Compartmental modeling to quantify α-linolenic acid conversion after longer term intake of multiple tracer boluses

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Abstract To estimate in vivo α-linolenic acid (ALA; C18:3n-3) conversion, 29 healthy subjects consumed for 28 days a diet providing 7% of energy from linoleic acid (C18:2n-6) and 0.4% from ALA. On day 19, subjects received a single bolus of 30 mg of uniformly labeled [^{13}C]ALA and for the next 8 days 10 mg twice daily. Fasting plasma phospholipid concentrations of ^{12}C- and ^{13}C-labeled ALA, eicosapentaenoic acid (EPA; C20:5n-3), docosapentaenoic acid (DPA; C22:5n-3), and docosahexaenoic acid (DHA; C22:6n-3) were determined on days 19, 21, 23, 26, 27, and 28. To estimate hepatic conversion of n-3 fatty acids, a tracer model was developed based on the averaged ^{13}C data of the participants. A similar tracee model was solved using the averaged ^{12}C values, the kinetic parameters derived from the tracer model, and mean ALA consumption. ALA incorporation into plasma phospholipids was estimated by solving both models simultaneously. It was found that nearly 7% of dietary ALA was incorporated into plasma phospholipids. From this pool, 99.8% was converted into EPA and 1% was converted into DPA and subsequently into DHA. The limited incorporation of dietary ALA into the hepatic phospholipid pool contributes to the low hepatic conversion of ALA into EPA. A low conversion of ALA-derived EPA into DPA might be an additional obstacle for DHA synthesis.—Goyens, P. L. L., M. E. Spilker, P. L. Zock, M. B. Katan, and R. P. Mensink. **Compartmental modeling to quantify α-linolenic acid conversion after longer term intake of multiple tracer boluses. *J. Lipid Res.* 2005.** 46: **1474–1483.**

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α-Linolenic acid (ALA; C18:3n-3) is an essential fatty acid of the n-3 family that is present in unhydrogenated canola and soybean oils and in foods prepared with these oils (1–3). Humans are unable to synthesize ALA because they lack the necessary Δ-15 desaturase enzymes (4–6). Therefore, ALA must be provided in adequate amounts through the diet. After consumption, ALA can be converted in the liver into longer and more unsaturated fatty acids such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). These latter two fatty acids, which are also present in fatty fish, play an essential role in many physiological processes (7). Whether ALA is a useful source for EPA and DHA synthesis depends on the efficacy of ALA conversion.

Several animal and human intervention studies have examined the effects of diets rich in ALA on n-3 fatty acid metabolism and accretion in tissues (8–10). Although the results indicate that ALA is converted, these studies only allow a qualitative or semiquantitative description of n-3 metabolism. In contrast, the use of stable isotopes offers a means to assess quantitatively the in vivo conversion of ALA (11). Nevertheless, quantification of the separate conversion reactions remains complex. The few studies that were performed with deuterated or ^{13}C-labeled ALA tracers mostly used area under the curve (AUC) values, which give a global impression of n-3 fatty acid conversion (12–15). Compartmental modeling, however, provides more accurate estimates of the various metabolic parameters underlying the n-3 fatty acid cascade. The first study to apply compartmental modeling to quantify the conversion of ALA in adults was published by Pawlosky et al. (16). In that study, the conversion of ALA was examined after oral administration of a single 1 g dose of deuterated

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Abbreviations: ALA, α-linolenic acid (C18:3n-3); AUC, area under the curve; CE, cholesteryl ester; DHA, docosahexaenoic acid (C22:6n-3); DPA, docosapentaenoic acid (C22:5n-3); En%, percent of energy; EPA, eicosapentaenoic acid (C20:5n-3); FAME, fatty acid methyl ester; LCPUFA, long-chain polyunsaturated fatty acid; TG, triacylglycerol; [U-^{13}C]ALA, uniformly labeled [^{13}C]α-linolenic acid.

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ALA, which was quite high compared with daily ALA intake. In addition, enrichments of n-3 fatty acids were measured in total plasma lipids, although the fatty acid composition of plasma phospholipids may more closely resemble hepatic n-3 fatty acid metabolism. Therefore, we decided to use compartmental modeling to quantify ALA conversion after ingestion of multiple trace amounts of uniformly labeled [^{13}C]ALA ([U-^{13}C]ALA) for 9 days, and enrichments of ALA and its long-chain polyunsaturated fatty acids (LCPUFAs) were measured in plasma phospholipids.

MATERIALS AND METHODS

Subjects

Thirty healthy volunteers (15 men and 15 women) participated in the study. One male subject was excluded before analysis of the results because of gastrointestinal complaints and related weight loss during the study. The mean ± SD age of the remaining 29 subjects was 49.9 ± 13.4 years (range, 21–63 years). Volunteers did not use medication, had a stable body weight for at least 3 months preceding the study (<3 kg loss or gain of weight), and had a body mass index of 24.3 ± 3.1 kg/m^2. The study protocol was approved by the Medical Ethics Committee of Maastricht University, and written informed consent was obtained from each participant.

Diet

This tracer study was part of a dietary intervention trial that studied the effects of polyunsaturated fatty acids on cardiovascular risk markers. For this part of the project, only data from the 4 week run-in period were used, during which all subjects received the same control diet. At the start of the study, each subject weighed and recorded his or her food intake for 3 consecutive days: 2 working days and 1 weekend day. Energy intake was calculated using the Dutch food composition table, and each subject was assigned a diet that met his or her energy requirement (17). To this end, nine different diets were formulated with varying amounts of energy (7.5–13.4 MJ). The target composition of the diet consumed during the first 4 weeks consisted of 15 percent of energy (En%) protein, 50 En% carbohydrates, and 35 En% fat [13 En% saturated fatty acids, 13 En% monounsaturated fatty acids, 7 En% linolenic acid (LA), and 0.4 En% ALA]. Thus, the ALA/LA ratio of the control diet was 1:19. EPA and DHA intakes were less than 0.1 En%. To achieve these intakes, subjects received each week products such as pies, pastry, and margarine that were made from an experimental fat (**Table 1**). The margarine as well as the experimental fat, which was composed of a mixture of 30.4% sunflower oil, 33.1% olive oil, 11.5% rapeseed oil, and 25% hardstock made from fully hydrogenated palm kernel and palm oils, were made by NIZO Food Research (Ede, The Netherlands). Depending on energy intake, the products supplied provided 62–71% of total fat intake or 22–25 En% of total energy. In addition, subjects were given strict written dietary guidelines concerning the preparation and consumption of other food items. The use of fish or seafood was prohibited during the entire study period. Body weight was monitored weekly. Subjects were assigned to another energy intake level whenever weight changed by >2 kg compared with the body weight at trial entry.

Tracer protocol and blood sampling

[U-^{13}C]ALA was purchased from Isotec, Inc. (Miamisburg, OH), as free fatty acid tracer with an isotopic purity of 99%. The total amount of [U-^{13}C]ALA was dissolved in olive oil and then pipetted into capsules with a volume of 0.3 ml, such that each capsule contained 10 mg of tracer.

TABLE 1. Fatty acid composition of the experimental fat

Fatty Acid	g/100 g Fatty Acids
Saturated fatty acids	29.3
Monounsaturated fatty acids	41.0
Polyunsaturated fatty acids	29.4
Linoleic acid	27.9
ALA	1.3

ALA, α-linolenic acid.

At day 19, 10 days before the end of the run-in period, a first blood sample was drawn (t = 0 h) after subjects had fasted for at least 12 h and abstained from alcohol for 24 h. Subjects then received an oral bolus of 30 mg of [U-^{13}C]ALA. For the next 8 days (days 20–27), subjects consumed one capsule at 8 AM and one capsule at 8 PM. On these days, total daily [U-^{13}C]ALA intake was 20 mg. Fasting blood samples were drawn at days 21 (t = 48 h after the first tracer administration), 23 (t = 96 h), 26 (t = 168 h), 27 (t = 192 h), and 28 (t = 216 h).

Sample analysis

Blood was collected into precooled EDTA tubes and centrifuged at 2,000 *g* (3,500 rpm) for 30 min at 4°C within 1 h after sampling. Aliquots from the midportion of the plasma were taken, snap-frozen in liquid nitrogen, and stored at −80°C until analysis. After the trial, all samples from one subject were analyzed in the same run.

Total lipids from plasma were extracted according to a modified procedure of the Folch method with 1,2-dinonadecanoyl phosphatidylcholine as an internal standard (18). Phospholipids were then isolated using an Extract-Clean NH_2-aminopropylsilyl column (500 mg, 4.0 ml; Alltech Associates, Inc., Deerfield, IL) and hydrolyzed and methylated into their corresponding fatty acid methyl esters (FAMEs) (19, 20). The FAMEs were separated and quantified by means of a gas chromatograph-flame ionization detector (Perkin-Elmer Autosystem, Norwalk, CT). For separation, a CP-Sil 88 capillary column (50 m × 0.25 mm, 0.20 μm film thickness; Chrompack, Middelburg, The Netherlands) was used with helium as the carrier gas (injector inlet pressure of 130 kPa) as described previously (21). Both the injection and the detector temperature were set at 300°C. The temperature of the oven started at 160°C and increased for 10 min to 190°C in steps of 3.2°C/min. It remained at 190°C for 15 min and then increased at 5°C/min to 230°C and was kept constant for 7 min.

Isotopic ^{13}C enrichments of the FAMEs of ALA, EPA, docosapentaenoic acid (DPA), and DHA were determined on a gas chromatograph-combustion-isotope ratio mass spectrometer (MAT 252; Finnigan, Bremen, Germany) with a BPX-70 column (50 m × 0.33 mm, 0.25 μm film thickness; SGE, Austin, TX) and helium as the carrier gas (injector inlet pressure of 124 kPa). The sample injection temperature was 250°C. Two different temperature programs were used to obtain a complete separation of the peaks of interest. For ALA, EPA, and DHA, the oven was programmed at 185°C for 35 min and increased at 30°C/min up to 260°C for 33 min. For DPA, the oven was programmed at 205°C for 20 min and increased at 30°C/min up to 260°C for 25 min.

^{13}C enrichments of the FAMEs were expressed as tracer-to-tracee ratios as follows (22).

$$\mathrm{TTR} = \left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right)\mathrm{sample} - \left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right)\mathrm{background} \qquad \textit{(Eq. 1)}$$

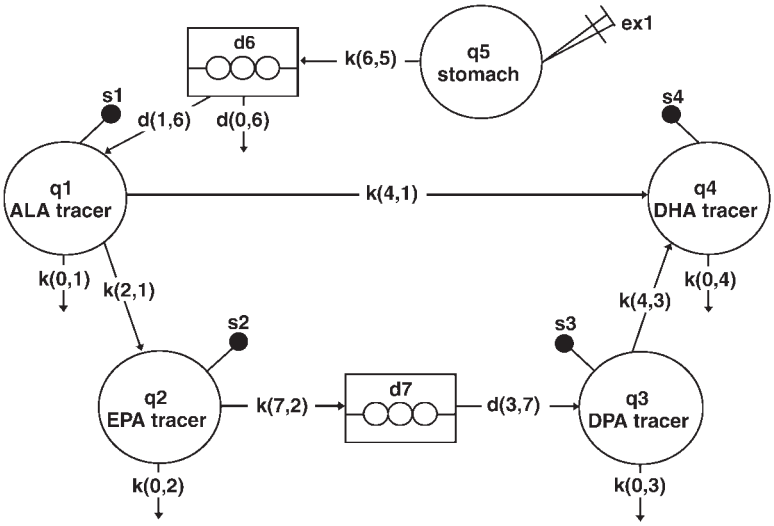

Fig. 1. Tracer model for n-3 conversion. Circles denoted q1, q2, q3, and q4 represent the plasma α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) tracer phospholipid compartments, respectively. Delay compartments, which were introduced to account for the delayed appearance of the tracer, are depicted by the rectangular boxes d6 and d7. The exogenous oral tracer input ex1 into compartment q5 is symbolized by a syringe. The small closed circles s1, s2, s3, and s4 attached to each compartment represent the blood samples that were taken to measure the concentration of ^{13}C-labeled n-3 fatty acids in plasma phospholipids.

The tracer-to-tracee ratios were adjusted for the extra carbon atoms added to the ^{13}C-labeled fatty acids through elongation and methylation. It was assumed that these additional carbon fragments were not enriched above background. At each time point (t), the above background concentrations of the labeled (C_{TRACER}) and unlabeled (C_{TRACEE}) fatty acids were derived according to the following formulas (23):

$$C_{TRACER}(t) = C_{TOTAL}(t) \times \left(\frac{TTR(t)}{1 + TTR(t)}\right) \quad \textit{(Eq. 2)}$$

and

$$C_{TRACEE}(t) = C_{TOTAL}(t) \times \left(\frac{1}{1 + TTR(t)}\right) \quad \textit{(Eq. 3)}$$

where $C_{TOTAL}(t)$ is the total concentration (^{12}C + ^{13}C) in mmol/l of a given fatty acid as determined by gas chromatography-flame ionization detection at time point (t).

Model input parameters such as daily ALA consumption and the ^{12}C and ^{13}C n-3 fatty acid concentrations in the plasma phospholipid pool followed a normal distribution. Quantification of n-3 fatty acid conversion was carried out using compartmental modeling by means of the SAAM II version 1.2 software package (SAAM Institute, Inc., Seattle, WA). A constant standard deviation error model (SD = 0.001) was specified for each fatty acid and implemented as "data-relative" within the SAAM II modeling framework (24). The structural model developed from the mean data was applied to each subject's data individually using PopKinetics software (SAAM Institute) and Bayesian parameter priors. The analysis resulted in maximum a posteriori Bayesian estimates of the parameters.

MODEL DEVELOPMENT AND RESULTS

General modeling strategy

The main objective of a tracer study is to obtain information on tracer kinetics, which can be used to describe the kinetic behavior of the unlabeled or tracee substances (25). Information on the kinetics of the tracer is obtained by structuring the system into a mathematical model from which the exchange, input, and output of tracer material can be resolved (26). For our study, a structural model (**Fig. 1**) was developed based on the mean tracer data of the 29 participants. Mean values were used to reduce the influence of random noise during model development. Thereafter, a structurally comparable tracee model (**Fig.**

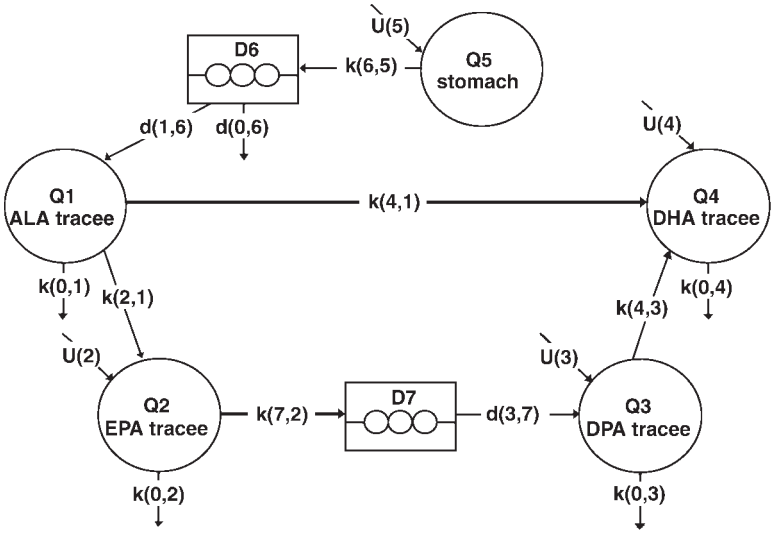

Fig. 2. Tracee model for n-3 conversion. Circles denoted Q1, Q2, Q3, and Q4 represent the plasma ALA, EPA, DPA, and DHA tracee phospholipid compartments, respectively. U(5) represents the daily dietary intake of tracee ALA. The direct endogenous inputs into the EPA, DPA, and DHA tracee phospholipid compartments are given by U(2), U(3), and U(4), respectively.

2) was set up that incorporated the kinetic parameters derived from the tracer system, the averaged values of the tracee measurements of the 29 subjects, and their mean daily dietary ALA intake. The tracer and tracee models were then solved simultaneously to estimate the incorporation of [U-^{13}C]ALA into plasma phospholipids.

Tracer model

The final tracer model is shown in Fig. 1. The experimental period was defined from 0 to 216 h. In agreement with the known conversions of ALA into its LCPUFAs, the main structure of the model consists of a string of four compartments (q1, q2, q3, and q4) that represent the total amounts (μmol) of ^{13}C-labeled ALA, EPA, DPA, and DHA, respectively, in plasma phospholipids. Because the exogenous [^{13}C]ALA tracer was administered orally, it enters the system via the gastrointestinal tract, which causes a delay (d6) before its appearance into plasma phospholipids as ALA. The ALA tracer is then either irreversibly lost [k(0,j)] from the delay compartment or proceeds through the conversion cascade [k(i,j)], as represented by the arrows and their corresponding transfer rate constants. The rate constants, k(i,j), denote the fraction of tracer from the plasma phospholipid pool that is transferred per unit of time from compartment j to i. The fraction of tracer that is irreversibly lost from each compartment j is denoted k(0,j). Tracer n-3 fatty acid concentrations (μmol/l) were measured in plasma phospholipids as designated by the closed circles (si). Because our results were expressed in units of concentration (μmol/l) and compartmental modeling is based on the principle of mass balance, the volume of distribution, which was assumed to be the plasma volume, was incorporated. As plasma volume was not measured, it was assumed to be 4.5% of body weight (27).

The details of the various model structures and assumptions that were tested before arriving at the final model are described below.

Tracer uptake. The oral boluses of the tracer (ex1) first enter the stomach, compartment q5. The assumption was made that there was no irreversible loss of tracer from the stomach. A gastric residence time of 30 min was assumed by setting the rate of gastric emptying, k(6,5), at 2 h^{-1}. The tracer then enters a delay compartment (d6), which depicts all metabolic processes between the arrival of [U-^{13}C]ALA in the small intestine and the appearance of the tracer in the ALA plasma phospholipid compartment (q1). In our model, the delay consisted of a series of five compartments. Several models were analyzed with a physiologically relevant delay time between 3 and 5 h. Because the model was insensitive to the length of the delay time, the delay was set at 5 h. After the delay compartment, [U-^{13}C]ALA can follow two different routes: it is either irreversibly lost or it arrives in the plasma phospholipid pool. The irreversible loss, which is denoted d(0,6) = 1 − incorp, includes the fraction of ALA tracer that is not absorbed as well as the fraction of ALA tracer that is not incorporated into ALA plasma phospholipids after intestinal uptake. The tracer, for example, can be oxidized or incorporated into nonplasma phospholipids, membranes, and tissues. The fraction of ingested [U-^{13}C]ALA that, per unit of time, appears in the plasma phospholipid compartment is defined as d(1,6) = incorp. Because this appearance could not be determined as an adjustable parameter, different values for it were tested. When appearance values were close to 5%, model convergence was obtained. In contrast, values much greater than 5% resulted either in the nonconvergence of the model or in too high estimates of the tracee masses. Therefore, it was decided to fix the appearance parameter temporarily at 5% to solve the tracer model. This constraint of 5% was later replaced by a constraint equation, as detailed in the description of the tracee model.

ALA to EPA. The [U-^{13}C]ALA tracer present in the plasma phospholipid pool was described by a single compartment, q1. The fraction of the ALA tracer irreversibly lost from q1 is depicted by k(0,1), and the fraction that was further elongated and desaturated into EPA is represented by k(2,1). The rate constant, k(0,1), was fixed at zero, as its value was close to zero, when solving the model. The plot of the predicted values and the averaged ALA tracer data is shown in **Fig. 3**. The cyclical nature of the dosing protocol is clearly visible in the predicted curve. Analyzed values were on or near the nadirs of the fitted curve. This was as expected, because blood was sampled in the morning after an overnight fast and before the intake of the next capsule.

EPA to DPA. The structure of the model implied that all newly formed [^{13}C]EPA originated from the plasma ALA phospholipid compartment. As observed for ALA, the predicted values also exhibited a cyclic pattern (**Fig. 4**). However, the amplitudes were smaller and, because of the time needed to synthesize EPA from ALA, shifted to the right on the *x* axis. Unlike for ALA, the observed EPA values increased along the curve until at the end of the study an apparent plateau was reached, which was between the maximum and minimum of the predicted values. This finding can be explained by a slow conversion of EPA into DPA or by an influx of labeled EPA from the nonphospholipid [U-^{13}C]ALA pool. The latter possibility was explored by introducing an additional input, d(2,6), into the EPA com-

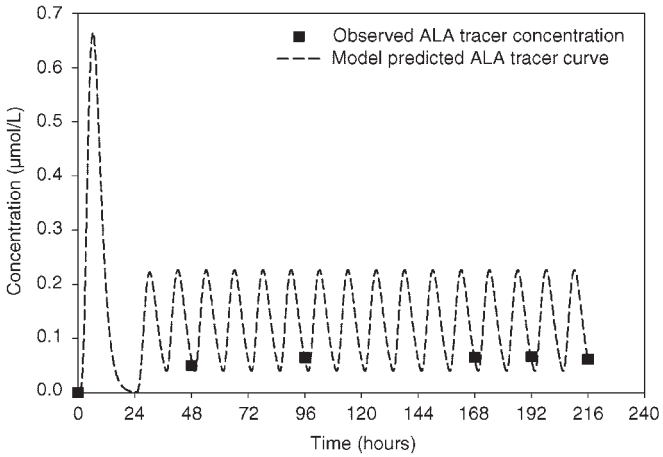

Fig. 3. Model predicted fit through the observed ALA tracer data obtained with the final tracer model.

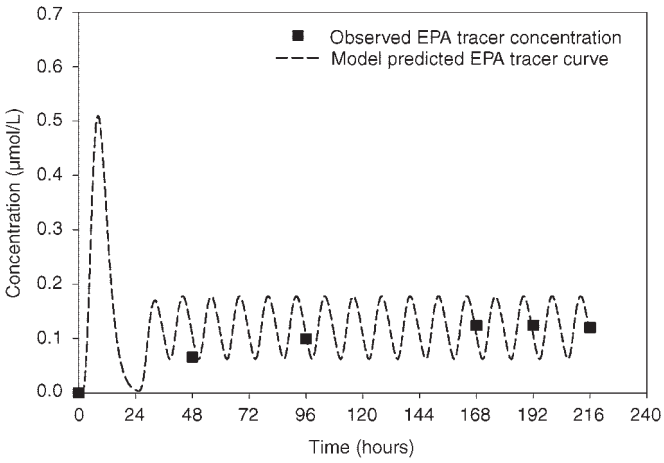

Fig. 4. Model predicted fit through the observed EPA tracer data obtained with the final tracer model.

partment from the delay compartment. This input d(2,6) was estimated by testing several models in which d(1,6) was set as a fixed or adjustable parameter and k(2,1) was set as adjustable or equal to zero. Model convergence was obtained only when d(1,6) was fixed at 5%, k(2,1) was fixed at zero, and k(0,1) was set as adjustable. This model was not considered further, as it was not physiologically meaningful and did not describe the EPA data significantly better than the initial model. Although not evident from our data, it cannot be ruled out that some influx of labeled EPA from the nonphospholipid [U-^{13}C]ALA pool did take place. However, combined with the information from the DPA data, as described below, the most likely explanation for the increase in observed [^{13}C]EPA over time is that the turnover of EPA to DPA is slower than that of ALA to EPA.

DPA to DHA. Compartment q3 represents the ^{13}C-labeled DPA in plasma phospholipids, derived from [^{13}C]EPA through elongation. The irreversible loss from DPA was indistinguishable from zero, and k(0,3) therefore was fixed at zero. The plot of the predicted values (**Fig. 5A**) shows that DPA was still increasing steeply by the end of the experiment, which may be one explanation for why k(0,3) was close to zero. The curve of the predicted DPA values was also very different from those of ALA and EPA. Not only was the curve still increasing, the cyclical shape resulting from the multiple input dosing protocol was reduced to minute oscillations. This indicates that the conversion of EPA into DPA is much slower than the conversion of ALA into EPA. Furthermore, the curve of the predicted values overestimated the first two data points, whereas it slightly underestimated the final points. As this indicated that too much material was introduced too fast into the DPA compartment, a delay (d7) was inserted between the EPA and DPA compartments. The time of the delay was fixed at 30 h, because this resulted in the best possible description of the data (Fig. 5B).

DHA compartment. The amount of DHA in the plasma phospholipid compartment q4 was first described by a single inflow and a single outflow of tracer. The irreversible loss, k(0,4), was small but could be estimated. The plot

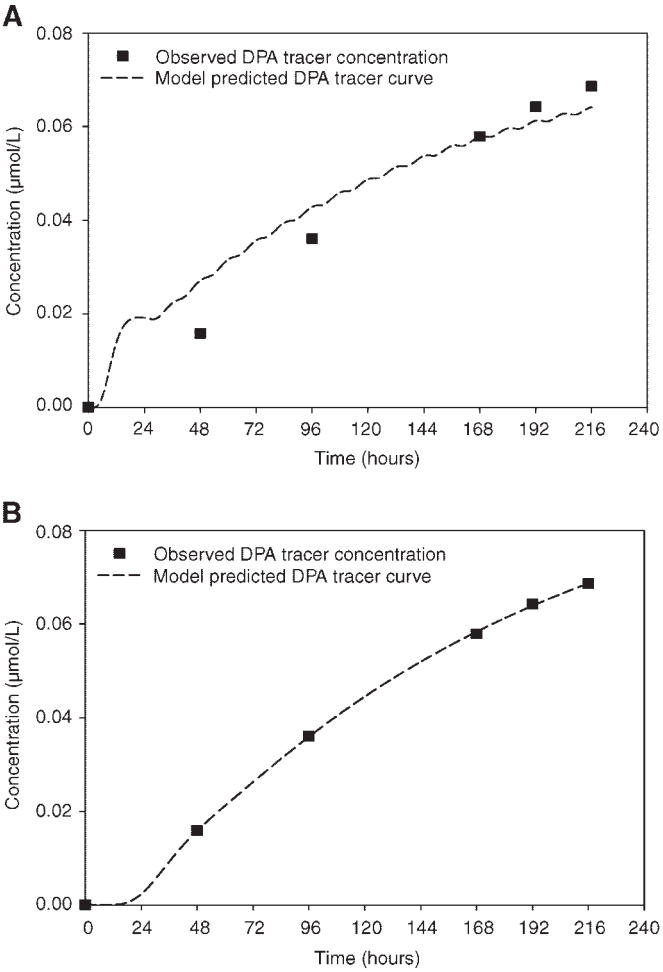

Fig. 5. A: Model predicted fit through the observed DPA data obtained with a model without the delay compartment d7. B: Model predicted fit through the observed DPA data obtained with the final tracer model.

of the predicted DHA curve, however, showed that this model underestimated the amount of DHA during the initial phase of the study (**Fig. 6A**). This implied that additional input into the DHA compartment was required to better describe the data. Therefore, two other models were examined. One model included a pathway, k(4,1), from ALA to DHA, and the other model included a pathway, k(4,2), from EPA to DHA. As k(4,1) and k(4,2) were very small, other transfer rates were not changed or were changed only slightly. As the direct conversion in the liver from ALA to DHA seemed more physiological than the conversion of plasma EPA to DHA, we formulated the final model with k(4,1). This resulted in excellent agreement between the predicted and observed values for the [^{13}C]DHA tracer (Fig. 6B).

Tracer results. The rate constants describing the final model are presented in **Table 2**. The small coefficients of variation show that estimations were quite precise.

Tracee model

The rate constants obtained from the tracer model were used to calculate conversions and fluxes of the tracee

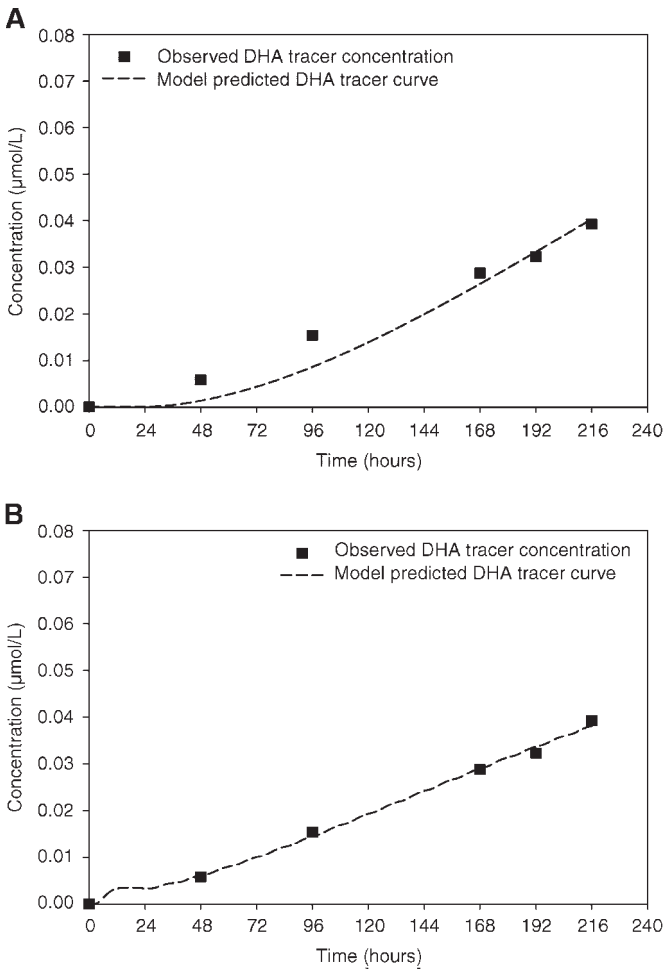

Fig. 6. A: Model predicted fit through the observed DHA data obtained with a model without the rate constant k(4,1). B: Model predicted fit through the observed DHA data obtained with the final tracer model.

data. If the tracee system is in a steady state and the transfer rates are known from the tracer model, the differential equations that describe the tracee n-3 fatty acid system become algebraic equations that can be solved. In the present study, concentrations of the tracee n-3 fatty acids did not change systematically during the tracer experiment, as evident from the results of the GC analyses (data not shown).

As can be seen from Fig. 2, the tracee model is structurally identical to the tracer model (Fig. 1) and has the same k(i,j) values. The various compartments associated with tracee fatty acids are comparable to those of the ^{13}C-labeled tracer fatty acids (Fig. 1), except that now uppercase letters (e.g., Qi, Di) are used. A main difference between the tracee and tracer models is that the tracee model contains additional input arrows denoted U(i). The arrow U(5) into compartment Q5 (stomach) of the tracee model represents the daily consumption of tracee ALA and is expressed as a continuous input (μmol/h). Also, three other direct endogenous inputs, U(2), U(3), and U(4), were created into the EPA, DPA, and DHA compartments, respectively. Furthermore, to estimate the incorporation of dietary ALA into the ALA plasma phospholipid compartment, a constraint equation was incorporated, which could be solved by resolving the tracer and tracee models simultaneously. The derivation of the constraint equation and the rationale for introducing the endogenous inputs U(i) are described below.

TABLE 2. Results of the tracer model parameters and derived variables

Transfer Rates (h^{-1})	Value	Coefficient of Variation
k(6,5)	2	
d(0,6)	0.93041	0.4
d(1,6)	0.06959	5.7
k(2,1)	0.44981	5.7
k(4,1)	0.00081	16.4
k(0,2)	0.48015	9.0
k(7,2)	0.00496	8.9
d(3,7)	1	
k(4,3)	0.00613	3.4
k(0,4)	0.00916	11.3

Appearance constraint. Recall that for the development of the tracer model, the appearance of the tracer in the ALA compartment d(1,6) was temporarily fixed at 5%. To obtain a more accurate estimate for incorporation into the ALA plasma phospholipid compartment, additional information was provided by the known daily intake of dietary ALA and the concentration of tracee n-3 fatty acids into plasma phospholipids. The constraint equation was based on the concept that, to keep the tracee system in a steady-state situation, the maximal input of tracee ALA into the ALA compartment is equal to the sum of tracee ALA that is lost from this compartment.

Tracee ALA in the plasma phospholipid pool can originate from dietary ALA as well as from ALA stored in endogenous depots. It was not possible to estimate both inputs at the same time, and there was no quantitative information on the fraction of endogenous input or the proportion of the endogenous input and the dietary absorption. Therefore, we assumed that all of the tracee ALA came from the diet. The adopted constraint equation was derived as described below.

First, the total ALA output from compartment Q1 was calculated as

$$ALA_{OUTPUT} = Q_1 \times [k(2,1) + k(0,1) + k(4,1)] \quad \textit{(Eq. 4)}$$

or equivalently

$$ALA_{OUTPUT} = C_{ALA} \times V_{ALA} \times [k(2,1) + k(0,1) + k(4,1)] \quad \textit{(Eq. 5)}$$

where Q_1 = the mass of the ALA tracee compartment, C_{ALA} = the concentration (μmol/l) of tracee ALA in plasma phospholipids, and V_{ALA} = plasma volume (liters), estimated as 4.5% of body weight.

The total dietary ALA input into the plasma ALA phospholipid compartment (Q1) was given by

$$ALA_{UPTAKE} = d(1,6) \times U(5) \quad \textit{(Eq. 6)}$$

As the fractional output d(1,6) from the delay compartment into compartment Q1 was defined as

$$d(1,6) = \text{incorp} \quad \textit{(Eq. 7)}$$

the ALA uptake could be rewritten as

$$ALA_{UPTAKE} = \text{incorp} \times U(5) \quad \textit{(Eq. 8)}$$

Furthermore, because at steady state the input into Q1 equaled the output from Q1, ALA_{UPTAKE} could be presented as

$$ALA_{UPTAKE} = ALA_{OUTPUT} \quad \textit{(Eq. 9)}$$

or alternatively

$$\text{incorp} \times U(5) = C_{ALA} \times V_{ALA} \times [k(2,1) + k(0,1) + k(4,1)] \quad \textit{(Eq. 10)}$$

The tracer and tracee models were then linked by the following constraint equation

$$\text{incorp} = \frac{ALA_{OUTPUT}}{U(5)} = \frac{C_{ALA} \times V_{ALA} \times [k(2,1) + k(0,1) + k(4,1)]}{U(5)} \quad \textit{(Eq. 11)}$$

and both models were then solved simultaneously.

Endogenous inputs. Initially, when the tracee model was solved, it was assumed that the tracee fatty acids EPA, DPA, and DHA in plasma phospholipids originated directly from Q1. However, the compartmental masses of these LCPUFAs were then underestimated, which indicated that the EPA, DPA, and DHA compartments were also fed via other routes. Therefore, endogenous input routes U(2), U(3), and U(4) were introduced into the model.

Calculations

The steady-state masses in Qi were obtained by multiplying the ^{12}C-labeled fatty acid concentrations with 4.5% of the average body weight of the 29 participants, which was 73.3 kg. The tracee flux (μmol/h) from compartment j to compartment i was calculated by multiplying the compartmental tracee mass Qj with the rate constant k(i,j). Similarly, the irreversible flux out of compartment j was calculated by multiplying compartmental tracee mass Qj with the rate constant k(0,j).

The proportion of fatty acid transferred from compartment j to compartment i was defined as follows:

$$\text{frac\%\_i,j} = \frac{k(i,j)}{k(j,j)} \times 100\% \quad \textit{(Eq. 12)}$$

where k(j,j) represented the sum of all losses from compartment j.

The relative contribution of the endogenous flux U(i) (mass/h) could be determined for EPA, DPA, and DHA. Because the total tracee influx into compartment Q(i) was equal to the sum of the separate influxes, the fractional contribution of U(i) was calculated as

$$\text{frac\%\_U(i)} = \frac{U(i)}{U(i) + \sum_{\substack{j=1 \\ j \neq i}}^{n} Q_j \times k(i,j)} \times 100\% \quad \textit{(Eq. 13)}$$

RESULTS

The steady-state compartmental masses of the tracee n-3 fatty acids, the tracee fluxes, as well as the endogenous inputs are shown in **Table 3**. The incorporation of dietary ALA into plasma phospholipids and the subsequent conversion to its n-3 fatty acid derivatives, expressed in percentages and absolute amounts of dietary ALA, are given in **Table 4**.

Values were first calculated using averaged data from the 29 subjects. The average daily ALA intake equaled 1,129 mg or 4,056 μmol. Approximately 78.5 mg or 6.96% of dietary ALA was incorporated as ALA into plasma phospholipids. Of this ALA pool, 99.98% was converted to EPA, which corresponded to 6.95% (78.4 mg) of ALA intake. The remaining 0.18% of the ALA plasma phospholipid pool, which was equivalent to 0.013% (0.14 mg) of ALA in the diet, was converted directly to DHA in the liver, before it appeared in the plasma phospholipids as DHA. Only 1% of the EPA in plasma phospholipids, which was equivalent to 0.07% (0.80 mg) of ALA consumption, was converted to DPA. All DPA from the plasma phospholipid pool was converted to DHA. Thus, the total amount of ALA from the diet that was ultimately converted to DHA was 0.08% (0.94 mg). As can be seen from Table 4, the estimates based on the individual values were essentially similar.

Table 5 shows that there was considerable endogenous input of each of the ^{12}C-labeled tracee fatty acids into the EPA, DPA, and DHA plasma phospholipid pools. For example, U(2) corresponded to 33.09 μmol/h ^{12}C-labeled EPA, or 73.8% of the total flux into the EPA plasma phospholipid pool that was not derived from the ALA plasma phospholipid pool. This EPA could have originated from seafood, which had to be consumed before the start of the study, as intake of these products was prohibited during the study. It is also possible that this EPA was partly derived from ALA, which was consumed before or during the tracer study and stored in tissues other than plasma phospholipids. Similarly, the endogenous input [U(3)] explained 34.1% of the total inflow into the DPA plasma phospholipid pool, whereas 80.3% of total inflow into the DHA compartment was DHA obtained from nonphospholipid sources [U(4)]. Except for the mean endogenous inflow into the DHA compartment, the estimates based on individual values were very comparable to the estimates based on the averaged data. This discrepancy (64.3% vs. 80.3%) may be attributable to the individually estimated values for k(0,4) derived from the tracer data. In some individuals, this parameter was close to zero. When this happened, U(4) [and in some cases U(3)] resulted in a small negative number to satisfy the mass balance requirement. For these subjects, U(4) was set to zero. Furthermore, k(0,4) values close to zero could result from measurement variability or from individuals exhibiting slow clearance of tracer DHA, so that a loss from the DHA compartment [k(0,4)] is difficult to estimate.

DISCUSSION

Using compartmental modeling, we found that nearly 7% of daily ALA consumption was incorporated into

TABLE 3. Results of the tracee model parameters and derived variables

Parameter		Value	Coefficient of Variation
Compartmental tracee mass (μmol)			
Q5	Stomach	84.51	
Q1	ALA	26.10	
Q2	EPA	92.41	
Q3	DPA	113.55	
Q4	DHA	397.41	
Tracee fluxes (μmol/h)			
Flux(6,5)	Stomach → delay d6	169.010	
Flux(1,6)	Delay → ALA compartment	11.761	5.72
Flux(0,6)	Loss from delay	157.249	0.43
Flux(2,1)	ALA → EPA	11.740	5.73
Flux(4,1)	ALA → DHA	0.021	16.36
Flux(7,2) = Flux(3,7)	EPA → delay → DPA	0.459	8.89
Flux(0,2)	Loss from EPA compartment	0.021	9.03
Flux(4,3)	DPA → DHA	0.696	3.39
Flux(0,4)	Loss from DHA compartment	3.642	11.34
Endogenous input (μmol/h)			
U(5)	Into stomach	169.01	
U(2)	Into EPA	33.09	11.7
U(3)	Into DPA	0.24	19.6
U(4)	Into DHA	2.92	13.7

ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.

plasma phospholipids. Approximately 99.8% of ALA from this pool was subsequently converted to EPA, whereas only 1% of the EPA plasma phospholipid pool was converted to DPA. Essentially all DPA was used for the synthesis of DHA. Usually, the limiting step within the n-3 cascade is considered to be the Δ-6 desaturation that is necessary for the conversion of ALA to C18:4n-3 (28–31). However, our study suggests that this is not the limiting step, as nearly all EPA in the plasma phospholipid pool was derived from the ALA plasma phospholipid pool. Thus, entry into the hepatic phospholipid pool is a limiting factor for ALA conversion to DHA.

Only a few studies have used stable isotopes to quantitatively study ALA metabolism in adults (12–16, 32, 33). From their pioneering studies, Emken, Adlof, and Gulley (15) estimated that conversion of ALA to its LCPUFAs was 11% on a diet rich in saturated fatty acids and 18.5% on a polyunsaturated-rich diet. We estimated that only ~7% of dietary ALA was converted to its LCPUFAs. The higher n-3 conversion values observed by Emken, Adlof, and Gulley (15) may have been attributable to the use of AUCs. Indeed, Demmelmair et al. (34) have shown that AUC-derived calculations, compared with compartmental modeling, resulted in two times and four to five times higher estimates for the conversion of LA into dihomo-γ-linolenic acid and arachidonic acid, respectively. Compared with AUC values, compartmental modeling is considered to be more accurate, as it accounts for the appearance and disappearance rates of ALA and its various metabolites.

For our modeling framework, we chose plasma phospholipid fatty acid composition to reflect hepatic conversion. Plasma triacylglycerol (TG), which contains even more ALA than phospholipids, could also have been an option. Data from the stable isotope studies of Burdge and colleagues (12, 13), however, showed that only trace amounts of the labeled elongation products of ALA are incorporated into plasma TG. In the present study, we also analyzed in 78 samples the ^{13}C-labeled n-3 fatty acid en-

TABLE 4. Incorporation of dietary ALA into plasma phospholipids and subsequent conversion to its n-3 fatty acid derivatives expressed in percentage and absolute amounts of dietary ALA

	Based on Averaged Data of 29 Subjects	Based on Individual Data of 29 Subjects			
Incorporation and Conversion	Value	Mean	SD	Minimum	Maximum
Dietary ALA intake or U(5) (mg/day)	1129	1129	124	907	1369
Incorporation of dietary ALA into ALA compartment (%)	6.96	7.72	3.25	1.88	15.69
Percentage of dietary ALA converted from					
ALA → EPA	6.946	7.705	3.24	1.88	15.68
ALA → DHA	0.013	0.016	0.03	0.00	0.16
ALA → EPA → DPA → DHA	0.071	0.073	0.04	0.02	0.20
Incorporation of dietary ALA into ALA compartment (mg)	78.54	86.28	35.31	20.81	173.27
Absolute amount (mg) of dietary ALA converted from					
ALA → EPA	78.39	86.11	35.25	20.73	173.23
ALA → DHA	0.14	0.17	0.31	0.00	1.54
ALA → EPA → DPA → DHA	0.80	0.82	0.43	0.22	2.05

TABLE 5. Percentage fractional contribution of endogenous inputs and percentage fractional transfers

Contribution and Transfer	Based on Averaged Data of 29 Subjects	Based on Individual Data of 29 Subjects			
	Value	Mean	SD	Minimum	Maximum
Percentage fractional transfer					
incorp%: into ALA compartment	6.96	7.72	3.25	1.88	15.69
frac%_02: loss from EPA compartment	98.98	98.95	0.52	97.90	99.69
frac%_21: ALA → EPA	99.82	99.81	0.29	98.85	100.00
frac%_41: ALA → DHA	0.18	0.19	0.29	0.00	1.15
frac%_72: EPA → DPA	1.02	1.05	0.52	0.33	2.10
frac%_43: DPA → DHA	100.00	100.00	0.00	100.00	100.00
Percentage fractional contribution of U(i)					
Frac%_U(2): endogenous input into EPA compartment	73.81	73.78	4.70	65.21	82.43
Frac%_U(3): endogenous input into DPA compartment	34.11	34.10	25.30	0.00	80.06
Frac%_U(4): endogenous input into DHA compartment	80.31	64.29	38.30	0.00	97.10

richments of plasma TG, which were isolated by thin-layer chromatography. Enrichments in the long-chain n-3 fatty acids were too low to be measured reliably.

Except for phospholipids and TG, plasma total lipids also consist of free fatty acids, chylomicrons, and cholesteryl esters (CEs). We believe that neither of these lipid fractions reflects hepatic n-3 conversion reliably. From the studies of Burdge and colleagues (12, 13), it can be estimated that in women only 2.1% of the total [^{13}C]ALA plasma pool was found in plasma free fatty acids over a period of 21 days. For EPA, DPA, and DHA, these values were 0.3, 0.0, and 1.5%, respectively (13). For men, 4.8% of total plasma [^{13}C]ALA was found in the plasma free fatty acid fraction, whereas other ^{13}C-labeled n-3 fatty acids were not traced at all (12). Chylomicrons are also not representative for hepatic n-3 fatty acid conversion, as they contain a fraction of [^{13}C]ALA that has not yet been taken up and metabolized by the liver. Thus, if ^{13}C-labeled n-3 LCPUFAs are present in chylomicrons, these fatty acids may be derived from conversion within the enterocyte and not from conversion within the liver. Plasma CEs are formed from cholesterol released from peripheral tissues through the action of LCAT, which transfers a fatty acid from a phospholipid to free cholesterol. CEs are also synthesized in the intestine and liver. If the CE-derived n-3 LCPUFAs originate from plasma phospholipids, the CE pool will not provide new information on hepatic ALA conversion, because these fatty acids are part of the loss parameters, leaving from the various plasma phospholipid compartments. If the CEs are of intestinal origin, the CE-derived ^{13}C-labeled n-3 LCPUFAs, if any, are produced by the enterocyte and therefore do not represent n-3 conversion in liver. Only CEs produced by the liver itself might reflect hepatic ALA conversion, but, as for TG, plasma CEs are rather poor in n-3 LCPUFAs (35). Therefore, including masses of any other n-3 fatty acid lipid pool in the model would mainly increase the mass of the ALA compartment. Masses of the other compartments would be minimally affected. As a result, the increased mass of the ALA compartment would be reflected by an increased loss from the ALA compartment rather than by changed fluxes into the other compartments.

Pawlosky et al. (16) were the first to use compartmental modeling to quantify n-3 conversion in adults. In contrast to our results, their findings suggested that only 0.2% of ALA from plasma total lipids was used for EPA synthesis. Approximately 63% from the plasma total lipid EPA pool was converted to DPA, and 37% of DPA was converted to DHA. As already discussed, plasma total lipids may not be the most appropriate lipid fraction for the quantification of hepatic n-3 fatty acid conversion. The discrepancies between our results and those of Pawlosky et al. (16) may further be attributable to other differences in experimental protocols. In our study, tracer intake on the first day (30 mg/day) was ~2.7% of dietary ALA intake, and on the other 8 days (20 mg/day) it was 1.8%. In contrast, Pawlosky et al. (16) gave a single bolus of 1,000 mg of deuterated α-linolenate ethyl ester (d5-C18:3n-3), dissolved in a low-fat yogurt, followed by a morning meal and a lunch 4 h later. This tracer bolus constituted as much as ~89% of the dietary ALA consumption during the study period. Because isotope intake clearly exceeded trace amounts, perturbation of the kinetic behavior of the ^{12}C-labeled n-3 fatty acids cannot be ruled out (25). Another difference between our study and that of Pawlosky et al. (16) is the mode of administration of the stable isotopes. As in most other studies, Pawlosky et al. (16) administered the ALA tracer as a single bolus, whereas we provided the tracer in fractionated portions over time. We chose this approach to mimic the longer term supply and metabolism of ALA and to ensure measurable values for DHA. It has already been demonstrated by Vermunt et al. (33) that after administration of a single bolus of [^{13}C]ALA, enrichment of DHA increased only marginally and slowly. Burdge, Jones, and Wootton (12) also could not detect any significant DHA enrichment over a period of 21 days in plasma phosphatidylcholine, chylomicron TG, or nonesterified fatty acids after administration of a single bolus of [^{13}C]ALA. It is further known that, at least for LA, the mode of tracer administration influenced the modeling outcomes (34). The estimated conversions of LA to dihomo-γ-linolenic acid and of LA to arachidonic acid after a fractionated intake of LA tracer were higher compared with the estimated conversions after a single bolus of the same tracer amount.

One possible limitation of our model is that it mirrored the hepatic conversion of dietary ALA. Although it is gen-

erally accepted that in humans the liver is the most significant site for n-3 fatty acid conversion, studies with primates suggest that other tissues, such as nervous tissue, are also able to convert ALA (36). Hence, the total n-3 fatty acid conversion might have been underestimated. However, from the present findings, we can conclude that the limited incorporation of dietary ALA into the phospholipid pool contributes to the low hepatic conversion of dietary ALA to EPA. We further found that the conversion of EPA to DPA, which involves an elongation reaction, might be an additional bottleneck within the hepatic n-3 cascade.

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