Physiological compartmental analysis of α-linolenic acid metabolism in adult humans

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Abstract **A physiological compartmental model of α-linolenic acid metabolism was derived from the plasma concentration-time curves for d5-18:3n-3, d5-20:5n-3, d5-22:5n-3, and d5-22:6n-3 in eight healthy subjects. Subjects received a 1-g oral dose of an isotope tracer of α-linolenate (d5-18:3n-3 ethyl ester) while subsisting on a rigorously controlled beef-based diet. By utilizing the Windows Simulation and Analysis Modeling program, kinetic parameters were determined for each subject. Half-lives and mean transit times of the n-3 fatty acids in the plasma were also determined. The model predicted plasma values for the n-3 fatty acids in good accordance with the measured steady state concentrations and also predicted dietary linolenic acid intake for each subject in accordance with values determined by lipid analysis of the diet. Only about 0.2% of the plasma 18:3n-3 was destined for synthesis of 20:5n-3, approximately 63% of the plasma 20:5n-3 was accessible for production of 22:5n-3, and 37% of 22:5n-3 was available for synthesis of 22:6n-3. The inefficiency of the conversion of 18:3n-3 to 20:5n-3 indicates that the biosynthesis of long-chain n-3 PUFA from α-linolenic acid is limited in healthy individuals. In contrast, the much greater rate of transfer of mass from the plasma 20:5n-3 compartment to 22:5n-3 suggests that dietary eicosapentaenoic acid may be well utilized in the biosynthesis of 22:6n-3 in humans.**—Pawlosky, R. J., J. R. Hibbeln, J. A. Novotny, and N. Salem, Jr. **Physiological compartmental analysis of α-linolenic acid metabolism in adult humans.** ***J. Lipid Res.*** **2001.** 42: **1257–1265.**

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The American Heart Association has recommended increasing the dietary intake of fish, especially "fatty" fish containing long-chain ω-3 fatty acids docosahexaenoic acid (22:6n-3) and eicosapentaenoic acid (20:5n-3), because of their beneficial effects on cardiovascular health (1–7). Other potential health benefits may include reductions in risks of common cancers (8, 9), improvements in infant neurodevelopmental outcomes (10–13), as well as possible psychiatric benefits (14).

Several species of marine fish, such as herring, sardines, salmon, and fresh tuna, offer a rich dietary source of 20:5n-3 and 22:6n-3 (15, 16). However, these foods do not form a regular part of a "typical" diet for most Americans (17). For the majority of Americans, the biosynthesis of long-chain n-3 PUFA from α-linolenic acid (18:3n-3) may be a major contributor to the body's supply of 20:5n-3 and 22:6n-3 (18). An accurate model of α-linolenic acid metabolism in humans based on direct data obtained from an isotope tracer would be valuable in assessing the contribution of biosynthesis in the maintenance of long-chain n-3 PUFA in the body.

Although several human studies have been carried out by using stable isotope-based approaches to determine the conversion of essential fatty acids to long-chain PUFA (19–24), little quantitative information exists that utilizes compartmental modeling to assess metabolism of 18:3n-3 in humans (25). A goal of the mathematical analysis used in this study is to determine the quantitative contributions of each of the dietary fatty acids to the maintenance of long-chain n-3 PUFA homeostasis in plasma. To accurately assess the biochemical and physiological interactions involved in maintaining plasma n-3 PUFA concentrations within a given population, specific dietary and metabolic information must be taken into account. Three sets of information should be evaluated: *1*) dietary fatty acid intake from each individual collected over a specified time, *2*) kinetic parameters (isotopic tracer data) that describe the flux of the distinct biosynthetic intermediates from each subject, and *3*) accurate determinations of the masses of endogenous fatty acids in the precursor pools that are available for biosynthesis.

This study of eight healthy subjects satisfied the above criteria. Subjects were maintained on a well-defined diet, and after a period of dietary equilibration were given an

Abbreviations: GC-MS, gas chromatography-mass spectrometry; WinSAAM, Windows Simulation and Analysis Modeling.

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oral dose of a stable isotopic tracer (d5-18:3n-3 ethyl ester). The isotopic fatty acid data used for determining the plasma kinetic profiles were obtained by mass spectrometric analyses and gas chromatography was used to determine the concentrations of the endogenous n-3 fatty acids. Using this information, a physiological compartmental model of α-linolenic acid metabolism was constructed with the Windows version of Simulation, Analysis, and Modeling program [WinSAAM; National Institutes of Health (NIH), Bethesda, MD]. The n-3 fatty acid kinetic parameters were determined for each subject and then the mean values were used to calculate the mean transit time (MTT) and half-life of the individual fatty acids in the plasma.

MATERIALS AND METHODS

Clinical procedures

Subjects. All subjects were evaluated at the clinical research unit of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the National Institutes of Health Clinical Center in Bethesda, Maryland. Subjects were given a physical examination and extensive clinical laboratory testing. Male and female subjects were included if they had no major medical problems; did not smoke or use tobacco products within the last 2 years; if they did not consume more than the equivalent of two glasses of beer or wine per day, use prescription medications within the last month or over the counter medications; and if they were judged to be reliable in maintaining the dietary requirements of the protocol. All healthy control subjects provided written informed consent and all clinical procedures were under continuous review by the NIAAA Institutional Review Board under protocol #92-AA-0194.

Excluded were subjects who persistently used vitamin E, vitamin C, multivitamins, or lipid supplements; herbal or home remedies of unknown composition; home cures or unusual vitamins; or other unusual dietary habits.

Subjects received a beef-based diet for 21 days. Diet nutrients were calculated from the USDA Nutrient Database (Handbook 8). The fatty acid composition of the diet is listed in **Table 1**. All meals were prepared and served by the research kitchen in the clinical research unit at the NIH. To increase acceptance, a 2-day rotating menu was provided during each experimental period. Food sources were consistent throughout the study. To limit the intake of 20:5n-3 and 22:6n-3 no seafood or seafood products were included in the dietary regimen.

Administration of the deuterated fatty acid and subsequent blood draws occurred during the final week of the dietary period. Subjects were admitted as inpatients, fasted overnight, and then administered 1 g of the deuterated fatty acid ethyl ester blended into low fat (1% fat) yogurt before being given a standardized morning meal. The isotope used was deuterated α-linolenate ethyl ester (d5-17, 17, 18, 18, 18-18:3n-3; Cambridge Isotope Laboratories, Andover, MA). A standardized lunch (a hamburger, an apple, and reduced fat milk) was provided 4 h later to ensure uniform absorption. Blood (40 ml) was drawn under fasting conditions (with the exception of the 8-h sample) from the forearm, at baseline (before dosing with the deuterated fatty acid) and at intervals over the following week (8, 24, 48, 72, 96, and 168 h), into a plastic tube containing sodium citrate as an anticoagulant. The blood was placed on ice and then separated immediately into platelet-poor plasma by centrifugation at 3,000 rpm (1,800 *g*) for 10 min in a clinical centrifuge. Plasma was transferred to a separate tube and frozen at −80°C until analysis.

TABLE 1. Fatty acyl composition of the control diet

Fatty Acid	Composition
	g/day
16:0	32.4 ± 4.3
18:0	8.3 ± 1.1
16:1	5.3 ± 0.4
18:1	28.2 ± 1.4
n-6	
18:2	5.3 ± 0.3
20:3	0.10 ± 0.004
20:4	0.24 ± 0.01
n-3	
18:3	0.72 ± 0.03
20:5	0.044 ± 0.001
22:5	0.092 ± 0.003
22:6	0.015 ± 0.001

The diet was formulated to contain beef, olive oil, and butter as the major source of fats. The values were determined from triplicate fatty acyl analysis of the diet and represent approximate intake values for the group based on a 2,700-kcal diet.

Diet formulation and analysis

Beef provided the major source of dietary fat in the control diet. The only other significant sources of fat were olive oil and butter. The Harrison-Benedict equation was used to calculate calorie requirements. The calculations were compared with the subject's ad libitum food record data and adjusted accordingly. Subject weights were monitored during the study and individual caloric intake was adjusted to maintain less than a 1-kg weight change. Subjects were counseled not to eat or drink any additional foods or beverages other than those provided by the research kitchen. No alcoholic beverages or smoking was allowed.

The fatty acyl content of the metabolic diet was analyzed directly. The foods from an entire day's menu were combined in a commercial 2-liter blender and homogenized, and aliquots were obtained and lipids extracted by the Folch, Lees, and Sloane-Stanley method (26). Gas chromatography analysis of the fatty acid methyl esters performed as described below.

Plasma lipid analysis by gas chromatography-flame ionization detection of fatty acid methyl esters

Lipid extraction. Lipid extraction was carried out by a modification of the Folch, Lees, and Sloane-Stanley method (26). Plasma (0.2 ml) was added to a tube containing 100 μl (10 μg) of 23:0 methyl ester internal standard and 1 ml of butylhydroxy toluene (BHT)-methanol solution (BHT, 50 mg/l of methanol). Chloroform (2 ml) and 0.2 M NaH_2PO_4 buffer (2 ml) were added and the tubes were vortexed for 1 min and centrifuged at 3,000 *g* for 2 min at 4°C. The lipid-containing lower phase was removed and the extraction was repeated by adding 2.0 ml of $CHCl_3$.

Preparation of fatty acid methyl esters

Transmethylation was performed by a modification of the Morrison and Smith method (27). An aliquot of the lower phase was evaporated to dryness under nitrogen and 1 ml of 14% BF_3-methanol was added along with 0.2 ml of hexane. Tubes were flushed with nitrogen, heated to 100°C for 1 h, and cooled. The methyl esters were extracted into hexane and the pooled extracts were concentrated to 50 μl under nitrogen, and analyzed by gas chromatography.

Gas chromatographic analysis

Samples were analyzed with a model HP-5890 gas chromatograph with flame ionization detector (Hewlett-Packard, Wil-

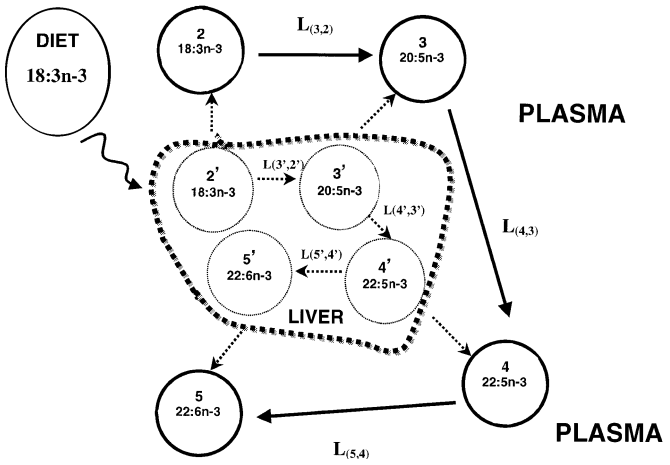

Fig. 1. An illustration of n-3 fatty acid metabolism in humans, beginning with dietary α-linolenic acid (circles designate various n-3 fatty acid compartments). The liver, site of biosynthesis of n-3 fatty acid intermediates, synthesizes VLDL that transport fatty acids in the plasma to tissues. Dotted arrows represent metabolic processes for which rate constants have yet to be determined. For purposes of the model it was assumed that the kinetic constants, $L_{(I,J)}$, determined in the plasma reflects similar values $L_{(I'J')}$ within the liver.

mington, DE) according to previously published procedures (28). The concentrations of the individual fatty acids were calculated by comparing the peak area counts with the internal standard.

Negative chemical ionization gas chromatography-mass spectrometry derivatization

Plasma samples (0.2 ml) were lipid extracted as described above after addition of 0.1 μg of methyl 23:0, hydrolyzed in 5% KOH-methanol as previously described (29), and reacted with 150 μl of the pentafluorobenzyl (PFB) reagent [acetonitrile–diisopropylethylamine–pentafluorobenzyl bromide 1,000:100:1 (v/v/v)]. Vials were incubated at 60°C for 12 min with mixing, cooled, and evaporated under a steam of nitrogen and resuspended in 100 μl of hexane.

Instrumental analysis

Gas chromatography-mass spectrometry (GC-MS) conditions were as described previously by Pawlosky, Sprecher, and Salem (29). Samples (1 μl) were injected onto a 60-m FFAP bonded phase capillary column (0.25-mm i.d., film thickness 0.25 mm; Quadrex, New Haven, CT) into a quadrupole GC-MS. Data were acquired in the selected ion mode, monitoring the M-PFB anion of the fatty acids, and converted to the absolute quantity of the deuterated metabolite by reference to the concentration of the internal standard, using an experimentally determined response factor for each of the fatty acids.

Compartmental analysis of α-linolenic acid

n-3 fatty acid metabolism and physiology. Compartmental analysis began by considering the existing knowledge of n-3 fatty acid metabolism and human physiology. The liver hepatocyte is a main site for the biosynthesis of 20- and 22-carbon PUFA from 18:3n-3 and for formation of lipoproteins that transport fatty acids in the plasma (see **Fig. 1**). Because liver biopsies were not performed and therefore liver specimens were not available for analyses, this study offers only indirect information regarding the concentrations of d5-fatty acid substrates available within the hepatocyte. Similarly, rate constants (see below) determined from the model represent kinetics of d5-fatty acids from their plasma pool concentrations alone and therefore only indirectly reflect liver metabolism (Fig. 1). It is assumed that there is a definite, yet undefined, relationship maintained between the appearance of the labeled fatty acids in the plasma and the availability of n-3 fatty acid substrates for metabolism within the liver.

Fractional transfer rates, flow rates, percentages, and turnover

A physiological compartmental model of n-3 fatty acid metabolism was constructed after a consideration of the well-known metabolic pathway for α-linolenic acid metabolism (30, 31) and the concentrations of fatty acids in the plasma pool (**Fig. 2**). The fractional transfer rate, $L_{(I,J)}$ (or "rate" constant), is the fraction of substrate that is transferred from substrate compartment J to

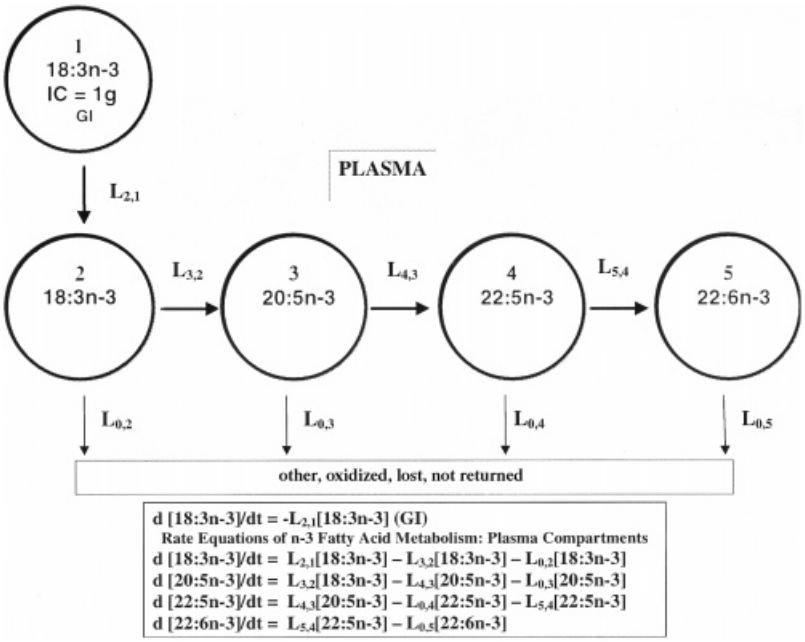

Fig. 2. Conceptual model of α-linolenic acid metabolism. The open circles represent separate n-3 fatty acid compartments in the metabolic scheme. Compartment 1 represents administration of the isotope (1 g) and intestinal absorption. Four compartments (2 through 5) represent n-3 fatty acid compartments in the plasma following on successive steps in desaturation and elongation of the label. The fractional transfer rates, $L_{(I,J)}$ are rate parameters derived from the model-fitted experimental data. The set of differential equations used in determining the rate parameters is given in the boxed area. GI, gastrointestinal tract.

product compartment I. The units are in time^{-1} (in this study, h^{-1}). In this analysis $L_{(I,J)}$ represents an assemblage of several independent enzymatic processes, each having a separate rate constant, for which no intermediates were isolated. The rate of flow, $R_{(I,J)}$, from substrate compartment J to product compartment I is obtained by multiplying the mass (M_J) of endogenous (unlabeled) fatty acid in compartment J by the fractional transfer rate $L_{(I,J)}$ and is given in micrograms per hour. The percentage of isotope that is transferred from J to I is given as $P_{(I,J)}$ and is a percentage. $P_{(I,J)}$ is the fraction of isotope that stays in the metabolic pathway as opposed to isotope taken up by tissues or irreversibly lost from the compartment. The half-life ($t_{1/2}$) of the n-3 fatty acids in the plasma was calculated from the sum of the fractional transfer rates leaving the compartment: $t_{1/2} = \ln 2/\Sigma L_{(I,J)} + L_{(0,J)}$. The MTT is the average interval of time during which a molecule of an n-3 fatty acid stays in that compartment during one pass through the compartment. It is equal to $1/\Sigma L_{(I,J)} + L_{(0,J)}$ and its units are in hours. Variances for the determined parameters are reported as the standard deviation (SD), the standard error of the mean (SEM), or the fractional standard deviation (FSD, equal to the standard deviation divided by the mean) where appropriate.

Model illustration and rate equations

The constructed model consisted of five compartments for which isotope data were obtained (Fig. 2). Compartment 1 represents the dose of the labeled fatty acid that was administered and absorption through the gastrointestinal tract. Compartments 2 through 5 denote plasma pools of the n-3 fatty acids (18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3). The five compartments are connected by arrows, which indicate flow along the path. Compartmental analysis of the experimental data did not require the inclusion of retroconversion kinetics to fit the data (e.g., the conversion of 22:5n-3 to 20:5n-3), thus arrows indicating retroconversion are not represented. Arrows drawn from each compartment J, directed toward the bottom of Fig. 2, represent losses of isotope from the system, that is, labeled fatty acids that do not remain in the metabolic pathway. These constants are designated as $L_{(0,J)}$. The rate equations (from which the kinetic parameters are derived) are defined by a set of differential equations that correspond to the flux (changes in the concentration) of the labeled fatty acid substrates through their respective compartments (Fig. 2).

Model limits and constraints

Because the caloric intake of the diet had been adjusted to the energy requirements of each subject and the dietary fatty acid composition had been experimentally determined, the daily n-3 fatty acid intake for each subject could be estimated and for purposes of this analysis upper and lower n-3 fatty acid limits were assigned. The mean predicted dietary intake values determined from the model were then compared with the estimated values for the cohort.

The plasma steady state fatty acid concentrations had been determined for each subject at each of the blood sampling time points. Inasmuch as the variability of dietary intake was minimized by the standardized diet, there was little difference (±5%) in the concentrations of the individual n-3 fatty acids within each individual over this period. Therefore, each subject's mean concentration of each plasma n-3 fatty acid was used to represent the steady state mass of the endogenous substrate (M_J) available for biosynthesis (see Table 3). These values were held constant.

The standardized diet was similar to a typical American beef-based diet. Because the liver and plasma fatty acid pools are rapidly exchangeable, and as a liver biopsy was not possible in these subjects, it was assumed that the plasma and liver pools of endogenous fatty acids had come to equilibrium. Further, it was assumed that the major input of n-3 fatty acids into the system resulted from dietary intake. Other input, such as fat mobilization from adipose tissue or fats accessible from the liver stores, would be determined from the model calculations.

Model calculations, errors, and predicting dietary n-3 fatty acid intake

The initial estimates of the fractional transfer rates $L_{(I,J)}$ and percentages $P_{(I,J)}$ of isotope transfer for this compartmental model were derived by the WinSAAM program from the concentration-time curves that were generated from the experimental data. Values assigned to kinetic parameters were then adjusted to compensate for individual variances in the plasma data until the model prediction gave the best fit to the experimental determinants. Final values were determined by using the WinSAAM iterative nonlinear least-squares routine. The error model for this analysis included the assumptions of independence, constant variance, and normal distribution about zero. Data points were weighted by assigning a fractional standard deviation of 0.1 to each measurement, which is consistent with the precision of the methods for the determination of the labeled and unlabeled fatty acids in the plasma. Steady state mass (M_J) values determined from the plasma fatty acid compartments were used to calculate the flow of n-3 fatty acid substrates to fatty acid products $R_{(I,J)}$. These values were used to predict the n-3 fatty acid intake for each subject and then compared with estimated dietary intake values for model validation.

RESULTS

Subject characteristics, dietary fat intake, and plasma composition

All eight subjects (four male and four female) in the protocol completed the study. Subjects had a mean age of 27.8 years (22–37 years), a height of 174 cm (116–187 cm), body weight of 71.7 kg (53–83 kg), and a body mass index of 23 (19–25). The energy and macronutrient group mean values for the controlled diet were adjusted to a level of 2,700 kcal/day and 102 (16% energy), 98 (33%), and 364 (51%) g/day

TABLE 2. Mean steady-state plasma fatty acyl concentrations in eight subjects after 3 weeks of dietary equilibration on a control diet

Fatty Acid	Concentration[a]
	μg/ml
16:0	356 ± 21
18:0	130 ± 7
18:1n-9	340 ± 22
n-6	
18:2	507 ± 32
20:3	34 ± 2
20:4	154 ± 12
22:4	5.5 ± 0.4
22:5	4.4 ± 0.3
n-3	
18:3	7.3 ± 0.8
20:5	9.1 ± 1.1
22:5	10.9 ± 0.7
22:6	27.1 ± 2.4

The values represent averages, for the purpose of compartmental analysis; individual steady-state concentrations from each subject were used as the substrate mass.

[a] Data are expressed as means ± standard error for n = 8 subjects.

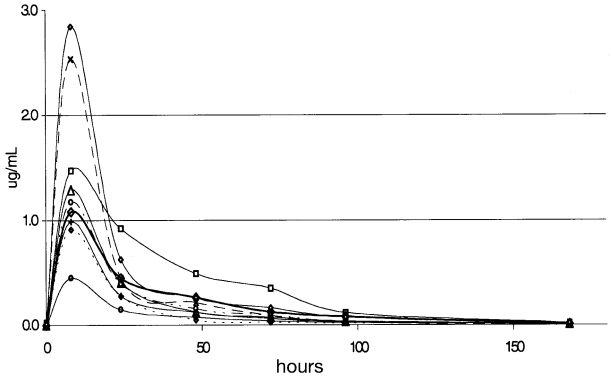

Fig. 3. The plasma concentration-time curves of d5-18:3n-3 in eight subjects after receiving oral administration of d5-18:3n-3 ethyl ester. Blood was sampled 0, 8, 24, 48, 72, 96, and 168 h after dosing. Concentrations were determined by GC-MS and are plotted here as a function of time.

of protein, fat, and carbohydrate, respectively. The total saturates, total monounsaturates, linoleate, and α-linolenate were all well balanced (Table 1). The mean concentrations of plasma fatty acids for this cohort at the end of the dietary period (168 h) are presented in **Table 2**.

Compartmental model and absorption of the labeled fatty acid

The compartmental model shown in Fig. 2 is a simplification of physiological reality in that the liver was not isolated as a separate compartment from the plasma. The model presumes that each rate constant reflects several steps of metabolism that occur within the liver. The model-determined fractional transfer rates are a reflection of those processes. This assumption implies that the individual $L_{(I,J)}$ constants, as determined by the model, incorporate a kinetic function that involves a transport step of fatty acids from the liver to the plasma (Fig. 1). It is assumed that this kinetic function is similar for the appearance of all the fatty acids measured in the plasma.

Two 18:3n-3 compartments are included in the model: one for the isotope administration and gastrointestinal tract and the second for the appearance of the fatty acid in the plasma. It was presumed that maximum absorption of the isotope as chylomicrons into the blood stream occurs between 2 and 4 h after consuming the morning meal (19). Chylomicrons formed during absorption are partially hydrolyzed in the blood stream to form chylomicron remnants, which are later taken up by the liver. The concentrations of d5-18:3n-3 in the plasma (**Fig. 3**) determined at 8 h probably result from a mixture of remnants and VLDL synthesized by the liver. No attempt was made to determine the exact mass of the isotope absorbed by each subject, but for the purposes of this analysis it was assumed that fat absorption was essentially complete (98%). However, a cursory inspection of intersubject variance of the concentration-time curves of d5-18:3n-3 shown in Fig. 3 suggests that a more detailed analysis of absorption should be considered in future studies. The mean value calculated from the area under the curve (AUC ± SEM) of the plasma d5-18:3n-3 for this group was 229.5 ± 18.5 mg·h.

Concentration-time curves for n-3 fatty acids

Figure 4 illustrates the experimentally determined concentrations of the labeled n-3 fatty acids as a function of time for d5-18:3n-3 (Fig. 4A), d5-20:5n-3 (Fig. 4B), d5-22:5n-3 (Fig. 4C), and d5-22:6n-3 (Fig. 4D) from a typical subject. Time curve lines, calculated from the proposed

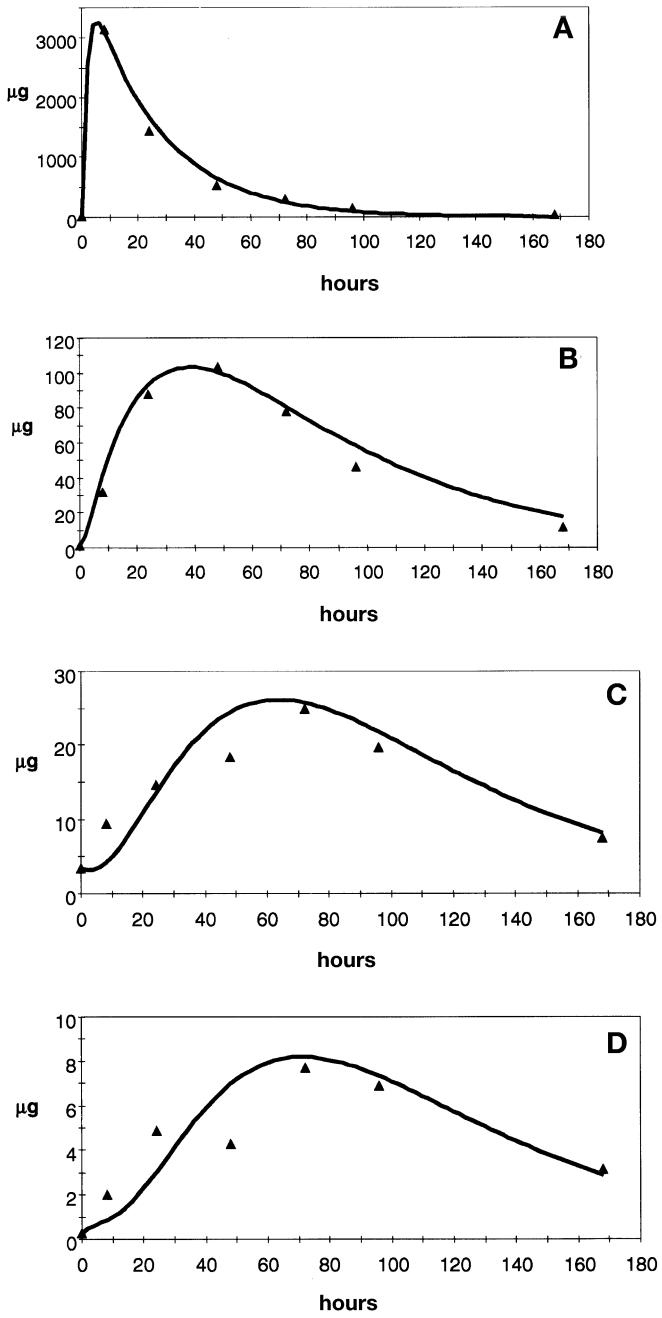

Fig. 4. Graphical analysis of the experimentally determined (solid triangles) isotope data and the best-fit line as determined from the physiological model (from Fig. 2) for a typical subject, using the WinSAAM program. Concentration-time curves for (A) d5-18:3n-3, (B) d5-20:5n-3, (C) d5-22:5n-3, and (D) d5-22:6n-3. Portions of the model were adjusted until the curve gave the best fit to the plasma data. Final values were obtained with the WinSAAM nonlinear least-squares routine. Kinetic parameters, half-lives of n-3 fatty acids in the plasma, and mean transfer time (MTT) were determined from the model.

model in Fig. 2, that best fit the experimental data from this subject are given for each of the n-3 fatty acid time courses. The individual kinetic parameter estimates resulting from compartmental curve fitting were optimized by using the least-squares routine for each subject before determining the mean kinetic values. In a few cases, it was observed that a residual amount of isotope appeared in some of the n-3 fatty acid compartments at the 0-h time point, for example, in Fig. 4C (22:5n-3) and in Fig. 4D (22:6n-3). These residues were the result of a prior dose of the label that the subjects had ingested 3 weeks before the present experiment. The residual levels of the isotope (although minor compared with the later synthesized concentrations), when present, were integrated into the model so that the initial conditions were altered to reflect availability of the labeled substrates at early time points.

The individual model-determined kinetic parameter estimates from each subject and the plasma fatty acid substrate masses are presented in **Table 3**. The fractional transfer rates $L_{(I,J)}$ are the kinetic constants of n-3 fatty acid metabolism for each of the subjects. The mean values of the individual parameter estimates were used to calculate the average values of the kinetic parameters for this cohort (**Table 4**). Table 4 summarizes these kinetic parameters $L_{(I,J)}$, $P_{(I,J)}$, and $R_{(I,J)}$ and the half-lives of n-3 fatty acids ($t_{1/2}$) and their MTT values in the plasma for this group. The mean fractional transfer rates, $L_{(I,J)}$, determined for this group of subjects are given in h^{-1}. Transfer rates having a subscript $_{(0,0J)}$ equal the sum of the kinetic constants $L_{(I,J)}$ leaving compartment J. "Turnover" of n-3 fatty acids in the compartments are calculated from the $L_{(0,0J)}$ constants for each subject and are represented here by the mean intervals for $t_{1/2}$ and MTT. Similarly, mean flow rates designated as $R_{(0,0J)}$ represent the total mass of fatty acid leaving compartment J in $\mu g\ h^{-1}$. The constants that pertain to the transfer of mass from compartment J to compartment I have the designation $R_{(I,J)}$. In Table 4, for example, the mean flow rate out of the 18:3n-3 compartment into 20:5n-3 [$R_{(3,2)}$] equals 34.5 $\mu g\ h^{-1}$ (or 0.83 mg day^{-1}); whereas the mean flow rate out of 20:5n-3 into 22:5n-3 [$R_{(4,3)}$] is given as 361 $\mu g\ h^{-1}$ (or 8.66 mg day^{-1}). Thus, there is a 10-fold greater rate of transfer of mass of 20:5n-3 to 22:5n-3 than there is from 18:3n-3 to 20:5n-3.

The proportion of the plasma n-3 fatty acids $P_{(I,J)}$ directed toward biosynthesis was determined by the model (Table 4). The percentage of 18:3n-3 destined for synthesis of 20:5n-3 was only about 0.2%. In contrast, the average fraction of 22:6n-3 synthesized from 22:5n-3 was 37% and the amount of 20:5n-3 available for synthesis of 22:5n-3 was 63%. The initial step in the biosynthesis (transfer of fatty acid from the 18:3n-3 compartment to 20:5n-3 compartment) is the most restrictive in this sequence and appears to strongly limit the production of 20- and 22-carbon fatty acids from 18:3n-3.

The half-life ($t_{1/2}$) of 18:3n-3 reflects a distinctly different destiny of this fatty acid compared with that of the long-chain n-3 PUFA in the plasma (Table 4). Having a

TABLE 3. Individual kinetic constants ($L_{I,J}$) of in vivo α-linolenic acid metabolism in eight subjects receiving a controlled diet

	Subject Number									
$L_{I,J}$	1	2	3	4	5	6	7	8		
$L_{2,1}$	0.026 ± 0.0003	0.03 ± 0.0008	0.024 ± 0.0005	0.029 ± 0.00056	0.028 ± 0.0012	0.05 ± 0.0003	0.03 ± 0.0002	0.025 ± 0.0005		
$L_{0,2}$	5.22 ± 0.04	5.87 ± 0.12	23.1 ± 0.67	12.9 ± 0.35	10 ± 0.26	7.05 ± 0.32	7.04 ± 0.12	3 ± 0.5		
$L_{3,2}$	0.00047 ± 0.000018	0.00089 ± 0.00003	0.0024 ± 0.00015	0.0017 ± 0.00008	0.0019 ± 0.00001	0.0033 ± 0.00001	0.0012 ± 0.00003	0.0017 ± 0.00007		
$L_{0,3}$	0 ± 0.00026	0.023 ± 0.0015	0 ± 0.0025	0 ± 0.00022	0.0017 ± 0.00012	0.02 ± 0.0012	0.009 ± 0.0017	0.005 ± 0.0016		
$L_{4,3}$	0.018 ± 0.0015	0.018 ± 0.0009	0.016 ± 0.0009	0.014 ± 0.009	0.019 ± 0.0002	0.017 ± 0.0012	0.0065 ± 0.0007	0.017 ± 0.0001		
$L_{0,4}$	0 ± 0.0012	0.03 ± 0.0031	0.005 ± 0.007	0.053 ± 0.006	0.06 ± 0.0004	0.047 ± 0.0043	0 ± 0.012	0.05 ± 0.004		
$L_{5,4}$	0.034 ± 0.009	0.0054 ± 0.001	0.036 ± 0.0004	0.0013 ± 0.00009	0.0088 ± 0.0003	0.0059 ± 0.0004	0.029 ± 0.011	0.0008 ± 0.0003		
$L_{0,5}$	0.064 ± 0.021	0.015 ± 0.0009	0.054 ± 0.007	0.029 ± 0.0023	0.029 ± 0.010	0.026 ± 0.0021	0.28 ± 0.11	0 ± 0.0017		
	Subject Number									
M_J	1	2	3	4	5	6	7	8	Mean	SEM
M_2	45,825	81,824	59,091	43,777	94,524	61,797	44,354	47,407	59,825	±3,401
M_3	74,131	61,682	91,817	72,717	102,570	76,464	50,958	55,897	73,280	±3,128
M_4	82,218	113,295	69,544	72,611	162,906	92,312	74,551	67,218	91,845	±5,795
M_5	265,524	232,884	154,544	155,083	228,270	212,384	184,025	283,024	214,467	±8,056

($L_{I,J}$) values represent model-determined kinetic constants for transfer of fatty acid between and out of compartment J. Steady state mass values for the fatty acid compartments (M_J) (in mg) are presented for compartments 2, 3, 4, and 5.

TABLE 4. Summary of kinetic parameters, half-lives, and mean transit times of n-3 fatty acid metabolism in eight healthy adult subjects, determined by compartmental analysis

Parameter	Value	Variance
Mean fractional transfer rate $L_{(I,J)}$	h^{-1}	$\pm SD$
$L_{(3,2)}$	0.0016	0.0004
$L_{(4,3)}$	0.013	0.0021
$L_{(5,4)}$	0.023	0.009
$L_{(0,01)}$ Σ $L_{(I,J)}$ leaving 1	0.007	0.0003
$L_{(0,02)}$ Σ $L_{(I,J)}$ leaving 2	10.8	3.3
$L_{(0,03)}$ Σ $L_{(I,J)}$ leaving 3	0.007	0.003
$L_{(0,04)}$ Σ $L_{(I,J)}$ leaving 4	0.021	0.010
$L_{(0,05)}$ Σ $L_{(I,J)}$ leaving 5	0.084	0.043
Mean rate of flow, $R_{(I,J)}$	$\mu g\ h^{-1}$	*FSD*
$R_{(3,2)}$	34.5	0.553
$R_{(4,3)}$	361	0.464
$R_{(5,4)}$	701	0.895
$R_{(0,02)}$	243,000	0.783
$R_{(0,03)}$	265	0.607
$R_{(0,04)}$	1,030	0.981
$R_{(0,05)}$	6,320	0.901
$P_{(I,J)}$ % FA transferred from (J) to (I)	%	$\pm SD$
$P_{(3,2)}$	0.002	0.0002
$P_{(4,3)}$	0.64	0.069
$P_{(5,4)}$	0.37	0.061
Half-lives	*h*	$\pm SD$
(18:3n-3) $t_{1/2}$	1.0	0.2
(20:5n-3) $t_{1/2}$	67	14
(22:5n-3) $t_{1/2}$	58	19
(22:6n-3) $t_{1/2}$	20	5.2
Plasma mean transit times	*h*	$\pm SD$
(18:3n-3)	1.2	0.3
(20:5n-3)	98	19
(22:5n-3)	83	23
(22:6n-3)	22	7.5

Values for the kinetic parameters are calculated as the mean values in their respective units and variances are reported as estimated error of the fractional standard deviation (FSD) or standard deviation of the mean (SD).

mean $t_{1/2}$ of about 1 h, 18:3n-3 had the shortest half-life of all the n-3 fatty acids, which contrasted with an average value of 67 h for 20:5n-3. The durations of the 20- and 22-carbon n-3 fatty acids were several-fold greater than that of 18:3n-3. The MTT, the average duration that a molecule of an n-3 fatty acid resides within a compartment, also reflects the different characteristics of 18:3n-3 compared with the long-chain PUFA. Interestingly, 20:5n-3 has the longest MTT (98 h) of the long-chain n-3 fatty acids, reflecting a relatively "stable" fatty acid pool with a slow turnover rate (Table 4).

Comparison of dietary n-3 fatty acid intake values to model predictions

In applying the derived kinetic constants $L_{(I,J)}$ to the constrained steady state fatty acid concentrations, the model calculated a dietary n-3 fatty acid intake that would be required to maintain n-3 PUFA plasma homeostasis for each subject. These predictions are given in **Table 5**. The daily "actual" intake amounts listed in Table 5 are estimated values as well, in that they were calculated from the fatty acid analysis of the diet and the energy intake of each subject. The model prediction for 18:3n-3 intake and the actual amount for this group were in good agreement (there was a 7% difference). It is interesting to note that the model predicted somewhat lower dietary intake values for 18:3n-3, 20:5n-3, and 22:5n-3 than the estimated concentrations. This indicates that the actual intake levels may be an overestimation. It also suggests that for these three fatty acids dietary equilibrium had been attained during this period, in that the n-3 fatty acid intake was sufficient to support n-3 PUFA plasma concentrations. The exception to this finding was 22:6n-3, where the predicted intake of 22:6n-3 was 2.2-fold greater than the estimated value. This strongly suggests that while subjects subsisted on this diet body stores of n-3 fatty acids are being utilized to support the mass of 22:6n-3 in the plasma. Alternatively, it is possible that biosynthesis of 22:6n-3 in the liver is greater than that predicted by the model; however, this conjecture contrasts with the good agreement of the other predictions.

TABLE 5. Model-determined predicted and actual intake of n-3 fatty acids in eight healthy subjects

	Mean	SD(±)
	g/day	
18:3n-3		
Predicted	1.04	0.40
Actual	1.12	0.15
20:5n-3		
Predicted	0.021	0.004
Actual	0.025	0.002
22:5n-3		
Predicted	0.046	0.013
Actual	0.061	0.005
22:6n-3		
Predicted	0.163	0.049
Actual	0.050	0.007

Predicted dietary intake values were calculated from model-derived kinetic constants $L_{(I,J)}$ and the plasma steady state concentrations. Actual fatty acid intake values were estimated from fatty acid analysis of the diet and adjusted for caloric intake of each subject.

DISCUSSION

This study was carried out to describe the in vivo metabolism of n-3 fatty acids, using isotope tracer methodology to determine plasma steady state fatty acid masses in healthy humans subsisting on a controlled beef-based diet. It was theorized that a diet of this type would stimulate the biosynthesis of long-chain n-3 PUFA in these subjects inasmuch as the diet provided only small amounts of long-chain PUFA (the major source of fat was from beef, butter, and olive oil). A goal of this investigation was to utilize model-determined kinetic parameters to quantify the in vivo biosynthesis of long-chain n-3 PUFA from their substrates, beginning with α-linolenic acid.

Data obtained from the analysis of isotopes in the plasma were used to determine in vivo kinetic constants of n-3 fatty acid metabolism, employing multicompartmental analysis. Kinetic constants, determined from the rate equations of the flux of the isotope tracer through the different

plasma compartments (i.e., from 18:3n-3 to 20:5n-3, from 20:5n-3 to 22:5n-3, and from 22:5n-3 to 22:6n-3), were calculated for each subject separately and mean values were obtained for the entire group. Using the steady state fatty acid masses as substrate concentrations available for the biosynthesis, the rate of flow of mass through the n-3 fatty acid pathway was determined. It was observed that a high rate of flow of 18:3n-3 (on average 240 mg h^{-1}; see Table 4) exiting the biosynthetic pathway restricted the rate of long-chain PUFA biosynthesis in this group. A high rate of oxidation of α-linolenic acid in humans has been reported by other investigators, in concurrence with this finding, and may account for the loss of isotope from the system (32). Another avenue of significant loss of 18:3n-3 for metabolism is via its utilization by the skin (33). Also consistent with this observation are several reports indicating that 18:3n-3 does not support neural levels of DHA as well as a preformed source of DHA does (28, 34, 35).

The percentage of isotope that was transferred through the n-3 fatty acid compartments along the pathway was calculated for each intermediate and these values were used to determine the efficiency of the biosynthetic processes. It was concluded that only about 0.2% of plasma 18:3n-3 was destined for biosynthesis of 20:5n-3 in these subjects. In contrast, highly efficient metabolic processes were attributed to the biosynthesis of 22:5n-3 and 22:6n-3 from their immediate substrate precursors. This implied that dietary 20:5n-3 should be well utilized in maintaining the body supply of 22:6n-3 and that 18:3n-3 may be an inadequate substrate even under dietary conditions, which were intended to enhance long-chain PUFA production in healthy subjects. In contrast, it has been reported that adult humans consuming vegetable oils enriched with α-linolenic acid (flax seed oil) over a 4-week period had marked increases in plasma 20:5n-3 as well as increased amounts in the phospholipid fraction of neutrophils but no increases in DHA (36). Moreover, the high percentage of 22:5n-3 destined for synthesis of 22:6n-3 found in the present study contrasts with essential fatty acid metabolism studies in cats (37, 38) and dogs (39, 40).

The "turnover" rates of n-3 fatty acids in the plasma deduced from their half-lives and mean transit times indicated that 18:3n-3 was rapidly removed from the plasma, which was consistent with its diminished biosynthetic prospect. The disappearance rates of other n-3 fatty acids in the blood were on the order of several hours and the longer transition times reflect greater stability in the plasma.

The model was used to predict the amount of dietary n-3 fatty acid intake consistent with the plasma n-3 fatty acid concentrations. These values were compared with the estimated dietary n-3 fatty acid intake values for each subject. The results from the model prediction were similar to the estimated values for 18:3n-3 (−7%), 20:5n-3 (−16%), and 22:5n-3 (−23%). Interestingly, the model predicted a much greater dietary intake of 22:6n-3 (+220%) than that estimated for this group of subjects subsisting on this diet. It appears that a high percentage of n-3 fatty acids from body reservoirs (liver or adipose tissue) contributes to the maintenance of plasma 22:6n-3. This suggests that as these reservoirs of n-3 fatty acid substrates subside this will be reflected in diminished concentrations of plasma 22:6n-3. Alternatively, the continuous demand for 22:6n-3 in the body may stimulate a greater rate of biosynthesis from its precursors.

A physiological compartmental analysis endeavors to account for metabolic homeostasis, using derived kinetic parameters, substrate masses, and nutrient intake. The current work is an attempt to establish an in vivo paradigm of n-3 fatty acid metabolism in human subjects, using plasma kinetic profiles, endogenous fatty acid substrate concentrations, and dietary fatty acid intake data. One limitation of the current model is in the integration of the plasma kinetics with liver metabolism. As further refinements to the model are developed (e.g., isotopic analysis of lipoproteins) this will result in the improved integration of these two components. Until this is realized, the results from the current analysis confirm the highly transitory nature of α-linolenic acid in humans as compared with other n-3 fatty acids and the limited potential of 18:3n-3 toward the biosynthesis of long-chain n-3 PUFA.

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REFERENCES

1. Krauss, R. M., R. H. Eckel, B. Howard, L. J. Appel, S. R. Daniels, R. J. Deckelbaum, J. W. Erdman, Jr., P. Kris-Etherton, I. J. Goldberg, T. A. Kotchen, A. H. Lichtenstein, W. E. Mitch, R. Mullis, K. Robinson, J. Wylie-Rosett, S. St. Jeor, J. Suttie, D. L. Tribble, and T. L. Bazzarre. 2000. AHA Dietary guidelines. Revision 2000: a statement for healthcare professionals from the nutrition committee of the American Heart Association. *Circulation.* **102:** 2296–2311.
2. Dolecek, T. A. 1992. Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. *Proc. Soc. Exp. Biol. Med.* **200:** 177–182.
3. Burchfiel, C. M., D. M. Reed, J. P. Strong, D. S. Sharp, P-H. Chyou, and B. L. Rodriguez. 1996. Predictors of myocardial lesions in men with minimal coronary atherosclerosis at autopsy. The Honolulu Heart Program. *Ann. Epidemiol.* **6:** 137–146.
4. Ascherio, A., E. B. Rimm, E. L. Giovannucci, D. Spiegelman, M. Stampfer, and W. C. Willett. 1996. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. *Br. Med. J.* **313:** 84–90.
5. Nair, S. D., J. W. Leitch, J. Falconer, and M. L. Garg. 1997. Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanisms of action. *J. Nutr.* **127:** 383–393.
6. Pepe, S., and P. L. McLennan. 1996. Dietary fish oil confers direct antiarrhythmic properties on the myocardium of rats. *J. Nutr.* **126:** 34–42.
7. Kang, J. X., and A. Leaf. 1996. The cardiac antiarrhythmic effects of polyunsaturated fatty acid. *Lipids.* **31(Suppl.):** S41–S44.
8. Rose, D. P., and J. M. Connolly. 1999. Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacol. Ther.* **83:** 217–244.

9. Sauer, L. A., R. T. Dauchy, and D. E. Blask. 2000. Mechanism for the antitumor and anticachectic effects of n-3 fatty acids. *Cancer Res.* **60:** 5289–5295.
10. Gibson, R. A., and M. Makrides. 2000. n-3 polyunsaturated fatty acid requirements of term infants. *Am. J. Clin. Nutr.* **71(Suppl.):** 251S–255S.
11. Birch, E. E., D. G. Birch, D. R. Hoffman, L. Hale, M. Everett, and R. Uauy. 1993. Breast-feeding and optimal visual development. *J. Pediatr. Ophthalmol. Strabismus.* **30:** 33–38.
12. Birch, E. E., D. R. Hoffman, R. Uauy, D. G. Birch, and C. Prestidge. 1998. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatr. Res.* **44:** 201–209.
13. Carlson, S. E., A. J. Ford, S. H. Werkman, J. M. Peeples, and W. W. Koo. 1996. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Pediatr. Res.* **39:** 882–888.
14. Hibbeln, J. R. 1998. Fish consumption and major depression. *Lancet.* **351:** 1213.
15. Bang, H. O., J. Dyerberg, and N. Hjorne. 1976. The composition of food consumed by Greenland Eskimos. *Acta Med. Scand.* **200:** 69–73.
16. Holland, B., J. Brown, and D. H. Buss. 1993. Fish and fish products. 3rd supplement to McCance and Widdowson's the Composition of Foods. 5th edition. Royal Society of Chemistry, Cambridge.
17. Lands, W. E., T. Hamazaki, K. Yamazaki, H. Okuyama, K. Sakai, Y. Goto, and V. S. Hubbard. 1990. Changing dietary patterns. *Am. J. Clin. Nutr.* **51:** 991–993.
18. Lands, W. E., B. Libelt, A. Morris, N. C. Kramer, T. E. Prewitt, P. Bowen, D. Schmeisser, M. H. Davidson, and J. H. Burns. 1992. Maintenance of lower proportions of (n-6) eicosanoid precursors in phospholipids of human plasma in response to added dietary (n-3) fatty acids. *Biochim. Biophys. Acta.* **1180:** 147–162.
19. Emken, E. A., R. O. Adlof, S. M. Duval, and G. J. Nelson. 1999. Effect of dietary docosahexaenoic acid on desaturation and uptake in vivo of isotope-labeled oleic, linoleic, and linolenic acids by male subjects. *Lipids.* **34:** 785–791.
20. Salem, N., Jr., B. Wegher, P. Mena, and R. Uauy. 1996. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc. Natl. Acad. Sci. USA.* **93:** 49–54.
21. Salem, N., Jr., R. J. Pawlosky, B. Wegher, and J. R. Hibbeln. 1999. In vivo conversion of linoleic acid to arachidonic acid in human adults. *Prostaglandins Leukot. Essent. Fatty Acids.* **60:** 407–410.
22. Uauy, R., P. Mena, B. Wegher, S. Nieto, and N. Salem, Jr. 2000. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. *Pediatr. Res.* **47:** 127–135.
23. Carnielli, V. P., D. J. L. Wattimena, I. Luijendijk, A. Boerlage, H. J. Degenhart, and P. P. J. Sauer. 1996. The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. *Pediatr. Res.* **40:** 169–174.
24. Sauerwald, T. U., D. L. Hachey, C. L. Jensen, H. Chen, R. E. Anderson, and W. C. Heird. 1997. Intermediates in endogenous synthesis of C22:6ω3 and C20:4ω6 by term and pre-term infants. *Pediatr. Res.* **41:** 183–187.
25. Sauerwald, T. U., D. L. Hachey, C. L. Jensen, H. Chen, R. E. Anderson, and W. C. Heird. 1996. Effect of dietary α-linolenic acid intake on incorporation of docosahexaenoic and arachidonic acids into plasma phospholipids of term infants. *Lipids.* **31S:** S131–S135.
26. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
27. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **5:** 600–608.
28. Woods, J., G. Ward, and N. Salem, Jr. 1996. Is docosahexaenoic acid necessary in infant formula? Evaluation of high linolenate diets in the neonatal rat. *Pediatr. Res.* **40:** 687–694.
29. Pawlosky, R. J., H. W. Sprecher, and N. Salem, Jr. 1992. High sensitivity negative ion GC-MS method for detection of desaturated and chain elongated products of deuterated linoleic and linolenic acids. *J. Lipid Res.* **33:** 1711–1717.
30. Sprecher, H. 1972. Regulation of polyunsaturated fatty acid biosynthesis in the rat. *Fed. Proc.* **31:** 1451–1457.
31. Voss, A., M. Reinhart, S. Sankarappa, and H. Sprecher. 1991. The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *J. Biol. Chem.* **266:** 19995–20000.
32. Vermunt, S. H., R. P. Mensink, M. M. Simonis, and G. Hornstra. 2000. Effects of dietary alpha-linolenic acid on the conversion and oxidation of ^{13}C-alpha-linolenic acid. *Lipids.* **35:** 137–142.
33. Fu, Z., and A. Sinclair. 2000. Novel pathway of metabolism of α-linolenic acid in the guinea pig. *Pediatr. Res.* **47:** 414–417.
34. Abedin, L., E. L. Lien, A. J. Vingrys, and A. J. Sinclair. 1999. The effects of dietary alpha-linolenic acid compared with docosahexaenoic acid on brain, retina, liver, and heart in the guinea pig. *Lipids.* **34:** 475–482.
35. Bowen, R. A. R., and M. T. Clandinin. 2000. High dietary 18:3n-3 increases the 18:3n-3 but not the 22:6n-3 content in the whole body, brain, skin, epididymal fat pads, and muscles of suckling rat pups. *Lipids.* **35:** 389–394.
36. Mantzioris, E., M. J. James, R. A. Gibson, and L. G. Cleland. 1994. Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *Am. J. Clin. Nutr.* **59:** 1304–1309.
37. Pawlosky, R., A. Barnes, and N. Salem, Jr. 1994. Essential fatty acid metabolism in the feline: relationship between liver and brain production of long-chain polyunsaturated fatty acids. *J. Lipid Res.* **35:** 2032–2040.
38. Waldron, M. K., A. L. Spencer, and J. E. Bauer. 1998. Role of long-chain polyunsaturated n-3 fatty acids in the development of the nervous system of dogs and cats. *J. Am. Vet. Med. Assoc.* **213:** 619–22.
39. Bibus, D. M., and P. A. Stitt. 1998. The return of ω3 fatty acids into the food supply. I. Land-based animal food products and their health effects. *World Rev. Nutr. Diet.* **83:** 186–198.
40. Anderson, R. E., M. B. Maude, G. Acland, and G. D. Aguirre. 1994. Plasma lipid changes in PRCD-affected and normal miniature poodles given oral supplements of linseed oil. Indications for the involvement of n-3 fatty acids inherited retinal degenerations. *Exp. Eye Res.* **58:** 129–137.