Kinetics of $^{13}$C-DHA before and during fish-oil supplementation in healthy older individuals$^{1–3}$

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ABSTRACT

Background: Docosahexaenoic acid (DHA) kinetics appear to change with intake, which is an effect that we studied in an older population by using uniformly carbon-13–labeled DHA ($^{13}$C-DHA).

Objective: We evaluated the influence of a fish-oil supplement over baseline compared with 3.0 mg $^{13}$C-DHA in older persons.

Design: Thirty-four healthy, cognitively normal participants (12 men, 22 women) aged between 52 and 90 years were recruited. Two identical kinetic studies were performed, each with the use of a single oral dose of 40 mg $^{13}$C-DHA. The first kinetic study was performed before participants started taking a 5-mo supplementation that provided 1.4 g DHA/d plus 1.8 g eicosapentaenoic acid (EPA)/d (baseline); the second study was performed during the final month of supplementation (supplement). In both kinetic studies, blood and breath samples were collected ≤8 h and weekly over 4 wk to analyze $^{13}$C enrichment.

Results: The time × supplement interaction for $^{13}$C-DHA in the plasma was not significant, but there were separate time and supplement effects ($P < 0.0001$). The area under the curve for plasma $^{13}$C-DHA was 60% lower while subjects were taking the supplement than at baseline ($P < 0.0001$). The uniformly carbon-13–labeled EPA concentration was 2.6 times as high 1 d posttracer while patients were taking the supplement as it was at baseline. The mean (±SEM) plasma $^{13}$C-DHA half-life was 4.5 ± 0.4 d at baseline compared with 3.0 ± 0.2 d while taking the supplement ($P < 0.0001$). Compared with baseline, the mean whole-body half-life was 61% lower while subjects were taking the supplement. The loss of $^{13}$C-DHA through β-oxidation to carbon dioxide labeled with carbon-13 increased from 0.085% of dose/h at baseline to 0.208% of dose/h while subjects were taking the supplement.

Conclusions: In older persons, a supplement of 3.2 g EPA + DHA/d increased β-oxidation of $^{13}$C-DHA and shortened the plasma $^{13}$C-DHA half-life. Therefore, when circulating concentrations of EPA and DHA are increased, more DHA is available for β-oxidation. This trial was registered at clinicaltrials.gov as NCT01577004. Am J Clin Nutr 2014;100:105–12.

INTRODUCTION

From currently available dose-response studies, it has been generally accepted that the amount of DHA in plasma total lipids or phospholipids tends to level off when the intake of DHA and EPA is >1000 mg/d (1), whereas at <1000 mg/d, the DHA plasma dose-response relation is generally linear (2, 3). One explanation for the plateau effect of DHA and EPA doses >1000 mg/d is that DHA is potentially more β oxidized, but to our knowledge, this possibility has not previously been shown experimentally. Indeed, regardless of the dietary intake of DHA and EPA, the kinetics of DHA in humans are still poorly understood.

In humans, the kinetics of DHA in plasma and its β-oxidation can be evaluated by using an oral dose of uniformly carbon-13–labeled DHA ($^{13}$C-DHA)$^4$. Plasma $^{13}$C-DHA kinetics in humans were first reported more than a decade ago (4–6). In one study, a single oral dose of 250–280 mg $^{13}$C-fatty acid mixture in which $^{13}$C-DHA represented 44% of the total labeled fatty acids was given in the form of triglyceride (5). Two hours postdose, $^{13}$C-DHA reached a maximum in plasma triglycerides in 3 healthy men (5). The apparent retroconversion of $^{13}$C-DHA to uniformly carbon-13–labeled EPA ($^{13}$C-EPA) was estimated to be equivalent to 1.4% of the plasma concentration of $^{13}$C-DHA (5). Neither β-oxidation nor the $^{13}$C-DHA half-life were reported in these studies (4–6). We recently reported the metabolism of a single 50-mg dose of 99% pure $^{13}$C-DHA methyl ester given to 6 young and 6 older humans (7). We showed that 4 h after $^{13}$C-DHA intake, older participants had a 4-fold higher $^{13}$C-DHA concentration in plasma total lipids compared with that of the young participants (7). In older persons supplemented with 323 mg EPA + 680 mg DHA for 3 wk, the study showed that...

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$^{4}$Abbreviations used: PDR, percentage of dose recovered; $^{12}$C-DHA, uniformly carbon-12–labeled DHA; $^{13}$C-CO$_2$, carbon dioxide labeled with carbon-13; $^{13}$C-DHA, uniformly carbon-13–labeled DHA; $^{13}$C-EPA, uniformly carbon-13–labeled EPA.

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unlabeled DHA in plasma total lipids reached a plateau 7 d earlier than in young adults (8). Therefore, DHA homeostasis seems to be disturbed in older persons, which is a situation that could both influence risk of cognitive decline (9, 10) and, in turn, be influenced by a preexisting cognitive age. The objective of the current study was to evaluate the influence of fish-oil supplementation on 13C-DHA kinetics in healthy, cognitively normal older persons.

SUBJECTS AND METHODS
Participants were >50 y old, which was an age chosen to represent the population that could potentially benefit from DHA intake in preventing cognitive decline and cardiovascular diseases (11, 12). All participants completed the Mini-Mental State Examination before and 4 mo after starting the supplement and were cognitively normal for their age (13). Exclusion criteria were diabetes, a cancer diagnosis in the past 6 mo, low serum albumin, liver or renal disease, uncontrolled hyperthyroidism or hypothyroidism, an autoimmune disorder, or C-reactive protein concentration >10 mg/L. Women had to be postmenopausal to be enrolled in this study. Because we previously showed that 13C-DHA kinetics are not the same in APOE3 and APOE4 carriers (14), we excluded APOE4 carriers from the current study. Other exclusion criteria included smoking, the use of n-3 PUFA supplements, overt heart disease or a cardiac event 6 mo before the study, and use of long-action benzodiazepines, warfarin, Coumadin, or a fibrate. At baseline, all participants reported having consumed ≤2 portions fatty fish/wk (eg, of salmon, herring, or sardines). At baseline, the mean percentage of DHA in plasma total lipids was 1.6 ± 0.5%, which indicated that DHA intake was ≤150 mg/d (15, 16). During the study, participants recorded fatty fish consumption in a logbook.

All participants gave informed written consent before starting the study. The study was approved by the Human Ethics Research Committee of the Health and Social Sciences Center–Sherbrooke University Geriatrics Institute.

Experimental design
The 13C-DHA tracer used in this study was uniformly 13C labeled (>98%) and of high chemical purity (99%). The 13C-DHA was synthesized by using microalgae grown in the presence of 13C-glucose according to the method of Le et al (17). Each dose comprised 40 mg 13C-DHA methyl ester that, before use, was stored in an individual glass ampoule sealed under argon.

The design of the overall study and the 2 embedded kinetic studies is presented in Figure 1. There were 2 matching 28-d kinetic studies that used 13C-DHA as follows: one study took place before subjects started taking the supplement (ie, baseline), and one study took place during the last month of supplementation with EPA and DHA (ie, supplement). The supplement consisted of 4 × 1.3-g capsules of fish oil that provided 1.8 g EPA/d plus 1.4 g DHA/d in ethyl ester (Ocean Nutrition Canada). We have shown that at least a 28-d follow-up is needed for the plasma 13C-DHA concentration to return to near baseline after 13C-DHA has been consumed orally (7, 14). The 28-d kinetic study started by the collection of fasting blood and breath samples (0 h), after which each participant received a single oral dose of 40 mg 13C-DHA.
13C-DHA kinetics in older persons

Analytic methods

Concentrations of 13C-DHA and 13C-EPA were measured in plasma total lipids. Plasma total lipids were analyzed instead of separating by lipid classes (phospholipids, triglycerides, cholesterol esters, and free fatty acids) because of the absence of a significant modification in concentrations of triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol while subjects were taking the supplement compared with the baseline (Table 1). Total lipids were extracted from 0.25 mL plasma by using Folch’s method (18). Heptadecanoate was added as an internal standard to quantify fatty acids. The plasma total lipid extract was saponified using 1 mol/L KOH in methanol and heated at 90°C for 1 h, which released fatty acids from cholesterol esters and glycerolipids. After cooling to room temperature, hexane and saline were added. The hexane phase that contained cholesterol was discarded and the remaining saline plus fatty acid salt mixture was acidified with hydrogen chloride to obtain free fatty acids; the latter were extracted with hexane. Fatty acid methyl esters were generated by adding boron trifluoride/methanol (14% Sigma-Aldrich) to the free fatty acids and heated at 90°C for 30 min. Fatty acid methyl esters were analyzed by using a gas chromatograph (model 6890; Agilent) equipped with a 50-m BPX-70 fused capillary column (SGE). The injection and flame-ionization detection were performed at 250°C, and the oven-temperature program was 50°C for 2 min, increased to 170°C at 20°C/min, held there for 15 min, finally increased to 210°C at 5°C/min, and held there for 7 min. Helium was the carrier gas, and the inlet pressure was 233 kPa at 50°C. Standard mixtures of fatty acids were used to identify individual fatty acids [NuChek 68A, NuChek 411, and NuChek 455 (NuChek Prep Inc) plus a custom mixture of SFAs]. The 13C-DHA enrichment in plasma total lipids was performed by using gas chromatography—combustion isotope ratio mass spectrometry as previously described (19). 13C:12C values at each time were compared with deposited on a piece of toast consumed at breakfast. Breakfast comprised 2 pieces of whole-wheat toast with peanut butter, one scrambled egg, one apple, 35 g mozzarella cheese, and 250 mL orange juice. The macronutrient composition of this 670-kcal breakfast was 25.5 g fat, 78 g carbohydrate, and 29 g protein. The breakfast including the tracer was consumed by all participants within 15 min. Four hours after breakfast, participants were given a lunch consisting of store-bought lasagna with 200 mL vegetable juice and a cereal bar. The macronutrient composition of this 500-kcal lunch was 15 g fat, 88 g carbohydrate, and 23 g protein.

Blood and breath samples were collected at 0, 1, 2, 4, 6, and 8 h on day 0 and days 1, 7, 14, 21, and 28 postdose (Figure 1). A catheter was installed in each participant’s forearm to collect the first 6 samples during day 0. Blood samples were collected by using a 5-mL syringe (Becton Dickinson) and transferred into 4 mL EDTA-coated tubes (Becton Dickinson). The tubes were centrifuged at 2300 × g for 15 min at 4°C, and plasma was stored in 3.0 mL Eppendorf tubes at −80°C until additional analysis. Alveolar breath samples at rest were collected by having subjects breath into a device that consisted of a perforated plastic bag attached to a mouthpiece (Easysampler; Quintron Instrument Co) to which an evacuated tube could be inserted to collect a sample of the exhaled breath (7, 14). These breath samples were used to follow the appearance of carbon dioxide labeled with carbon-13 (13C-CO2) that came from 13C-DHA β-oxidation.

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Anthropometric characteristics and blood biochemistry of healthy older persons at baseline and while taking the supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n = 34)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>72.4 ± 8.91</td>
</tr>
<tr>
<td>Sex (M/F) (%)</td>
<td>12/22</td>
</tr>
<tr>
<td>MMSE (30)</td>
<td>28.7 ± 1.3</td>
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<tr>
<td>Blood biochemistry</td>
<td></td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>4.7 ± 0.6</td>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.4 ± 0.8</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.9 ± 0.8</td>
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<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.5 ± 0.4</td>
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<td>LDL cholesterol (mmol/L)</td>
<td>2.8 ± 0.7</td>
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<tr>
<td>HDL cholesterol</td>
<td>3.5 ± 0.9</td>
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13C-DHA at baseline (t = 0 of day 0) to calculate the 8 per mil values, which were designated thereafter as the atom percent excess. The actual 13C enrichment in plasma DHA and EPA was calculated from atom percent excess values according to Brossard et al (5).

The enrichment of 13C in expired-breath CO2 was analyzed by using isotope ratio mass spectrometry (Europa 20–20; Sercon Ltd) as previously described (20). Helium (Praxair) was the carrier gas, and 5% CO2:N2 was used as the reference gas. The ratio of 13C:12C was used to calculate the β-oxidation of 13C-DHA recovered in the breath in the form of 13C-CO2. The percentage of dose of 13C-DHA recovered in the breath as 13C-CO2 was calculated as previously described (21) except that basal metabolism was evaluated by using indirect calorimetry (CCM/D; Medgraphics Corp) to measure the volume of CO2 and O2 exhaled by participants over 30 min as previously described (22). The cumulative 13C-DHA β-oxidation was calculated as the AUC of the percentage of dose recovered (PDR) at each time point (GraphPad Prism 5 for Windows software, version 5.03; GraphPad). Specific activities of plasma DHA were calculated by using the ratio of 13C-DHA (nmol/mL plasma) to uniformly carbon-12–labeled DHA (12C-DHA; nmol/mL plasma) times 100.

The 13C-DHA half-life in plasma was calculated as previously described (14). The whole-body 13C-DHA half-life was estimated by using cumulative β-oxidation curves from days 1–28 postdose (14). For some participants, a cumulative β-oxidation of 50% was not reached within 28 d, and thus in those cases, it was assumed that cumulative 13C-CO2 data >28 d would be linear (see Figure 4). With the use of cumulative 13C-CO2 data at time points 1, 7, 14, 21, and 28 d postdose, a linear equation was calculated for each participant to estimate the whole-body half-life defined as the time needed to reach a 50% cumulative β-oxidation of the 13C-DHA dose recovered as 13C-CO2. Correlations between plasma 13C-DHA and the percentage of dose of 13C-DHA recovered as breath 13C-CO2 were performed for each participant by using hierarchical linear models (measured within persons).

The slope of the correlation between plasma 13C-DHA and the percentage of dose of 13C-DHA recovered as breath 13C-CO2 were calculated and compared between baseline and while subjects were taking the supplement.

Statistics were performed by using a paired t test. There was no significant difference (P < 0.05) between when subjects were taking the supplement and at baseline for any of the blood biochemistry markers.

Mean ± SD (all such values).

MMSE, Mini-Mental State Examination.

n = 22 subjects taking the supplement.

13C:12C at baseline (t = 0 of day 0) to calculate the 8 per mil values, which were designated thereafter as the atom percent excess. The actual 13C enrichment in plasma DHA and EPA was calculated from atom percent excess values according to Brossard et al (5).
Statistics

To calculate the sample size, we used the AUC of plasma $^{13}$C-DHA as the primary endpoint. From a previous published article that used $^{13}$C-DHA in older person, the mean AUC was 230 ± 63 nmol·L$^{-1}$·h$^{-1}$ (7). We did not have data to estimate the AUC while subjects were taking the supplement, and thus, we set the effect size at 0.5, and the $\delta$ SD was set at 63 nmol·L$^{-1}$·h$^{-1}$ with the assumption that the correlation ($r$) of the AUC at baseline compared with when subject were taking the supplement was equal to 0.5, which was conservative. We also set the power at 80% by using a paired $t$ test with a 0.05 two-sided significance. With these data, the number of participants was $n = 28$, but we anticipated a dropout of 20%, and thus, the number of participants recruited was $n = 34$. During the study, there were no dropouts. See supplemental Figure 1 under “Supplemental data” in the online issue for a presentation of a Consolidated Standards of Reporting Trials—like flowchart that shows the flow of data through the kinetic studies.

All data from plasma and breath are presented as means ± SEMs. Data from blood and breath samples collected during kinetic studies were analyzed by using the PROC MIXED procedure implemented in SAS 9.2 software (SAS) (14). Instead of a classical 2-factor ANOVA, the PROC MIXED procedure was used to optimize the use of all data over time and maintain statistical power. This procedure allowed for the testing of the effect of time as a repeated measure, supplementation as a fixed factor (baseline compared with supplement), and the interaction time × supplement. Student paired $t$ tests were performed for plasma and whole-body half-lives and to detect significant differences in anthropometric characteristics and the blood biochemistry of participants while they were taking the supplement than at baseline (SPSS 17.0; SPSS Inc). AUCs were calculated with GraphPad Prism 5 for Windows software (version 5.03; GraphPad). Hierarchical linear models (measured within persons) were performed with HLM for Windows v 7.0. This multilevel model addresses within-subject and between subject variability simultaneously from a pair of submodels (23). The dependent variable was the percentage of dose of $^{13}$C-DHA recovered as $^{13}$C-CO$_2$ in the breath per hour. Independent variables were $^{13}$C-DHA in plasma and the supplement. See supplemental Text under “Supplemental data” in the online issue for a presentation of the mathematical modeling. Statistical significance was set at $P \leq 0.05$.

RESULTS

Participants

There were 12 men and 22 women with an age range of 52–90 y in the study (Table 1). Their mean (±SEM) score on the Mini-Mental State Examination was 29 ± 1 both at baseline and while subjects were taking the supplement, which confirming that subjects were cognitively normal throughout the study. Blood biochemistry was performed on 34 subjects at baseline and 22 subjects while they were taking the supplement. Initially, it was intended that blood biochemistry be performed to evaluate the eligibility of participants only at baseline. Thereafter, the protocol was amended to include a second evaluation of blood biochemistry while participants were taking the supplement, but the first 12 participants had already completed the study. Blood glucose, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, and total:HDL cholesterol did not significantly change while subjects were taking the supplement than at baseline (Table 1). During the 5 mo that subject were taking the supplement, all participants reported the consumption of <2 portions fatty fish/wk. At baseline, means of plasma EPA and DHA were 117 ± 52 and 183 ± 70 nmol/mL, respectively, which represented 0.95 ± 0.38% and 1.60 ± 0.53% of total fatty acids, respectively (see supplemental Table 1 under “Supplemental data” in the online issue). One month after subject started supplementation, EPA and DHA plateaued in total lipids of plasma at concentrations between 383 and 589 nmol/mL for EPA and between 290 and 376 nmol/mL for DHA (Figure 2).

$^{13}$C-DHA kinetics

At baseline, plasma $^{13}$C-DHA peaked 6 h postdose at 2.0 ± 0.2 nmol/mL, whereas it peaked 4 h postdose at 1.6 ± 0.1 nmol/mL when subjects were taking the supplement (Figure 3A). The time × supplement interaction was not significant, but there were separate time and supplement effects ($P < 0.0001$) such that, over the follow-up period of 28 d, the mean difference of $^{13}$C-DHA at each time was not significantly lower while subjects were taking the supplement than at baseline. However, the AUC of plasma $^{13}$C-DHA while subjects were taking the supplement was 60% lower than at baseline ($P < 0.0001$; Figure 3A). The rising slope of plasma $^{13}$C-DHA was the same at baseline as while subjects were taking the supplement (Figure 3A).

Plasma tracee ($^{13}$C-DHA; nmol/L) was stable over the 28-d follow-up period and was ~70% higher while subjects were taking the supplement than at baseline. The specific activity is presented in Figure 3B and defined as $^{13}$C:$^{12}$C-DHA. At baseline, the peak specific activity was 1.12 ± 0.08% compared with 0.53 ± 0.04% while subjects were taking the supplement, with a significant time × supplement interaction ($P < 0.0001$) that showed that the kinetics of specific activity over 28 d differed significantly in participants while they were taking the supplement than at baseline. The specific activity was 2.5–7-times higher at baseline than while subjects were taking the supplement throughout the metabolic follow-up period 1–28 d postdose (Figure 3B).

There was a significant time × supplement interaction for plasma $^{13}$C-EPA with 2.0–2.8-times higher plasma $^{13}$C-EPA from 8 h to 7 d postdose while subjects were taking the supplement than at baseline (Figure 3C). The specific activity of plasma $^{13}$C-EPA was significantly different over time ($P < 0.0001$), but there was no supplement effect and no interaction (Figure 3D).

![FIGURE 2. Mean (±SEM) DHA in plasma total lipids over the 5-mo supplementation period. One month after subjects started taking the supplement, the concentration of DHA doubled in plasma total lipids and reached a plateau (n = 34).](https://academic.oup.com/ajcn/article-abstract/100/1/105/4576428)
The cumulative β-oxidation of 13C-DHA to 13C-CO2 over 28 d was 1.9-times higher while subjects were taking the supplement than at baseline (Figure 4). The peak 13C-CO2 enrichment occurred 4 h postdose and reached 0.48% of dose of 13C-DHA/h while subjects were taking the supplement compared with 0.31% of dose per hour at baseline (data not shown). The enrichment of 13C-CO2 returned almost to baseline 28 d postdose at baseline and while subjects were taking the supplement (data not shown). The cumulative β-oxidation at 28 d was 35.1 ± 6.7% of the tracer dose at baseline compared with 65.7 ± 9.1% while subjects were taking the supplement (Figure 4).

DHA half-life

The cumulative β-oxidation of 13C-DHA to 13C-CO2 over 28 d was 1.9-times higher while subjects were taking the supplement than at baseline (Figure 4). The peak 13C-CO2 enrichment occurred 4 h postdose and reached 0.48% of dose of 13C-DHA/h while subjects were taking the supplement compared with 0.31% of dose per hour at baseline (data not shown). The enrichment of 13C-CO2 returned almost to baseline 28 d postdose at baseline and while subjects were taking the supplement (data not shown). The cumulative β-oxidation at 28 d was 35.1 ± 6.7% of the tracer dose at baseline compared with 65.7 ± 9.1% while subjects were taking the supplement (Figure 4).

The mean plasma half-life of 13C-DHA was 4.5 ± 0.4 d at baseline compared with 3.0 ± 0.2 d while subjects were taking the supplement (P < 0.0001; Figure 5A). The mean whole-body half-life of 13C-DHA was 140 ± 27 d at baseline compared with 54 ± 12 d while subjects were taking the supplement (P = 0.0107; Figure 5B). Neither the plasma nor whole-body 13C-DHA half-life differed between men and women, and there was not a significant correlation with the age of participants.

Hierarchical linear model

The final estimation of the HLM model is shown in Figure 6. At baseline, 13C-DHA was positively related to PDR as 13C-CO2 with a slope of 0.0847, whereas while subjects were taking the supplement, the slope was much steeper at 0.2076 (P < 0.001), which showed that, for a similar concentration of 13C-DHA in blood, the β-oxidation of 13C-DHA to 13C-CO2 was higher while subjects were taking the supplement than at baseline. Plasma 13C-DHA explained 50% of the PDR variation in the model. The intersubject variation (u_i) associated with random effect (b_j) was tested by using age, sex, education, and BMI as covariates (see supplemental Text for a description of the mathematical model under “Supplemental data” in the online issue). BMI was the only covariate to be significantly involved in explaining the intercept and contributed to ~5% of the variation in the relation between PDR and plasma 13C-DHA.

FIGURE 3. Mean (±SEM) concentrations of 13C-DHA (A), 13C:12C-DHA (B), 13C-EPA (C), and 13C:12C-EPA (D) in plasma total lipids over 28 d at baseline and while subjects were taking the supplement (n = 34). Statistics were performed by using the PROC MIXED procedure implemented in SAS 9.2 software (SAS) and tested the effect of time as a repeated measure, supplementation as a fixed factor (baseline compared with supplement), and the interaction of time × supplement. When there was an interaction, comparisons at each time were performed by using a paired t test. For the AUC in panel A, a paired t test was performed. In panel A, the 13C-DHA concentration varies significantly over time, and there was an independent supplement effect. In panels B and C, there was a time × supplement interaction. In panel D, 13C:12C-EPA varied significantly over time only. *P < 0.05. 12C-DHA; uniformly carbon-12–labeled DHA; 12C-EPA, uniformly carbon-12–labeled EPA; 13C-DHA, uniformly carbon-13–labeled DHA; 13C-EPA, uniformly carbon-13–labeled EPA.
DISCUSSION

In this study, we report that supplementation with an EPA + DHA supplement significantly modified the kinetics of 13C-DHA in healthy older persons without cognitive decline such that its plasma and whole-body half-lives were lower and its β-oxidation to 13C-CO2 was higher than at baseline. Hence, the plateauing of plasma DHA at higher intakes of EPA + DHA appears to be a function of increased β-oxidation.

Despite the higher β-oxidation of DHA while subjects were taking the supplement, the cumulative β-oxidation of 13C-DHA remained low compared with what has typically been reported for other long-chain fatty acids. For example, 24 h postdose, the β-oxidation of 13C-DHA was <7% while subjects were taking the supplement and at baseline (Figure 4), whereas the β-oxidation over the same period and under essentially the same conditions was 29% for 13C-oleic acid, 21% for 13C–linoleic acid, and 31% for 13C-α-linolenic acid (20). These results were potentially because, in contrast to PUFAs with <20 carbons, PUFAs with >20 carbons require the previous peroxisomal β-oxidation to shorten the fatty acid chain before entry to the mitochondrial β-oxidation spiral (24, 25). However, the relative contribution of peroxisomal compared with mitochondrial β-oxidation to the whole-body production of 13C-CO2 from 13C-DHA in humans is unknown. The affinity of carnitine palmitoyl transferase is also much lower for DHA than long chain fatty acids with 1–3 double bonds (26). Thus, DHA is relatively efficiently conserved, probably because of its structural importance in cell membranes (27) and as a precursor to signaling molecules (28).

Our 13C-CO2 breath results did not account for the 13C that was converted to carbon-13-labeled acetyl-CoA but then recovered in other metabolites rather than entering the Krebs’ cycle and being converted to 13C-CO2. However, during our gas chromatography–combustion isotope ratio mass spectrometry analysis, there was no measurable 13C enrichment in any other plasma fatty acids except EPA, which suggested that, once 13C-DHA starts to be β-oxidized, the process goes mostly to completion (ie, quantitatively to 13C-CO2).

The whole-body half-life was calculated from the 13C-CO2 recovery in the breath to estimate the time needed to β-oxidize 50% of the 13C-DHA dose without regard to compartments. In this calculation, it was assumed that the quantity of 13C-CO2 going from peripheral compartments to plasma and then to breath remained constant after the 28th d for subjects who did not reach 50% of cumulative 13C-CO2. Hence, the calculated whole-body half-life represents the best estimate of the time needed for one-half of the 13C-DHA dose to be exhaled in the form of 13C-CO2 by participants, with the recognition that this is probably an underestimate because some β-oxidized 13C-DHA could theoretically get incorporated into other compounds synthesized via carbon-13-labeled acetyl-CoA.

The lower postprandial specific activity of plasma 13C-DHA while subjects were taking the supplement can largely be explained by the 70% tracer dilution compared with at baseline (Figure 3B). 13C-DHA kinetics may also have been affected because the supplement was not pure DHA but rather a combination of EPA + DHA. The provision of pure DHA results in an

FIGURE 4. Mean (±SEM) cumulative percentage of dose of 13C-DHA β-oxidized and recovered in the breath as 13C-CO2 at baseline (gray squares) and while subjects were taking the supplement (black triangles) (n = 34). Statistics were performed using a PROC MIXED procedure implemented in SAS 9.2 software (SAS) and tested the effect of time as a repeated measure, supplementation as a fixed factor (baseline compared with supplement), and the interaction of time × supplement. When there was an interaction, comparisons at each time point were performed by using a paired t test. There was a time × supplement interaction supporting the idea that the difference in cumulative 13C-CO2 expired while subjects were taking the supplement; baseline was not the same at each time point. *P < 0.05. 13C-DHA, uniformly carbon-13–labeled DHA.

FIGURE 5. Mean (±SEM) calculated half-life of 13C-DHA at baseline and while subjects were taking the supplement in plasma total lipids (n = 32 at baseline and n = 34 while subjects were taking the supplement) (A) and the whole body (n = 29 at baseline and n = 32 while subjects were taking the supplement) (B). Paired t tests were performed for comparison between when subjects were taking the supplement and at baseline. At baseline, blood samples collected at 14, 21, or 28 d had an enrichment of 13C over 12C below the enrichment at 0 h of day 0, and thus, it was not possible to calculate the plasma half-life for 2 participants. In 5 participants at baseline and 2 participants while taking the supplement, the cumulative β-oxidation of 13C-DHA recovered as carbon dioxide labeled with carbon-13 reached a plateau of <50% 7 d postdose, and thus, the calculation of the whole-body 13C-DHA half-life was not possible. While subjects were taking the supplement, plasma and whole-body half-lives were 33–61% lower than at baseline. *P ≤ 0.05. 13C-DHA, uniformly carbon-13–labeled DHA.
while subjects were taking the supplement than at baseline. BMI significantly increased from baseline to supplement (Table). The slope of curves was significantly different while subjects were taking the supplement than at baseline, which suggested that, for a similar dose of EPA and DHA, the specific activity of EPA derived from circulating DHA was higher when subjects were taking the supplement than at baseline. The post-hoc analysis of the mass of 13C-EPA during EPA + DHA supplementation was performed to assess a possible age effect. Participants in this study were of an age when cognitive decline could be starting, but they were all cognitively healthy as assessed by using the Mini-Mental State Examination. This fact is important because we wanted to eliminate lower cognitive performance as a confounder that might have influenced the results (10).

In conclusion, in older persons taking 3.2 g EPA + DHA/d, there is an increased β-oxidation of 13C-DHA and shortened plasma and whole-body 13C-DHA half-life. Therefore, when circulating concentrations of EPA + DHA are increased, more DHA appears to be available for β-oxidation.

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The authors’ responsibilities were as follows—MP and SCC: designed the research; RC-W, MP, MF, CR-P, JT-M, MTMD, M-JA, and PP: conducted experiments and collected data; MP, RC-W, JTB, and SCC: analyzed data or performed the statistical analysis; MP, JTB, and SCC: wrote the manuscript; MP: had primary responsibility for the final content of the manuscript; YZ, PL, and JTB: performed the 13C-fatty acid analysis; DL: conducted the cognitive testing; M-CV: performed the genotyping; and all authors: read and approved the final version of the manuscript. None of the authors had a conflict of interest.

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FIGURE 6. Mean (±SEM) correlations between 13C-CO2 and plasma 13C-DHA concentrations at baseline and while subjects were taking the supplement separated by the median BMI (in kg/m2) ≤26 or >26. The Hierarchical linear model test was used to plot mean correlation curves from individual curves of 34 participants (see supplemental Text under “Supplemental data” in the online issue for the description of the mathematical model). The slope of curves was significantly different while subjects were taking the supplement than at baseline, which suggested that, for a similar concentration of 13C-DHA in blood, the β-oxidation of 13C-DHA expressed as the percentage of dose of 13C-DHA recovered as 13C-CO2/h was higher while subjects were taking the supplement than at baseline. BMI significantly explained ~5% of the variation in the relation between the percentage of dose of 13C-DHA recovered as 13C-CO2/h and plasma 13C-DHA. 13C-CO2, carbon dioxide labeled with carbon-13; 13C-DHA, uniformly carbon-13-labeled DHA.

apparently linear increase in EPA in plasma phospholipids (16). Therefore, if the supplement had been pure DHA, it is possible that the 13C-EPA concentration in plasma would have been higher than we observed, but this possibility is necessarily speculative. Similarly, had the supplement provided a dose of EPA + DHA closer to the international recommendation (rather than 6 times above the recommendation as was the case in this study), the effect on 13C-DHA kinetics would likely have been attenuated.

Unlike the specific activity of plasma 13C-DHA, the specific activity of plasma 13C-EPA was not significantly lower while subjects were taking the supplement than at baseline (Figure 3D) despite the EPA + DHA supplement having raised plasma EPA 5-fold. Changes in the mass of 13C-EPA during EPA + DHA supplementation may originate via several routes, including the retroconversion from DHA, carbon recycling that provides carbon-13–labeled acetyl-CoA for stearidonic acid elongation, or the sparing of EPA from β-oxidation. No change in the specific activity of plasma EPA suggested that EPA sparing was significant while subjects were taking the supplement. Because of the bigger pool of circulating 13C-DHA, the apparent rate of retroconversion to EPA or displacement of EPA from nonplasma pools appears to have been unchanged by the supplement. In contrast, the slope of 13C-DHA β-oxidation increased with higher circulating DHA while participants were taking the supplement than at baseline (Figure 6). These data suggest that the partitioning of DHA between β-oxidation and retroconversion to EPA are probably regulated separately.

In the current study, there was no difference in plasma 13C-DHA, plasma DHA, or the 13C-DHA half-life in whole body or


