Compartmental analyses of $^{2}H_5$-α-linolenic acid and ^{13}C-U-eicosapentaenoic acid toward synthesis of plasma labeled 22:6n−3 in newborn term infants[1–3]

Yu Hong Lin, Adolfo Llanos, Patricia Mena, Ricardo Uauy, Norman Salem Jr, and Robert J Pawlosky

ABSTRACT

Background: During early postnatal development, the nervous system accretes docosahexaenoic acid (DHA; 22:6n−3), a highly unsaturated n−3 (omega-3) fatty acid (FA) used in the formation of neural cell membranes. DHA, which is present in human breast milk, may also be biosynthesized from n−3 FAs such as 18:3n−3 [α-linolenic acid (ALA)] or 20:5n−3 [eicosapentaenoic acid (EPA)]. An important concern is to what extent these precursors can supply DHA to the developing infant.

Objective: We analyzed measurements of fractional percentages of plasma $^{2}H_5$-ALA and ^{13}C-U-EPA directed toward the synthesis of labeled 22:6n−3 in 11 newborn infants by using compartmental modeling procedures.

Design: One-week-old infants received doses of $^{2}H_5$-ALA and ^{13}C-U-EPA ethyl esters enterally. We drew blood from the infants periodically and analyzed the plasma for endogenous and labeled n−3 FAs. From the time-course concentrations of the labeled FAs, we determined rate constant coefficients, fractional synthetic rates, and plasma turnover rates of n−3 FAs.

Results: In infants, ≈0.04% of the $^{2}H_5$-ALA dose converted to plasma $^{2}H_5$-EPA. Plasma $^{2}H_5$-EPA and $^{2}H_5$-22:5n−3 [docosapentaenoic acid (DPA)] efficiently converted to $^{2}H_5$-DPA and $^{2}H_5$-DHA, respectively. The percentage of plasma ^{13}C-U-EPA directed toward the synthesis of ^{13}C-DHA was lower than the percentage of plasma $^{2}H_5$-EPA that originated from $^{2}H_5$-ALA.

Conclusions: Endogenously synthesized EPA was efficiently converted to DHA. In comparison, preformed EPA was less efficiently used for DHA biosynthesis, which suggests a differential metabolism of endogenous EPA compared with exogenous EPA. However, on a per mole basis, preformed EPA was 3.6 times more effective toward DHA synthesis than was ALA. Newborns required an intake of ≈5 mg preformed DHA · kg^{-1} · d^{-1} to maintain plasma DHA homeostasis. *Am J Clin Nutr* 2010;92:284–93.

INTRODUCTION

In recent years, some infant formulas marketed in North America and elsewhere have been fortified with arachidonic acid (20:4n−6) and docosahexaenoic acid (DHA; 22:6n−3), which are long-chain n−6 (omega-6) and n−3 (omega-3) polyunsaturated fatty acids (PUFAs) that are present in human breast milk. Evidence from human studies (1, 2) indicated that 20:4n−6 (3–5) and 22:6n−3 (6) play important roles in the development of the human nervous system, which is supported by experimental evidence from animal studies (7–9). DHA may be biosynthesized from n−3 fatty acids (FAs) such as 18:3n−3 [α-linolenic acid (ALA)] or 20:5n−3 [eicosapentaenoic acid (EPA)]. It is unclear to what degree infants have the capability to biosynthesize long-chain n−3 FAs from ALA during the early postnatal period. Consequently, no precise amounts or appropriate ratios of n−6 and n−3 FAs have been established for infant formulas on the basis of nutritional needs or the biosynthetic capabilities of the newborn. In addition, human adults have a relatively low capacity to convert ALA to DHA on the basis of evidence from analyses of the plasma time-course concentrations of isotopically labeled n−3 FAs (10–12). However, a recent study in adult men suggested that the liver has a greater biosynthetic capacity for production of long-chain n−3 PUFAs than that inferred from the plasma FA time-course data (13).

We and other authors carried out human studies that used stable isotopes to determine the conversion of ALA to DHA in infants (14–17). However, very little quantitative information is available on the effects of the nutritional supply of ALA and EPA toward synthesis and maintenance of plasma DHA in infants (17).

The purpose of this study was to assess plasma n−3 FA kinetic variables and the quantitative contributions of dietary ALA and EPA toward the maintenance of plasma DHA during the first week of life in term and late preterm neonates. The research strategy involved the development of 2 independent compartmental models to assess the biosynthetic capacity of ALA and EPA on the production and appearance of DHA in the plasma. Each of the models integrated the n−3 FA intake from infant

[1] From the Laboratory of Membrane Biochemistry and Biophysics (YHL and NS) and the Laboratory of Metabolic Control (RJP), National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD; the Institute of Nutrition and Food Technology, Santiago, Chile (AL, PM, and RU); the Neonatology Unit, Hospital Sótero del Rio, Santiago, Chile (AL and PM); and the London School of Hygiene and Tropical Medicine, London, United Kingdom (RU).

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[3] Address correspondence to RJ Pawlosky, Room 1S-22, 5625 Fishers Lane, Bethesda, MD 20892-9410. E-mail: bpawl@mail.nih.gov.

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TABLE 1
Subject weight, age, and feeding variables[1]

ID	Birth weight	GA	Sex	Age at entrance	Weight at entrance	Weight at end	Enteral feeding	Formula	Breast milk
	g	*wk*		*wk*	*g*	*g*	*d*	*mL/d*	*mL/d*
81	3310	39	M	2	3172	3245	3	118	45
82	3250	38	F	1	3400	3550	4	136	143
83	3890	42	M	1	3930	3920	2	38	390
84	3070	39	M	3	3080	3250	3	110	405
86	2350	36	M	2	2390	2170	4	0	70
87	3070	37	M	3	3060	2940	3	165	136
88	3310	39	M	4	3510	3510	4	180	0
89	3160	37	M	2	3410	3480	3	234	10
90	2540	35	M	1	2550	2270	2	0	60
91	4650	41	F	2	4580	4680	3	169	44
92	2490	37	M	2	2440	2350	3	0	130

[1] ID, subject identification number; GA, gestational age.

formula and/or breast-milk feeding with the n−3 FA plasma concentrations and the time-course concentrations of the ^{13}C-labeled n−3 FA (derived from ^{13}C-U-EPA) and the $^{2}H_5$-labeled n−3 FA (derived from $^{2}H_5$-ALA) for each subject.

SUBJECTS AND METHODS

Study subjects and clinical procedures

Neonates with gestational ages >34 wk were admitted to the neonatal intensive care unit (NICU) and had an umbilical line in place on the basis of clinical indication. Infants who were small for gestational age, with major malformations, or in evaluation for necrotizing enterocolitis or feeding intolerance were excluded from the study. Written informed consent was obtained from mothers, and the study protocol was approved by the National Institutes of Health Institutional Review Board (under protocol OH93-AA-N027), the Institute of Nutrition and Food Technology's ethics committee, and the research committee of the participating NICU (Hospital Sótero del Rio and Clínica Presbiteriana Madre-Hijo) in metropolitan Santiago, Chile. The gestational age of each infant was assessed from the mother's last menstrual period or by using the date of conception on the basis of early ultrasound and confirmed by the modified Ballard neonatal physical evaluation. Infants were classified as small for gestational age or appropriate for gestational age according to the Lubchenco standard (18). Birth weights of infants were between 2.35 and 4.65 kg (mean ± SE: 3.19 ± 0.66 kg), and gestational ages of infants were 34–41 wk (**Table 1**). Most subjects presented with mild forms of hypoxia or transient respiratory problems. Feeding was started generally ≤2 d after birth, and the type of feeding varied. If breast milk was unavailable, infants received a commercial brand infant formula

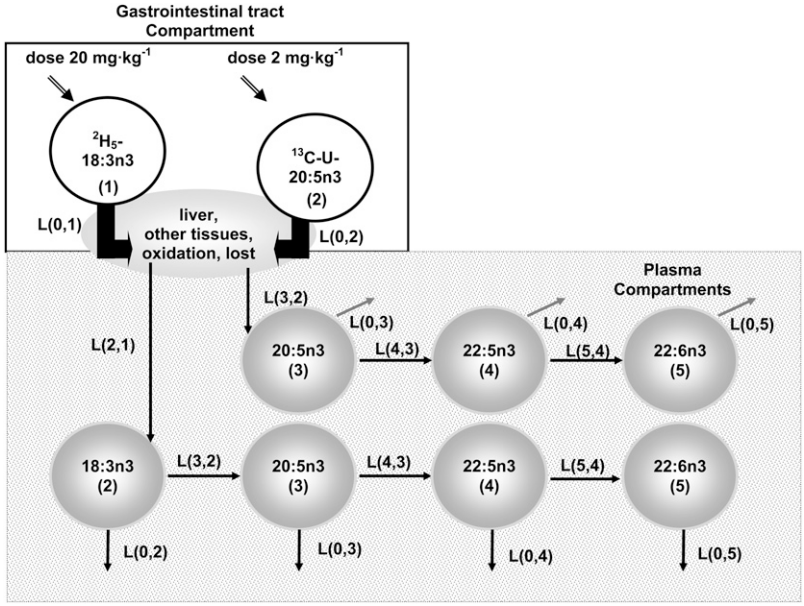

FIGURE 1. Diagram of the 18:3n−3 compartmental model and 20:5n−3 compartmental models. Open circles represent plasma and gastrointestinal compartments for n−3 fatty acids. $L_{I,J}$ values denote rate constant coefficients. $L_{0,J}$ values indicate the loss of isotopes from the pathway. Numbers in parentheses represent individual compartments.

TABLE 2
Plasma n−3 fatty acid values (in μg) for compartments (M_J) 2, 3, 4, and 5 in newborn infants[1]

	Subject identification number											
n−3 Fatty acid	81	82	83	84	86	87	88	89	90	91	92	Mean ± SE
M_2/18:3	1137	466	3473	1926	186	1566	370	988	196	1041	298	1059 ± 299
M_3/20:5	2026	2996	1446	1044	1152	1028	667	1081	1073	1760	768	1367 ± 204
M_4/22:5	1589	1901	1047	644	959	657	717	633	549	1672	681	1004 ± 148
M_5/22:6	19,219	22,192	14,335	8680	13,904	8749	7787	8993	9275	30,907	8811	13,896 ± 2245

[1] Mean values were measured from plasma concentrations of fatty acids over 6 d and multiplied by the total plasma volume from each subject.

(Ross Laboratories, Columbus, OH) that contained 18:3n−3 (77 mg 18:3n−3/100 mL) but was devoid of 20- and 22-carbon PUFAs. Subjects received a mixture of $^2H_5$-18:3n−3 (20 mg $^2H_5$-18:3n−3/kg) and deuterated ^{13}C-U-20:5n−3 (2 mg ^{3}C-U-20:5n−3/kg) with an enteral gastric tube. Blood was drawn (0.5 mL) from an umbilical catheter and a peripheral vein after the catheter had been removed into a tube containing EDTA. Blood was drawn at 0, 4, 8, 24, and 48 h and on days 4 and 7 after dosing; if possible, the time of drawing was made to coincide with the timing of sampling for clinical needs. Plasma was separated by centrifugation shortly after sampling and frozen at –80°C.

Infant feeding

Infants were nursed and/or fed expressed breast milk when available and/or an infant formula (Ross Laboratories) on demand. The quantity of expressed milk and the amount of formula consumed were recorded for each subject. Subjects that could not be initially fed enterally received parenteral glucose until they were capable of receiving enteral nutrition (100 mL $\cdot$ kg^{-1} $\cdot$ d^{-1}). Infants received no intravenous lipids during the study period.

Stable isotopes

Carbon-13 uniformly labeled eicosapentaenoate ethyl ester (^{13}C-U-20:5n−3; ^{13}C >95%) was obtained from Martek Biosciences Corp (Columbia, MD), and deuterium-labeled α-linolenate (17, 17, 18, 18, 18-$^2H_5$-18:3n−3; ^{2}H >95%) ethyl esters were obtained from Cambridge Isotope Laboratories (Andover, MA).

Lipid extraction and FA methyl esters

Plasma lipids were extracted by using a modified Folch procedure (19). Plasma (200 μl) was added to methanol (1 mL) that contained ethyl tricosanoate (0.13 nmol) as an internal standard and vigorously extracted twice with chloroform. One-half of the lipid extract was derivatized to methyl esters by using 14% boron trifluoride in methanol (20) and dissolved in hexane.

Gas chromatographic analyses

Gas chromatographic analyses were made with an Agilent 6890 system (Agilent Technologies, Santa Clara, CA) with flame ionization detection. Two microliters of the sample was injected on to a Durabond Free Fatty Acid Phase capillary column (30-m × 0.25-mm inside diameter and 0.25-μm film thickness; J&W Scientific, Folsom, CA) with hydrogen carrier gas. The inlet and detector temperatures were set at 250°C. The oven was programmed from 130°C to 175°C at 4°C/min and to 210°C at 1°C/min and then increased to 245°C at 30°C/min.

Pentafluorobenzyl derivatization

The lipid extract (100 μL) was evaporated and saponified with 5% methanolic KOH. Free FAs were extracted into hexane and derivatized to pentafluorobenzyl esters as described previously (21). Reagents were evaporated under a steam of nitrogen and resuspended in 100 μL hexane.

Gas chromatography–mass spectrometry analyses

Gas chromatography–mass spectrometry was carried out on an Agilent 6890 GC-5973 Mass Selective Detector system (Agilent Technologies) in the negative chemical ionization mode as previously described (21). Samples (1 μL) were injected in the splitless mode onto a Durabond Free Fatty Acid Phase column (J&W Scientific), and the oven was programmed from 125°C to 245°C at 8°C/min. Data were acquired by monitoring the M-pentafluorobenzyl anion of each analyte and converted to the absolute quantity with reference to the internal standard by using an appropriate response factor. Analyses for n−3 and n−6 FAs (23) were carried out at the same time.

Compartmental models

The n−3 FA compartmental model has been described previously and is only briefly detailed here (11). The hepatocyte is a main site for biosynthesis of desaturated and chain elongated PUFAs from 18:3n−3 and for the formation of lipoproteins. Because liver specimens were not available, rate constants represent kinetics of labeled FAs from their plasma pool and may only indirectly reflect liver metabolism. Two independent compartmental models of n−3 FA metabolism were developed by using the concentration-time courses of the labeled FAs and concentrations of endogenous FAs in plasma (**Figure 1**) with WinSAAM modeling software (version 3.0.7; http://www.winsaam.com).

The fractional transfer rate constant coefficient $L_{I,J}$ is the fraction of substrate transferred from substrate-compartment J to product-compartment I (and $L_{0,J}$ represents the isotopes lost from the path). The units are in hours. $L_{I,J}$ represents an assemblage of several independent enzymatic and transport processes, each having a separate rate constant, for which no intermediates were isolated. The rate of flow ($R_{I,J}$) from

TABLE 3
Concentrations of plasma fatty acids and variances in 11 infants over 168 h

	Subject identification number										
n−3 Fatty acid	81	82	83	84	86	87	88	89	90	91	92
18:3 (nmol/mL)	10.6 ± 0.76[1]	3.9 ± 0.23	14.8 ± 1.16	10.1 ± 0.62	2.1 ± 0.20	8.3 ± 0.71	3.0 ± 0.28	6.0 ± 0.54	3.4 ± 0.33	5.0 ± 0.20	2.7 ± 0.19
CV[2]	0.36	0.30	0.40	0.31	0.19	0.46	0.49	0.45	0.49	0.52	0.38
20:5 (nmol/mL)	12.4 ± 0.54	11.7 ± 0.73	6.5 ± 0.28	6.7 ± 0.14	7.1 ± 0.26	6.0 ± 0.17	4.6 ± 0.07	7.8 ± 0.21	8.8 ± 0.40	6.4 ± 0.26	6.1 ± 0.19
CV[2]	0.22	0.34	0.22	0.11	0.18	0.14	0.08	0.14	0.23	0.20	0.16
22:5 (nmol/mL)	11.7 ± 0.22	10.7 ± 0.26	7.2 ± 0.21	4.3 ± 0.05	5.9 ± 0.27	4.3 ± 0.21	5.1 ± 0.13	4.0 ± 0.07	4.8 ± 0.31	10.0 ± 0.22	5.6 ± 0.15
CV[2]	0.10	0.12	0.14	0.05	0.23	0.25	0.13	0.08	0.32	0.11	0.14
22:6 (nmol/mL)	148 ± 1.56	116 ± 3.94	91 ± 1.59	64 ± 0.78	93 ± 4.57	77 ± 1.19	61 ± 1.46	77 ± 2.60	73 ± 2.34	134 ± 2.37	83 ± 1.56
CV[2]	0.05	0.17	0.09	0.06	0.25	0.08	0.12	0.17	0.16	0.9	0.10

[1] Mean ± SE (all such values).
[2] CVs of fatty acid concentrations across the trial period.

substrate-compartment J to product-compartment I is obtained by multiplying the mass (M_J) of endogenous FAs in compartment J by $L_{I,J}$ and is given in micrograms per hour. The FA concentrations from the total lipid extract represent the endogenous amounts. The percentage of isotopes transferred from J to I is given as $P_{I,J}$ and is a percentage of the total flux of FAs leaving J. $P_{I,J}$ is the fraction of the isotopes remaining in the metabolic pathway as opposed to the isotopes taken up by tissues or in other ways irreversibly lost from the compartment. Variances for the measured variables are reported as either the SE or SD.

Model illustration and rate equations

Diagrams of the compartmental models for 18:3n−3 and 20:5n−3 are presented in Figure 1. The initial compartment represents the dose of the labeled FAs absorbed from the gastrointestinal tract into the system. Other compartments denote plasma pools of 18:3n−3, 20:5n−3, 22:5n−3, and 22:6n−3. Arrows connecting the 6 compartments indicate the transfer of isotopes along the path. The rate equations were defined by a set of differential equations that corresponded to the flux of the labeled FAs through each respective compartment and those that exit the system.

Approximating endogenous n−3 FA masses, predicting n−3 FA intake and the influence of n−6 FA intake on n−3 FA rate variables, and statistical comparisons

Mean concentrations of plasma n−3 FAs (over 168 h) for each subject were used to represent the mass of endogenous substrates (M_J) available for biosynthesis (**Table 2**). For purposes of this study, both deuterium- and ^{13}C-labeled FAs, which elute within the gas chromatographic peak envelope, were considered part of the endogenous pool because it was assumed that these FAs are biochemically equivalent to the unlabeled n−3 essential fatty acids (EFAs). These values were held constant. When the daily upper and lower n−3 FA intake limits were estimated, the FA content of the formula, availability of breast milk, and frequency of feeding for each subject were entered into the model. Intakes of n−3 FAs (U_i) by breastfed infants and/or those who received expressed breast milk were approximated from breast-milk samples obtained from mothers who lived in metropolitan Santiago, Chile (22). Initial n−3 FA intake approximations were further refined through successive iterations while constraining plasma n−3 EFA concentrations. In addition, the model was adjusted to compensate for low intake volumes during the first 48 h after birth with gradual increases in volume through the week. Because most subjects had low intakes during the first 24–48 h period, initial U_i estimates tended to be overestimated. On the basis of each subject's unique feeding regimen, the estimates were reduced by a similar factor. For instance, an ≈30% reduction of the initial U_i estimate would be applied to this value if an infant only began nursing on day 2 (≈48/168 h = ≈29%) to compensate for a 48-h period. There was no direct control incorporated in the modeling procedure to account for n−6 FA intake, and variable n−6 FA intake may have had an effect on n−3 FA rate variables. Differences between rate variables and the efficacy of each of the 2 precursors (18:3n−3 and 20:5n−3) toward synthesis of 22:6n−3 were compared by using a paired

Student's t test analysis with each subject serving as its own control. $P \leq 0.05$ was considered significant.

Calculations, errors, and predicting dietary n−3 FA intake

Initial $L_{I,J}$ and $P_{I,J}$ estimates, which were derived from the concentration-time curves, were adjusted to compensate for individual variances in plasma data until the model prediction gave the best fit to the experimental data. Final values were measured by using an iterative nonlinear least-squares routine. The error model included assumptions of independence, constant variance, and normal distribution of about zero. Consistent with the precision of analytic methods, data points were weighted by assigning a fractional SD of 0.1 to each measurement in the WinSAAM software (version 3.0.7; http://www.winsaam.com). The use of a fractional SD weighting factor was considered appropriate for this study because it assigns the highest weight to the lowest observation values and the lowest weight to the highest observation values.

RESULTS

Subject characteristics

Eleven infants (9 boys and 2 girls) completed the study protocol. Their sex, weights, gestational ages, and feeding regimens at birth and during the study are given in Table 1. Infants had mild forms of respiratory distress syndrome secondary either to meconium aspiration or perinatal asphyxia. Subjects were monitored in the NICU until respiratory function was normalized. Infants received supplemental feeding with breast milk and/or infant formula in increasing volumes during the study. Mean (±SE) plasma concentrations (in μg/mL) of the n−3 FA 18:3n−3, 20:5n−3, 22:5n−3, and 22:6n−3 for infants in this group were 6.55 ± 1.03, 9.76 ± 0.71, 7.17 ± 0.57, and 99.26 ±

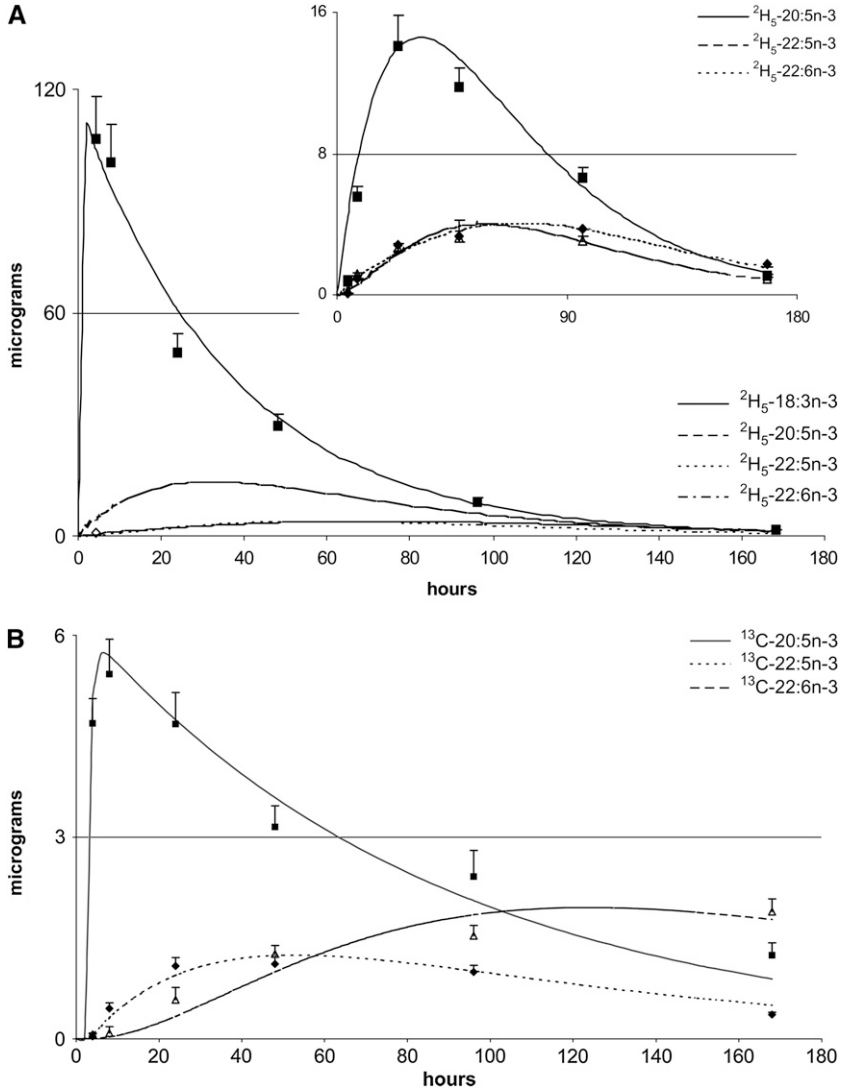

FIGURE 2. A: Mean (±SD) values for the experimentally measured plasma fatty acids $^2H_5$-18:3n−3, $^2H_5$-20:5n−3, $^2H_5$-22:5n−3, and $^2H_5$-22:6n−3 from 11 newborn infants who received an oral dose of $^2H_5$-18:3n−3 (20 mg $^2H_5$-18:3n−3/kg) ethyl ester. Time-course curves are model-determined best fits to the data by the modeling procedure developed from WinSAAM software (version 3.0.7; http://www.winsaam.com). B: Mean (±SD) values for the experimentally determined plasma fatty acids ^{13}C-20:5n−3, ^{13}C-22:5n−3, and ^{13}C-22:6n−3 from 11 newborn infants who received an oral dose of ^{13}C-20:5n−3 (2 mg ^{13}C-20:5n−3/kg) ethyl ester. Time-course curves are model-determined best fits to the data.

8.00, respectively. Generally, plasma FA masses for 20:5n−3, 22:5n−3, and 22:6n−3 varied over a narrow range (<20% from the mean value) through the study period (**Table 3**). However, plasma 18:3n−3 was more variable, and its concentration in the plasma increased with greater volume intake through the period.

Appearance of $^{2}H_5$-18:3n−3 and ^{13}C-U-20:5n−3 in plasma

From previous analyses of isotopes recovered from feces, it was estimated that infants absorbed ≈94% of the labeled EFAs (23). This value, similar to findings in other infants (24), was used to estimate the absorption of labeled-FA and dietary-EFA components. Mean (±SE) values for area under the concentration curve for $^{2}H_5$-18:3n−3 and ^{13}C-U-20:5n−3 in plasma were 26.2 ± 2.2 nmol $^{2}H_5$-18:3n−3 · h/mL and 6.5 ± 0.7 nmol ^{13}C-U-20:5n−3 · h/mL. When calculated as the percentage of dose of the 2 labeled FAs by using isotope values from the gastrointestinal and plasma compartments (Figure 1) only 0.13 ± 0.02% and 0.28 ± 0.01%, of the dosages of $^{2}H_5$-18:3n−3 and ^{13}C-U-20:5n−3, respectively, appeared in the plasma compartment.

Concentration-time curves for n−3 FAs and average weighted residuals

The mean best-fit model-derived concentration-time curves to the experimentally measured plasma concentrations for $^{2}H_5$-18:3n−3, $^{2}H_5$-20:5n−3, $^{2}H_5$-22:5n−3, and $^{2}H_5$-22:6n−3 from the 11 subjects is illustrated in **Figure 2**A. The best-fit curves for ^{13}C-20:5n−3, ^{13}C-22:5n−3, and ^{13}C-22:6n−3 are illustrated in Figure 2B. Changes in the mean values of the concentrations of each of the labeled FA in the plasma and the approximate concentrations at their peak appearance times throughout 168 h for all subjects are depicted in the figures. Model-derived kinetic variables were determined for each subject uniquely by using individual dosing regimens, plasma volumes, and the unique time-course concentrations for ^{13}C- and ^{2}H-labeled FAs. Mean values of the weighted residuals determined from differences between the experimentally measured deuterium-labeled (**Figure 3**A) and ^{13}C-labeled (Figure 3B) plasma n−3 EFAs and the best-fit values across the time course are presented in Figure 3. These values give an indication of the variability between the experimentally measured isotope data and the best-fit model-derived values.

Rate constant coefficients and *R* values

Fractional rate constant coefficient estimates ($L_{I,J}$) for in vivo metabolism of $^{2}H_5$-18:3n−3 and ^{13}C-20:5n−3 were optimized for each subject, and final values are given in **Tables 4** and **5**, respectively. The appearance and disappearance rates of an n−3 FA in the plasma [$R_{I,J}$ (in μg/h)] (**Table 6**) are presented as the mass per unit of time for a given FA as it exits a substrate compartment *J* and is either transferred to product compartment *I* or exits the biosynthetic pathway (0). From these calculations, the predicted daily mean whole-body turnover of 18:3n−3 in subjects ($R_{0,1}$) was 187 ± 56 mg 18:3n−3 · kg^{-1} · d^{-1}, whereas the mean turnover of 18:3n−3 in plasma was 1.37 ± 0.33 mg 18:3n−3/d for the group ($R_{0,2}$). The mean (±SE) turnovers for other n−3 FAs in the plasma were 0.67 ± 0.03 mg 20:5n−3/d, 1.49 ± 0.21 22:5n−3 mg/d, and 16.6 ± 1.6 mg 22:6n−3/d. The mean (±SE) replacement of plasma 22:6n−3 via its

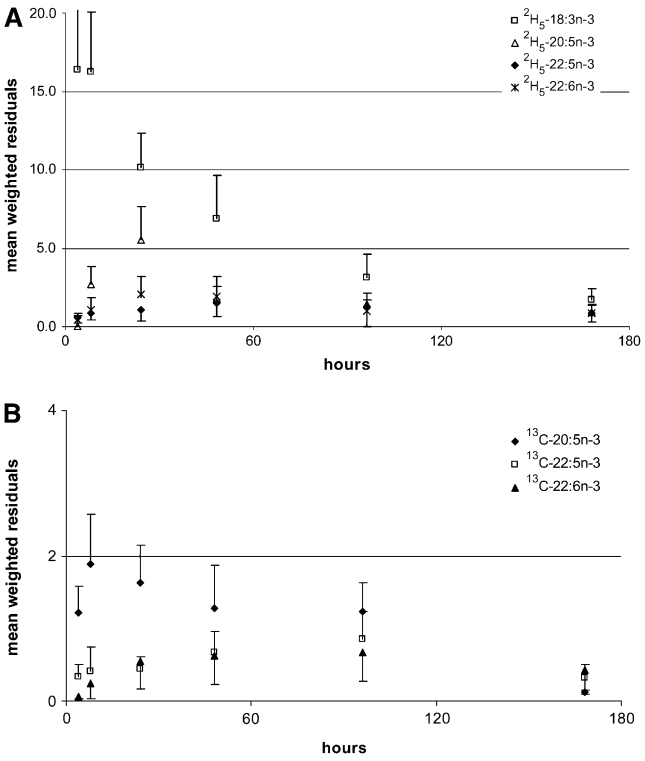

FIGURE 3. Mean (±SD) values for 11 subjects of weighted residuals calculated from the difference between the experimentally determined deuterium-labeled (A) and ^{13}C-labeled (B) n−3 essential fatty acids in plasma and compartmental model assigned values at various time points. Infants received oral doses of $^{2}H_5$-18:3n−3 (20 mg $^{2}H_5$-18:3n−3/kg) and ^{13}C-20:5n−3 (2 mg ^{13}C-20:5n−3/kg) ethyl esters, and plasma was sampled at 4, 8, 24, 48, 96, and 168 h. Along the ordinate, differences between experimental and calculated values are given for $^{2}H_5$-18:3n−3, $^{2}H_5$-20:5n−3, $^{2}H_5$-22:5n−3, and $^{2}H_5$-22:6n−3 (A) and for ^{13}C-20:5n−3, ^{13}C-22:5n−3, and ^{13}C-22:6n−3 (B).

synthesis from 22:5n−3 ($R_{5,4}$) was 1.5 ± 0.2 mg 22:6n−3/d (Table 6).

Percentages of the labeled plasma n−3 FA ($P_{I,J}$) directed toward biosynthesis were determined from the rate constant coefficients in the transfer of the label into and out of each FA compartment. These values for the ^{2}H- and ^{13}C-labeled FAs are given in Table 5 and **Table 7**, respectively. In most subjects, only ≈0.1% of $^{2}H_5$-18:3n−3 appeared in the plasma; however, a substantial percentage of plasma $^{2}H_5$-18:3n−3 (mean: 31 ± 4%; range: 3.7–85%) was directed toward the synthesis of $^{2}H_5$-20:5n−3. Consequently, the net mean daily biosynthetic output of 20:5n−3 in plasma was ≈200 μg. This amount, which was equivalent to ≈30% of the total plasma EPA (Table 4), was equal to approximately one-third of the daily plasma EPA turnover (Table 6). The mean percentages of plasma $^{2}H_5$-20:5n−3 and of $^{2}H_5$-22:5n−3 directed toward the synthesis of $^{2}H_5$-22:5n−3 and $^{2}H_5$-22:6n−3, respectively, were both >95% of the isotope use (Table 7). These highly efficient biosynthetic processes were consistent with the 10-fold difference in the amount of plasma DHA compared with that of EPA and docosapentaenoic acid (Table 2).

A comparatively smaller percentage of ^{13}C-U-20:5n−3 was used for synthesis of ^{13}C-22:5n−3 (mean: 59.6%) (Table 5) than $^{2}H_5$-20:5n−3, which suggests that preformed EPA may be somewhat less efficient for DHA biosynthesis than that derived

TABLE 4
Fractional transfer rate constant coefficients[1]

	Subject identification number											
	81	82	83	84	86	87	88	89	90	91	92	Mean ± SE
$L_{2,1}$	0.195	1.899	0.618	0.618	0.115	0.872	1.295	2.913	0.340	0.791	0.857	0.956 ± 0.263
Precision of estimates	0.012	0.023	0.052	0.363	0.133	0.151	0.077	0.036	0.295	0.128	0.109	—
$L_{3,2}$	8.0	4.7	4.4	4.2	25.5	14.3	4.5	9.4	6.6	11.4	2.6	8.7 ± 2.0
Precision of estimates	0.011	0.052	0.097	0.546	0.198	0.572	0.094	0.062	0.215	0.027	0.218	—
$L_{4,3}$	38.7	22.7	32.9	32.0	17.6	37.5	18.2	18.2	22.5	22.5	34.9	27.1 ± 2.5
Precision of estimates	0.012	0.153	0.197	0.101	0.124	0.113	0.124	0.124	0.114	0.124	0.145	—
$L_{5,4}$	120.0	41.4	56.6	107.8	62.4	108.3	20.4	53.5	71.1	50.2	36.4	66.2 ± 9.9
Precision of estimates	0.033	0.287	0.312	0.220	0.241	0.123	0.458	0.254	0.174	0.214	0.224	—
$L_{0,1}$	0.618	0.195	1.899	0.618	1.295	0.872	2.913	0.791	0.857	0.115	0.340	0.956 ± 0.255
Precision of estimates	0.015	0.129	0.1599	0.045	0.128	0.036	0.208	0.109	0.162	0.117	0.139	—
$L_{0,2}$	36.8	45.9	12.0	109.1	4.5	6.6	28.6	38.0	8.8	12.7	35.7	30.8 ± 9.5
Precision of estimates	0.048	0.031	0.048	0.478	0.064	0.155	0.038	0.027	0.082	0.052	0.028	—
$L_{0,3}$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.40	0.00	0.22 ± 0.23
Precision of estimates	0.023	0.092	0.115	0.038	0.049	0.085	0.056	0.038	0.045	0.046	0.027	—
$L_{0,4}$	0.98	0.11	0.35	0.61	4.97	0.98	4.86	4.56	0.33	0.63	6.47	2.26 ± 0.62
Precision of estimates	0.005	0.154	0.212	0.103	0.120	0.105	0.082	0.125	0.088	0.111	0.210	—
$L_{0,5}$	85.2	60.2	39.1	59.1	68.0	36.3	25.9	25.9	53.1	55.1	55.1	51.4 ± 5.8
Precision of estimates	0.036	0.244	0.246	0.119	0.158	0.091	0.237	0.211	0.112	0.177	0.122	—

[1] $L_{I,J}$ values represent the fractional transfer rate constant coefficients for the labeled n−3 fatty acid that was transferred between 2 adjoining compartments. For example, $L_{3,2}$ is the rate constant coefficient for the transfer of $^2H_5$-18:3n−3 from plasma compartment 2 to plasma compartment 3 (20:5n−3). Precision estimates are given as SDs of the estimates normalized to the values of the estimates expressed as percentages determined from the modeling procedure.

from ALA. This appears to be analogous to our previous observations on the metabolism of n−6 FA in the production of 20:4n−6 from 20:3n−6 (23). However, on a per mole basis, preformed EPA was 3.6 times more effective that ALA in DHA production (Tables 5 and 7). A direct side-by-side comparison of the n−6 from a previous report (23) and n−3 EFA metabolic variables in this group of infants is given in **Table 8**. *P* values for both n−6 and n−3 FA indicated uniformly high fractional synthetic rates for long-chain PUFAs in this group of infants.

n−3 FA intake

When integrating each subject's feeding regimen (Table 1) into each of the 2 compartmental models, an adjustment to n−3 FA intake values was necessary to account for the initial low intake volumes during the first 24–48 h after birth. FA intake for several subjects was negligible during this period. It was presumed that plasma EFA concentrations were maintained through mobilization of body-stored reserves. Individual and mean intake values for 18:3n−3, 20:5n−3, 22:5n−3, and 22:6n−3 are given

TABLE 5
Fractional transfer rates (*P*) and rate constant coefficients (in h × 1000) of ^{13}C-labeled n−3 fatty acids from 11 infants[1]

	Subject identification number											
	81	82	83	84	86	87	88	89	90	91	92	Mean ± SE
$P_{3,2}$	0.0004	0.002	0.002	0.008	0.004	0.002	0.002	0.008	0.001	0.001	0.002	0.003 ± 0.001
$P_{4,3}$	0.22	0.94	0.33	0.11	0.69	1.00	0.26	0.42	1.00	1.00	0.59	0.60 ± 0.11
$P_{5,4}$	1.00	0.92	1.00	0.77	0.55	1.00	0.05	0.99	1.00	0.82	0.12	0.75 ± 0.11
$L_{3,2}$	0.42	2.32	1.54	7.30	0.41	1.77	1.44	7.13	0.63	1.05	1.66	2.33 ± 0.75
Precision of estimates	0.066	0.008	0.503	0.059	0.051	0.008	0.010	0.002	0.024	0.014	0.009	—
$L_{4,3}$	5.1	12.8	10.6	6.4	10.4	13.6	6.0	11.8	10.0	12.7	14.4	10.3 ± 0.97
Precision of estimates	0.062	0.021	0.014	0.063	0.182	0.172	0.714	0.434	0.182	0.282	0.311	—
$L_{5,4}$	40.5	40.4	9.5	23.0	19.8	31.7	0.9	35.6	16.3	17.7	3.1	21.7 ± 4.3
Precision of estimates	0.203	0.219	0.098	0.046	0.136	0.136	0.265	0.137	0.131	0.165	0.211	—
$L_{0,3}$	17.6	0.8	21.3	52.5	4.7	0.0	17.5	16.2	0.0	0.0	10.1	12.8 ± 4.7
Precision of estimates	0.062	0.069	0.051	0.024	0.130	0.080	0.046	0.039	0.109	0.086	0.044	—
$L_{0,4}$	0.10	3.28	0.10	6.75	16.20	0.10	16.15	0.36	0.00	3.84	23.62	6.41 ± 2.54
Precision of estimates	0.072	0.068	0.085	0.040	0.277	0.213	0.397	0.187	0.413	0.086	0.252	—
$L_{0,5}$	72.5	119.5	45.1	53.2	29.0	8.4	0.0	38.0	38.3	31.4	29.4	42.2 ± 9.791
Precision of estimates	0.126	0.085	0.129	0.087	0.117	0.754	0.124	0.166	0.125	0.202	0.215	—

[1] $P_{I,J}$ values represent percentages of labeled fatty acid transferred between 2 adjoining compartments. $L_{I,J}$ values represent fractional rate constant coefficients for transfer of fatty acid between 2 adjoining compartments. Precision estimates for individual $L_{I,J}$ values are given as SDs of the estimates normalized to the values of the estimates expressed as percentages determined from the modeling procedure.

TABLE 6
Plasma appearance (from biosynthesis) and disappearance rates [R_J (in μg/h)] for n−3 fatty acids in newborn infants[1]

	Subject identification number											
	81	82	83	84	86	87	88	89	90	91	92	Mean ± SE
$R_{0,1}$	25,115	12,144	25,305	53,173	46,494	36,744	9066	15,393	8612	31,067	13,017	25,103 ± 4649
$R_{0,2}$	42	21	269	210	1	10	11	38	2	13	11	57 ± 28
$R_{3,2}$	9.1	2.2	10.6	8.0	4.7	22.4	1.7	9.3	1.3	11.9	0.8	7.5 ± 1.9
$R_{0,3}$	0	0	0	0	3	0	0	0	0	0	0	0 ± 0
$R_{4,3}$	78	71	18	33	20	39	12	29	24	39	27	36 ± 6.4
$R_{0,4}$	11	7.2	2	6	1	6	36	3	4	8	2	7.8 ± 3.0
$R_{5,4}$	191	79	14	69	60	71	15	34	39	84	25	62 ± 15.1
$R_{0,5}$	1638	1336	213	513	945	318	202	279	511	1074	579	692 ± 148

[1] $R_{I,J}$ values represent the rates of disappearance (not involved in synthesis) and appearance (synthesized from its precursor) of the n−3 fatty acids. Values were determined from the fractional rate constant coefficients and plasma masses for each fatty acid. For example, $R_{3,2}$ is the hourly amount of plasma 18:3n−3 involved in synthesis of 20:5n−3, and $R_{0,1}$ is the turnover of 18:3n−3 in each subject.

in micrograms per hour (**Table 9**). The mean (±SE) predicted daily intakes of 18:3n−3 and 22:6n−3 were 150 ± 92 mg 18:3n−3 · kg^{-1} · d^{-1} and 2.2 ±0.5 mg 22:6n−3 · kg^{-1} · d^{-1}. This amount of DHA intake was not sufficient to replace the plasma 22:6n−3 turnover. The combined input from synthesis (1.5 ± 0.2 mg DHA/d) and intake (≈7.08 ± 0.6 mg DHA/d) of DHA provided only about one-half of the 22:6n−3 needed to maintain plasma DHA homeostasis during the first week of life. In contrast, the predicted 18:3n−3 intake was well matched to its turnover in the system (187 ± 56 mg 18:3n−3 · kg^{-1} · d^{-1}).

DISCUSSION

Two compartmental models that used ^2H and ^{13}C isotopically labeled substrates were advanced to assess the contributions of dietary 18:3n−3 and 20:5n−3 toward the synthesis and maintenance of plasma 22:6n−3 in newborn infants. The current study continued our previous work that investigated n−6 EFA metabolism in the same group of subjects and, therefore, offered a unique opportunity to compare intrasubject n−3 and n−6 EFA metabolism during the first week of life (23). Clinical investigations that are intended to assess EFA use and metabolism in newborns, especially infants who present with variable levels of postnatal metabolic stress, have limitations. In particular, this group of neonates had delayed onset feeding, and the initial nutrient intake volumes were low, which may have contributed to nonideal plasma steady-state conditions for 18:3n−3. In addition, 6 infants lost some body weight (mean: 5%; range: 0.25–11%), however, 5 infants had an increase in body mass (mean: 2.8%; range: 2.1–4.4%) during the first week. Also, compartmental models that are based on plasma kinetics alone are somewhat limited in assessing true liver synthetic values (13). Further, because some infants received complex diets that contained both breast milk and infant formula, the variable n−3 and n−6 FA intake may have influenced the metabolism of labeled n−3 FA and n−3 FA rate variables. The modeling procedures provided a sufficient level of control to account for the varying intake of n−3 EFAs. However, the current version of this model does not extend similar control to a varying intake n−6 EFAs or n−6 long-chain PUFAs.

The efficiency of each biosynthetic step, which was calculated as a percentage of the isotope flux remaining in the biosynthetic pathway (P value), was measured from the rate constant coefficients for the transfer of label into and out of individual FA compartments. In infants there was about a 0.04% conversion of dietary ALA to plasma EPA (Table 7), whereas in adult subjects this value was 0.011% or a ≈4:1 ratio of infants to adults (11). Overall, the percentage conversion of dietary ALA to DHA was ≈14 times greater in infants than in adult humans, which signifies that DHA production was highly active during early developmental processes (11). The total amount of DHA supplied to plasma was 1.5 mg DHA/d. A high percentage of plasma 18:3n−3 was used for the biosynthesis of 20:5n−3 in most subjects (Table 7), which was of a similar magnitude as the percentage use of 18:2n−6 for the biosynthesis of PUFAs (≈10%) in the same group of infants (Table 8) (23). With the use of a similar isotope procedure in 3-wk-old infants, Sauerwald et al (17) estimated that the fractional rate of conversion (FRC) of plasma 18:3n−3–22:6n−3 (FRC is equivalent to the P value)

TABLE 7
Fractional transfer of labeled n−3 fatty acids (P)[1]

	Subject identification number											
	81	82	83	84	86	87	88	89	90	91	92	Mean ± SE
$P_{2,1}$	0.0002	0.0019	0.0006	0.0041	0.0001	0.0009	0.0013	0.0030	0.0004	0.0008	0.0009	0.0013 ± 0.0004
$P_{3,2}$	0.179	0.093	0.270	0.037	0.850	0.685	0.136	0.199	0.429	0.474	0.067	0.311 ± 0.081
$P_{4,3}$	1.00	0.60	1.00	1.00	0.88	0.95	1.00	1.00	0.97	1.00	1.00	0.945 ± 0.036
$P_{5,4}$	1.00	1.00	1.00	1.00	0.99	1.00	0.29	1.00	1.00	1.00	1.00	0.935 ± 0.065

[1] $P_{I,J}$ values represent the percentages of labeled fatty acid transferred between 2 adjoining compartments. For example, $P_{3,2}$ is the percentage of $^2H_5$-18:3n−3 transferred from plasma compartment number 2 (18:3n−3) to plasma compartment number 3 (20:5n−3).

TABLE 8
Use and synthetic rates, plasma turnover, and intake amounts of n−6 and n−3 essential fatty acids in newborn infants[1]

	Mean ± SE
n−6 EFA[2]	
Plasma (% of use)	
$P_{\%\ \text{dose in plasma}}$	0.54 ± 0.22
$P_{\%18:2\rightarrow20:3}$	3.83 ± 1.99
$P_{\%18:2\rightarrow18:3}$	1.24 ± 0.50
$P_{\%18:2\rightarrow20:2}$	3.02 ± 1.19
$P_{\%20:3\rightarrow20:4}$	71 ± 11
Plasma synthesis (mg/d)	
$R_{18:2\rightarrow20:3}$	0.29 ± 0.05
$R_{18:2\rightarrow18:3}$	0.52 ± 0.08
$R_{18:2\rightarrow20:2}$	0.25 ± 0.04
$R_{20:3\rightarrow20:4}$	0.94 ± 0.11
Turnover (mg/d)	
Whole body	
$R_{18:2}$	9471 ± 1746
Plasma (Σ)	
$R_{18:2}$	44 ± 9
$R_{18:3}$	0.41 ± 0.06
$R_{20:2}$	2.39 ± 0.34
$R_{20:3}$	1.67 ± 0.27
$R_{20:4}$	10.2 ± 2.4
Estimated intake (mg/d)	
$U_{18:2}$	7111 ± 1216
$U_{18:3}$	0.12 ± 0.02
$U_{20:2}$	1.36 ± 0.23
$U_{20:3}$	0.87 ± 0.11
$U_{20:4}$	7.43 ± 1.68
n−3 EFA[3]	
Plasma (% of use)	
$P_{\%\ \text{dose in plasma}}$	0.13 ± 0.04
$P_{\%18:3\rightarrow20:5}$	31 ± 9
$P_{\%20:5\rightarrow22:5}$	93 ± 10
$P_{\%22:5\rightarrow22:6}$	94 ± 11
Plasma synthesis (mg/d)	
$R_{18:3\rightarrow20:5}$	0.18 ± 0.05
$R_{20:5\rightarrow22:5}$	0.85 ± 0.15
$R_{22:5\rightarrow22:6}$	1.48 ± 0.36
Turnover (mg/d)	
Whole body	
$R_{18:3}$	602 ± 112
Plasma (Σ)	
$R_{18:3}$	1.55 ± 0.67
$R_{20:5}$	0.86 ± 0.17
$R_{22:5}$	1.67 ± 0.36
$R_{22:6}$	16.6 ± 3.5
Estimated intake (mg/d)	
$U_{18:3}$	482 ± 184
$U_{20:5}$	0.21 ± 0.05
$U_{22:5}$	0.21 ± 0.07
$U_{22:6}$	7.43 ± 1.68

[1] EFA, essential fatty acid.
[2] $n = 10$.
[3] $n = 11$.

varied from 1.5% to 4.2% and depended on the ALA content of the formula. Their analysis did not include isolation of any intermediates in the synthesis of DHA, and therefore, it is not possible to determine the efficiency associated with any individual biosynthetic processes. In the current study, the net mean FRC [net mean FRC = $(P_{5,4}) \times (P_{4,3}) \times (P_{3,2})$] for the conversion of plasma 18:3n−3–22:6n−3 was on the order of 28%. This higher FRC value may be due to several factors, including the somewhat earlier postnatal stage of our subjects and perhaps differences in each study's duration (24 compared with 168 h). Notably, the FRC from a bolus dose of labeled 18:3n−3–22:6n−3 in adult men and women who consumed their diets ad libitum was <1% (25). As characteristic of early life, it is supposed that the higher rate of biosynthesis may be related to the demand of the system for long-chain PUFAs during development. However, caution should be used when making cross comparisons (16).

In another earlier attempt to determine the conversion of 18-carbon EFA to long-chain PUFAs in infants, Demmelmair et al (26), who used changes in ratios of the ^{13}C:^{12}C isotopes of plasma FAs in subjects who received a corn-oil-based formula (a C-4 plant), took advantage of natural ^{13}C:^{12}C-isotope abundance differences that occur between C-3 and C-4 plant species. Carnielli et al (27) later used a modified version of this method in a long-term feeding study in premature infants. The former study (27) is more comparable with the current study design because it tracked changes in the time-course concentration ratios of plasma FA when the percentage conversion of 18:2n−6–20:4n−6 was computed. The black-box approach favored by Carnielli et al (27) inferred values for the percentage conversion of 18:3n−3 to DHA from single time-point assessments, lacked kinetic determinants, isolated no pathway intermediates, and required inclusion of several uncorroborated assumptions. Interestingly, both studies (26, 27) inferred a high percentage conversion of 18-carbon EFA to long-chain PUFAs from their data during the first few weeks of life. In the current study, we estimated that ≈28% of plasma ALA was converted to DHA by using a modeling paradigm that also accounted for FA turnover, dietary EFA intake, and the conversion of individual substrates to intermediates and final products. However, as the appearance of labeled ALA into the plasma was a very inefficient process, the overall conversion of dietary ALA to plasma DHA was only 0.04%.

The percentage conversion of ^{13}C-20:5n−3 to ^{13}C-22:6n−3 was significantly less than the percentage conversion of ^{2}H-20:5n−3 to ^{2}H-22:6n−3 (45% compared with 94%; $P < 0.004$, Student's t test). However, when compared on a per mole basis, dietary 20:5n−3 was ≈3.5 times more effective toward the synthesis of 22:6n−3 than was 18:3n−3.

The predicted mean intake of 18:3n−3 from the ALA compartmental model was ≈150 mg 18:3n−3 $\cdot$ kg^{-1} $\cdot$ d^{-1}, with a whole-body turnover of 187 mg 18:3n−3 $\cdot$ kg^{-1} $\cdot$ d^{-1}. This amount of ALA intake appeared to be sufficient to meet the newborn ALA requirements in contrast with a need for a greater amount of 18:2n−6 intake in the same group of subjects (23). The mean daily rates of synthesis and turnover of 22:6n−3 in the plasma of infants were estimated to be ≈1.5 mg 22:6n−3/d and 16.7 mg 22:6n−3/d, respectively. Although liver biosynthesis was not assessed directly on the basis of previous assessments of DHA biosynthesis in adults, it may be supposed that liver DHA biosynthesis was greater than that inferred from plasma analysis (13). From the predicted rates of turnover and synthesis of 22:6n−3 for the entire group, it was estimated that an additional intake of ≈2.5 mg 22:6n−3 $\cdot$ kg^{-1} $\cdot$ d^{-1} (or ≈8.1 mg 22:6n−3/d) was needed to maintain plasma DHA concentrations during the first week of life.

TABLE 9
Predicted n−3 fatty acid intake [U_J (in μg/h)] in 11 infants during the first week of life[1]

	Subject identification number											
	81	82	83	84	86	87	88	89	90	91	92	Mean ± SE
U_1/18:3	69,834	3382	70,426	14,843	12,927	10,224	2524	4292	20,092	8644	3622	20,073 ± 7680
U_2/20:5	21.2	21.0	2.2	7.8	5.6	4.9	3.2	6.0	8.8	8.1	8.0	8.8 ± 1.9
U_3/22:5	31.1	0.2	0.0	9.7	13.2	8.6	12.5	0.6	8.5	12.4	0.0	8.8 ± 2.8
U_4/22:6	661	92	574	202	404	113	85	112	297	452	253	295 ± 61

[1] U_J values were calculated for each subject and reflect low intake values in most subjects during the first 48 h after birth.

The results of this study, together with findings of n−6 EFA metabolism (23) in the same group of subjects, form a basis on which to hypothesize the effects of feeding a particular infant formulation on the metabolism and maintenance of plasma 18–20- and 22-carbon n−3 and n−6 essential FAs in newborn infants. Even with relatively high rates of conversion of plasma 18:3n−3 and 18:2n−6 (23) to long-chain PUFAs, overall low rates of utilization of dietary 18:2n−6 and 18:3n−3 necessitate the fortification of infant formula with preformed 20:4n−6 and 22:6n−3 to ensure the proper maintenance of plasma homeostatic concentrations of these FAs when breast milk is not available. Three infants (82, 84, and 92) appeared to have low conversions of plasma 18:3n−3–20:5n−3 (Table 7) compared with other individuals, and in such cases a formula with preformed 22:6n−3 is particularly critical for the maintenance of DHA status.

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