, n–3 PUFA half-life in plasma; J_{out}, n–3 PUFA turnover rate, y^0, y-intercept; Plateau, value of the best-fit curve at an infinite time; k, decay constant.

Values are means ± SD; n–3 PUFA plasma concentration used in J_{out} calculation.

[1] Fatty acid concentrations were previously reported in Gabbs et al. [22] from baseline, and days 1, 3, 7, 14, and 28 and here we present the mean value.

[2] t1/2 and k values were averaged subject-by-subject and J_{out} values were calculated individually before averaging and calculating SD.

The RMSE and Q-Q plot results show that the nonlinear 1-phase exponential decay models are adequate to a good fit across all 3 fatty acids of interest. The RMSE values for DHA, n–3 DPA, and EPA were 1.913, 3.118, and 1.687, respectively. Relative to the measurement scale, which is approximately −20 to −35, these errors support acceptable predictive accuracy. The Q-Q plots indicate that residuals are approximately normally distributed for all 3 outcomes, with only minor departures in the tails, consistent with no major violation of the Gaussian error assumption. The residual plots show residuals centered around 0 without a clear time-dependent structure, supporting the adequacy of the chosen model form for DHA, n–3 DPA, and EPA (See Supplemental Figure 2: Q-Q plot and residual plots for DHA fitting in Supplemental Materials).

Sex and age

To evaluate whether sex was associated with estimated half-life in the fish oil group, the investigators compared male and female subjects using Welch's 2-sample *t*-test, which is robust to unequal variances, and confirmed results with an equivalent linear model with sex as the sole predictor. The investigators found no evidence of a sex effect on half-life for DHA (Welch $P = 0.239$; linear model $P = 0.263$), EPA (Welch $P = 0.418$; linear model $P = 0.451$), or n–3 DPA (Welch $P = 0.580$; linear model $P = 0.529$). Moreover, the investigators also assessed whether age was associated with the half-life estimates using both correlation tests (Pearson and Spearman) and a simple linear regression model of the form half-life $= \alpha + \beta \times$ age. For DHA, there was no evidence of an age effect (Pearson's $r = -0.237$, $P = 0.540$; Spearman's $\rho = -0.597$, $P = 0.090$; regression $P = 0.540$ with 95% CI for β from −2.26 to 1.29). For EPA, results were similarly nonsignificant (Pearson's $r = -0.178$, $P = 0.623$; Spearman's $\rho = -0.318$, $P = 0.371$; regression $P = 0.623$ with 95% CI −0.63 to 0.40). For n–3 DPA, age was also not associated with half-life (Pearson's $r = -0.176$, $P = 0.650$; Spearman's $\rho = -0.094$, $P = 0.890$; regression $P = 0.650$ with 95% CI −1.44 to 0.96). Overall, age also did not show a statistically detectable association with half-life for any of the fatty acids of interest.

As the sample size was likely too small to detect significant effects of age and sex differences, in the future, appropriately powered studies will be able to investigate these covariates and others like genetics further.

Discussion

Preclinical studies have validated CSIA to trace n–3 PUFA metabolism and determine the half-life and turnover rate of DHA in the plasma, liver, adipose, brain, and other peripheral tissues of mice [13,14,16]. Synthesis rates, half-lives, and turnover rates are particularly important in n–3 PUFA metabolic research, as they help to provide insight into the rate at which fatty acids are incorporated into and lost from tissues [13,14,16]. Turnover rates in particular have been shown to be a primary determinant for tissue and blood n–3 PUFA levels and can help to determine the relationship between n–3 PUFA consumption, synthesis, and levels [19]. However, in clinical research settings to date, the use of CSIA and natural ^{13}C abundance as a tracer for n–3 PUFA metabolism has been minimal.

In 2007, Carnielli et al. [11] investigated n–3 PUFA synthesis over the course of 7 mo in preterm infants fed formula with PUFAs, including DHA. Using $\delta^{13}C$ signatures in the circulating plasma, DHA synthesis from metabolic precursors was investigated relative to the metabolizable dietary intakes and was determined to be 41.7% ± 14.9%, 10.5% ± 8.1%, and 7.4% ± 6.2% at 1, 3, and 7 mo old, respectively [11]. The half-lives and turnover rates for this study were not reported. Studies like these provide important information for n–3 PUFA research, as synthesis rates, half-lives, and turnover rates provide valuable insight into the metabolic consumption of these n–3 PUFAs during various life stages and bodily states, like stress or disease.

In the preclinical model, CSIA has been used to determine plasma and brain DHA half-lives in mice (4.7–6.4 d, diet dependent, and 46.2–46.9 d, respectively) [13]. Classical stable isotope methods in humans have been used to monitor uniformly labeled ^{13}C-DHA and ^{13}C-EPA metabolism with a single oral dose, and the half-life of plasma DHA was 4.5–4.6 d and plasma EPA (in males only) 1.7–2 d [20,21]. These methods used in humans are suitable for short-term measurements; however, when applied to longitudinal studies, they may be time consuming and expensive, limiting their feasibility in longer study durations with multiple administrations. The use of $\delta^{13}C$ via CSIA in studies has an advantage over classical short-term tracer studies, as it can be utilized for studies with a longer duration, allowing more time for equilibration between plasma pools and tissues [11]. This was done in our secondary analysis of a clinical trial over the course of 28 d with 6 time points.

For the first time, our study demonstrated the use of dietary natural ^{13}C abundance to determine the half-lives and turnover rates of n–3 PUFAs in humans. The investigators found that the DHA half-life in human plasma is 6.3 ± 8.9 d, which is consistent with previous estimates in preclinical models (4.7–6.4 d) as well as humans (4.5–4.6 d and 4.4–6.1 d, phospholipids only) [13,20,26]. The investigators also determined that the EPA half-life in human plasma is 3.4 ± 2.7 d, which is consistent with the previous estimate of 1.7–2 d in males by Léveillé et al. [21]. Furthermore, the half-life for plasma total EPA is in line with previous investigations of EPA half-lives in specific lipid pools, including phospholipids (1.97 d) and cholesteryl esters (3.27 d) [27]. Here, the investigators provided, to the best of their knowledge, the first estimation of n–3 DPA plasma half-life of 6.4 ± 5.3 d.

Additionally, the plasma turnover rates or J_{out} of EPA, n–3 DPA, and DHA were determined (61.9 ± 53.1, 6.0 ± 2.9, and 78.0 ± 44.5 nmol/mL/d, respectively; Table 2). Although plasma DHA turnover rates have only been reported in rodents, when expressed per mL at 86.0–116.3 nmol/mL/d Klievik et al. [13], they are broadly comparable to our values. As oxylipins contribute to the consumption of n–3 PUFAs, these turnover rates are interesting to consider in comparison

to the original trial where Gabbs et al. [22] showcased that DHA, in comparison to ALA, resulted in larger increases in n–3-derived oxylipins in healthy adults aged 19–34 y, with differences in response to DHA supplementation being larger and appearing sooner in females. Unfortunately, as the $\delta^{13}C$ signatures of ALA in the plasma and supplement were similar, a turnover rate could not be determined during the secondary analysis. To the best of our knowledge, this is the first study to use CSIA to report the half-lives and turnover rates for EPA, n–3 DPA, and DHA in human plasma. As turnover rates change independent of n–3 PUFA levels, using CSIA to test metabolic consumption in humans and whether dose, sex, age, disease, or life stages like pregnancy can alter turnover rates is something to further investigate.

The between-subject variability for the half-lives and turnover rates for the 12 subjects were considerable, as judged by the large SDs relative to the mean values. This variability could be related to unmodifiable factors, like sex, age, and genetics; modifiable factors, like background diet, including n–6 PUFA consumption, BMI, physical activity, alcohol consumption, and smoking; as well as bioavailability factors, like the form of supplements provided. Before commencing the study, subjects consumed varying levels of n–3 PUFAs, which are difficult to control for during analysis without FFQs or diet diaries. To account for this variability, the investigators used mixed-effects models to normalize data and account for these differences during statistical analyses. In addition, the dose of DHA provided will likely influence half-lives and turnover rates as showcased in preclinical research, particularly during states of n–3 PUFA deprivation [28–30]. The precise effect of varying doses and background diet on these values in humans should be investigated in a dose-response randomized controlled trial that is designed to calculate half-lives and turnover rates.

The estimated J_{out} value in mg/d for plasma DHA of 80.6 mg/d is interesting to consider, particularly in comparison to the DHA uptake rate of 3.8 mg/d in the human brain [31]. Our calculated J_{out} for DHA in plasma is $\simeq$20 times greater in comparison to the brain uptake rate, which appears reasonable, as the majority of DHA would need to be distributed to organs and tissues, like the heart, liver, adipose, and muscle. For example, in rats, different tissues were showcased to have tissue-specific n–3 PUFA compositions with DHA being the highest in muscle (45.9 mg), followed by liver (27.0 mg), carcass (19.7 mg), white adipose (13.0 mg), brain (6.3 mg), and bone (4.2 mg), suggesting a whole-body DHA requirement that far outpaces the needs of the brain alone [32]. Like the preclinical model, CSIA could also be used to determine turnover rates for other blood components like red blood cells or human tissues like skin, muscle, or adipose, particularly where repeat biopsies are available [33]. However, where access and assessment are limited for human tissues, preclinical research may need to be explored further. Importantly, to the best of our knowledge, the plasma DHA turnover and brain uptake rate are the only 2 rates that have been reported for DHA in humans.

As EPA, n–3 DPA, and DHA molecules are different structurally, have varying pool sizes, and are incorporated and used in tissues and cell membranes differently, it is likely that their half-lives and turnover rates will be different [33]. As discussed, the half-lives calculated based on the secondary analysis of this randomized clinical trial match previous literature for DHA and EPA half-lives [20,21], and n–3 DPA has not been previously determined. The doses for each n–3 PUFA in this trial were also different (4.3 g/d DHA, 1.0 g/d of EPA, and 0.2 g/d n–3 DPA), which has been shown to affect half-lives and turnover rates in preclinical studies but still needs to be confirmed in humans. Additional research with individual fatty acids at varying doses is needed to better understand the discrepancy between n–3 PUFAs' half-lives and rates.

As this is a secondary analysis, the generalizability of the results is limited to moderate due to factors including selection bias. The participants were healthy and young (19–34 y of age) individuals from Manitoba, Canada, who may not represent the real-world, diverse population. As the use of $\delta^{13}C$ is still novel in clinical trials, external validity of the results is limited; however, as discussed, the values listed above seem reasonable when compared with brain uptake and preclinical blood and tissue turnover studies [13,28]. Nevertheless, proof of concept of the method is demonstrated, indicating that our use of CSIA may be generalizable and potentially used widely in clinical research settings. Future directions would include varying doses of DHA, larger diverse populations, and additional covariates, including genetics. The method used here could also be used in dietary-focused clinical trials to determine food sources in the diet as well as compliance. This in turn could be important for validating FFQs or using them as biomarkers of consumption and providing insight for determining dietary recommendations or advice.

Limitations

In addition to being an exploratory secondary analysis where the results derived here were not the intended purpose of the study, the study was also not powered for testing potential effects of modifiers, such as age, sex, and genetics [34]. However, our study provides important preliminary data to inform future power calculations.

The $\delta^{13}C$ signature of ALA in the flax oil group's plasma and the flax oil supplement was similar at baseline (-33.2 and -33.3, respectively), and thus, as expected, there was no significant change in the plasma ALA signature over the course of the study for both groups. Therefore, an ALA half-life and turnover rate could not be calculated. Future studies should aim to look at ALA with a higher $\delta^{13}C$ signature to differentiate between food and supplement, as well as potential movement or conversion to DHA, which would be reflected in the DHA $\delta^{13}C$ signature.

The use of the mean DHA plasma concentration over the 28 d for C_{FA} while $\delta^{13}C$ signatures are approaching plateau is a limitation of this analysis as precise J_{out} values require steady-state conditions. However, as this was a secondary analysis, the investigators are limited to the study design provided. In this case, using the design and data provided, the mean DHA plasma concentration best represents what is happening over the course of the 28-d study. To improve upon these values, using a switch diet model similar to what was previously used in the preclinical work by Klievik et al. [13] would be ideal. This model showcased DHA being swapped for the same amount of DHA but of a differing $\delta^{13}C$ signature, therefore allowing turnover rates to be calculated during a steady state [13,16]. Future human studies should replicate this approach for calculating J_{out} values.

Moreover, in the future, it will be important when determining half-lives, particularly for n–3 DPA (where only 0.2 g/d was administered), to increase the amount of supplement given to improve the curves of best fit and minimize variability.

In conclusion, this method utilizes the natural variance of ^{13}C in marine life, offering a tracer-free and efficient repeated-measures biomarker for studying n–3 PUFA metabolism. Our findings indicate that $\delta^{13}C$ can effectively be used to measure n–3 PUFA levels, investigate metabolism, and determine half-lives and turnover rates. Specifically, our study found that plasma half-lives and turnover in

humans for EPA and DHA closely resemble those observed in the current literature in relation to humans and preclinical models, while adding new estimates of n–3 DPA. The investigators recommend future clinical trials where the primary outcome further investigates $\delta^{13}C$ as a natural tracer in n–3 PUFA metabolism to test if their half-lives or turnover rates are altered by sex, genetics, or disease. As this is a secondary analysis of a randomized clinical trial, it also demonstrates the power of this new approach to improve the identification of nutrients in human blood, something that can be done retroactively and has implications beyond metabolism, including for nutritional epidemiology.

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Author contributions

The authors' responsibilities were as follows — AS: conceptualization of secondary analysis, methodology, formal analysis, investigation, writing—original draft, revisions; DW: formal analysis, writing – review and editing; CC: technical support, writing – review and editing; MDV: investigation, writing – review & editing; PZ, CT: conceptualization of original study, investigation, writing – review and editing; HA: conceptualization of original study, writing – review and editing; DK: formal analysis, validation; AHM: conceptualization of secondary analysis, methodology, supervision; RPB: conceptualization of secondary analysis, methodology, supervision, project administration, resources, writing – review and editing, funding acquisition; and all authors: read and approved the final manuscript.

Conflict of interest

The funders had no role in the design, execution, or interpretation of the research. AHM is on the Board of Directors of the International Society for the Study of Fatty Acids and Lipids. AHM is also a Science Advisor for Benexia and Natures Crops International and was a coapplicant on a joint government/industry funded research grant with Natures Crops International. RPB has received industrial grants, including those matched by the Canadian government, and/or travel support from Arctic Nutrition, Bunge Ltd., DSM, The Dairy Farmers of Canada, Mead Johnson, Natures Crops International, Nestec Inc, Pharmavite, and Sansero Life Sciences Inc. RPB. has served as a consultant to Bunge Ltd., Fonterra and Red Abbey Labs. Moreover, RPB. was on the executive committee 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. The other authors report no conflicts of interest.

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Data availability

Raw data are available upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted technologies were used in the writing of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2026.101311.

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