Compartmental Analyses of Plasma ^{13}C- and ^{2}H-Labeled n-6 Fatty Acids Arising from Oral Administrations of ^{13}C-U-18:2n-6 and ^{2}H$_5$-20:3n-6 in Newborn Infants

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ABSTRACT: Efficacy of ^{13}C-U-18:2n-6 and ^{2}H$_5$-20:3n-6 toward synthesis of labeled-20:4n-6 was studied in newborn infants utilizing compartmental models of plasma labeled n-6 fatty acids (FA). Ten infants received oral doses of ^{13}C-U-18:2n-6 and ^{2}H$_5$-20:3n-6 ethyl esters (100 and 2 mg/kg, respectively). Rate constant coefficients and half-lives ($t_{1/2}$) of n-6 FA were determined from the time-course concentrations of labeled-FA. Plasma n-6 FA values approximated steady state concentrations. Synthetic and utilization rates were calculated. Eight percent (range, 2–21%) of plasma ^{13}C-U-18:2n-6 was used for synthesis of ^{13}C-18:3n-6, -20:2n-6, and -20:3n-6. Seventy percent of ^{13}C-20:3n-6 (mean, CV: 0.26) was available for synthesis of ^{13}C-20:4n-6. The percentage of ^{2}H$_5$-20:3n-6 converted to ^{2}H$_5$-20:4n-6 was lower (mean: 26%, $p < 0.02$) than the ^{13}C-labeled analogue. Turnover of 18:2n-6 in subjects and of 20:4n-6 in plasma was 4.2 g/kg/d (CV: 0.58) and 4.3 mg/kg/d (CV: 0.81), respectively. Intake of 18:2n-6 and 20:4n-6 were estimated to be 3.0 g/kg/d ($\pm$1.7) and 2.8 mg/kg/d ($\pm$ 2.2), respectively. Infants required additional 18:2n-6 and 20:4n-6 (mean: 1.2 g and 1.5 mg/kg/d) above predicted intake amounts to maintain plasma concentrations of 18:2n-6 and 20:4n-6, in order to spare FA from fat stores. (*Pediatr Res* **60: 327–333, 2006**)

Only recently did infant formulas marketed in North America contain long-chain PUFA, arachidonic (20:4n-6), and docosahexaenoic (22:6n-3) acid, both of which occur in human breast milk. Evidence from studies in human infants (1,2) indicate that together 20:4n-6 (3–5) and 22:6n-3 (6) are important during development and especially development of the nervous system. This is supported by evidence from animal studies (7,8). Although, it is now recognized as safe to add 20:4n-6 and 22:6n-3 to infant formula, it is still important to know how much 18:2n-6 and 20:3n-6 contribute to synthesis of 20:4n-6 (9,10), especially since neither precise amounts nor the optimal n-6 to n-3 fatty acid (FA) ratio have been established for infant formula based on dietary requirements.

Although several human studies have been carried out using stable isotope-based approaches to determine the conversion of linoleic acid (18:2n-6) to 20:4n-6 in infants (11–14), very little quantitative information is available using compartmental modeling to assess the effects of dietary 18:2n-6 and di-homo-γ-linolenic acid (20:3n-6) upon maintenance of plasma 20:4n-6 status in infants (14).

As reported here, two independent compartmental models were developed from the plasma masses of endogenous n-6 FA and isotopic tracer data of the ^{13}C-labeled n-6 (from ^{13}C-18:2n-6) and the ^{2}H$_5$-labeled n-6 (from ^{2}H$_5$-20:3n-6) FA using a compartmental modeling procedure. n-6 FA kinetic parameters were determined for each subject and mean values were calculated for the cohort. The $t_{1/2}$ of FA in plasma and quantitative contributions of dietary 18:2n-6 and 20:3n-6 toward maintenance of plasma 20:4n-6 during the first week of life were determined.

METHODS

Clinical procedures. Candidates with gestational ages >34 wk were admitted to the neonatal intensive care unit (NICU), and an umbilical line was inserted for monitoring. Infants that were small for gestational age, with major malformations, or in evaluation of necrotizing enterocolitis for feeding intolerance were excluded. Informed consent was provided from mothers and the protocol was approved by the National Institutes of Health Institutional Review Board under protocol #OH93-AA-N027 and approved by INTA's ethics committee and by the research committee of the participating NICU, Hospital Sótero del Rio, and Clínica Presbiteriana Madre-Hijo. Gestational age was assessed by last menstrual period or using date of conception based on early ultrasound and confirmed by the modified Ballard evaluation. Infants were classified as small for gestational age or appropriate for gestational age according to the Lubchenco standard (15). Most subjects presented with mild forms of hypoxic insult or transient respiratory problems. Feeding was started generally within 2 d after birth, and the type of feeding varied. If breast milk was unavailable, infants received Similac infant formula (Ross Labs, Abbott Park, IL), which contained 18:2n-6 (780 mg/100 mL) but devoid of 20:3n-6 and 20:4n-6. Subjects received a mixture of ^{13}C-U-18:2n-6 (100 mg/kg) and ^{2}H$_5$-20:3n-6 (2 mg/kg). Blood was drawn (0.5 mL) from an umbilical catheter or from a peripheral vein, into a tube containing EDTA. Blood was drawn at 0, 4, 8, 24, and 48 h and on d 4 and 7 after dosing. 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 Similac infant formula upon demand. The quantity of

Abbreviations: GC, gas chromatography; **GC/MS,** gas chromatography mass spectrometry; **FA,** fatty acid; **INTA,** Institute of Nutrition and Food Technology; **PUFA,** polyunsaturated fatty acid; **WinSAAM,** Windows Simulation, and Analysis Modeling

expressed milk and the amount of formula consumed were determined for each subject. Subjects that could not be initially fed enterally received parenteral glucose until they were capable of receiving enteral nutrition (100 mL/kg/d). None received intravenous lipids during the study period.

Stable isotopes. Carbon-13 uniformly labeled linoleate (^{13}C-U-18:2n-6, ^{13}C > 95%) and deuterium-labeled di-homo-γ-linoleate (19, 19, 20, 20, 20-^{2}H$_5$-20:3n-6, ^{2}H > 95%) ethyl esters were >95% chemical purity (Cambridge Isotope Laboratories, Andover, MA).

Lipid extraction and FA methyl esters. Plasma lipids were extracted using a modified Folch procedure (16). Plasma (200 μL) was added to methanol (1 mL) containing ethyl tricosanoate (0.13 nmol) as an internal standard and vigorously extracted twice with chloroform. Half of the lipid extract was derivatized to methyl esters using 14% boron trifluoride in methanol (17) and then dissolved in hexane.

GC analysis. GC analysis was performed on an Agilent 6890 system with flame ionization detection. Two microliters of the sample was injected onto a DB-FFAP capillary column (30 m × 0.25 mm I.D., 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, then to 210°C at 1°C/min, then increased at 30°C/min to 245°C.

Pentafluorobenzyl (PFB) derivatization. The lipid extract (100 μL) was evaporated and saponified with 5% of methanolic KOH. Free FA were extracted into hexane and derivatized to PFB esters as described previously (18). Reagents were evaporated under a steam of nitrogen and re-suspended in 100 μL of hexane.

GC/MS analysis. GC/MS was carried out on an Agilent 6890 GC-5973 Mass Selective Detector (Wilmington, DE) in the negative chemical ionization mode as previously described (18). Samples (1 μL) were injected in the splitless mode onto a DB-FFAP column and the oven was programmed from 125°C to 245°C at 8°C/min. Data were acquired by monitoring the M-PFB anion of each analyte and converted to the absolute quantity with reference to the internal standard using an appropriate response factor.

Compartmental models. Compartmental analysis began by considering existing knowledge of fat absorption, n-6 FA metabolism, and circulation of lipids in blood. The hepatocyte is a main site for biosynthesis of desaturated 18- and 20- PUFA from 18:2n-6 and for formation of lipoproteins (see Fig. 1). As liver specimens were not available, this study offers only indirect information regarding concentrations of labeled-FA substrates available within the hepatocyte. Similarly, rate constants represent kinetics of labeled-FA from their plasma pool and may only indirectly reflect liver metabolism. It is assumed that appearance of labeled n-6 FA in the plasma reflect availability of substrates within the liver. Two independent compartmental models of n-6 FA metabolism were developed using the concentration time-courses of the labeled-FA and concentrations of endogenous FA in plasma (Fig. 2) using WinSAAM (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 isotope lost from the path). The units are in h^{-1}. $L_{I,J}$ 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 substrate-compartment J to product-compartment I is obtained by multiplying the mass (M_J) of endogenous FA in compartment J by $L_{I,J}$ and is given in micrograms per hour. The percentage of isotope transferred from J to I is given as $P_{I,J}$ and is a percentage of the total flux of FA leaving J. $P_{I,J}$ is the fraction of isotope remaining in the metabolic pathway as opposed to isotope taken up by tissues or in other ways irreversibly lost from the compartment. The $t_{1/2}$ of the n-6 FA in plasma were calculated from a determination of the total transfer rates leaving each compartment:

$$t_{1/2} = \ln 2/\Sigma L_{I,J} + L_{0,J}.$$ Variances for the determined parameters are reported

as SD and coefficient of variance.

Model illustration and rate equations. The compartmental model for 18:2n-6 consisted of six compartments (Fig. 2). Compartment 1 represents the dose of the labeled-FA absorbed through the gastrointestinal tract. Compartments 2, 3, 4, 5, and 7 denote plasma pools of 18:2n-6, 20:3n-6, 20:4n-6, 18:3n-6, and 20:2n-6. Compartment 6 (not shown) is used to represent a time delay from the time of isotope administration to its appearance in the plasma. Arrows connecting the six compartments indicate flow along the path. Since 20:2n-6 may be converted to 18:2n-6, a rate constant, $L_{2,7}$, indicating conversion of 20:2n-6 to 18:2n-6 is also represented. The rate equations are defined by a set of differential equations corresponding to flux of labeled-FA through each respective compartment and those that exit the system (Fig. 2). The compartmental model for 20:3n-6 is presented in Figure 2B. Here, only two plasma compartments are considered.

Constraints, limits, validation, and statistical comparisons. Plasma n-6 FA concentrations, determined from mean values over 168 h for each subject, were used to represent the mass of endogenous substrates (M_J) available for biosynthesis (Table 1) and were held constant. For purposes of estimating a daily n-6 FA intake for each subject, the FA content of the formula, availability of breast milk, and frequency of feeding were entered into the model. Infants who were breast-fed and/or received expressed breast milk intake of n-6 FA were approximated from breast milk samples obtained from mothers living within the vicinity of Santiago (19). From these determinants, upper and lower n-6 FA limits were estimated for each subject. For purposes of model validation, estimated daily intake and synthesized amounts of n-6 FA were compared with daily turnover rate of each FA in the plasma. To determine differences between the efficacy of the two precursors (18:2n-6 and 20:3n-6) toward synthesis of 20:4n-6 or other rate parameters, a paired *t* test analysis was performed on values of the rate parameters using each subject as its own control. A *p* value of 0.05 or lower was considered significant.

Calculations, errors, and predicting dietary n-6 FA intake. Initial $L_{I,J}$ and $P_{I,J}$ estimates, 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 determined using an iterative nonlinear least squares routine. The error model included assumptions of independence, constant variance, and normal distribution about zero. Consistent with the precision of analytical methods, data points were weighted by assigning a fractional SD of 0.1 to each measurement. Daily dietary n-6 FA intake values (U_J) (Table 2) were estimated for each infant while constraining plasma FA masses to known limits. Additionally, the model was adjusted to compensate for low intake volumes during the first 48 h after birth with a gradual increase in volume over the next period.

RESULTS

Subject characteristics. Ten (8 male and 2 female) out of 11 infants completed the protocol. Patient characteristics at birth and relevant data at starting and ending the study are presented in Table 3. Infants had a mild form of respiratory distress syndrome secondary either to meconium aspiration or perinatal asphyxia. Subjects were monitored in the NICU until respiratory function normalized and most received supplemental feeding with breast milk and/or infant formula in increasing volume during the study. The mean concentrations (μg/mL ± SD) of plasma of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, and 20:4n-6 for this group of infants were 111 ± 14, 2.9 ± 0.4, 6.9 ± 2.9, 19.8 ± 11.9, and 94.6 ± 12.0. Other than
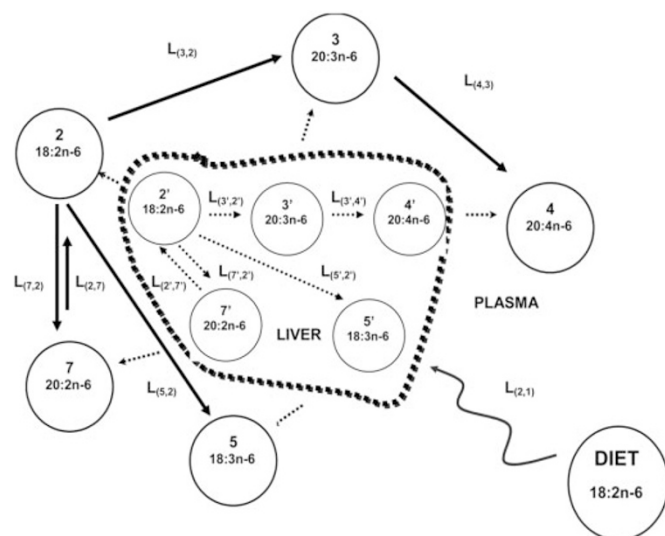
Figure 1. 18:2n-6 metabolism. Circles represent individual n-6 FA compartments, five plasma compartments (2, 3, 4, 5, and 7) and a compartment for 18:2n-6 intake (#1). The liver is shown in dotted outline. Dashed arrows represent portions of the pathway for which rate constants were not determined. Solid arrows represent portions of the pathway for which rate constants were determined from plasma kinetics.
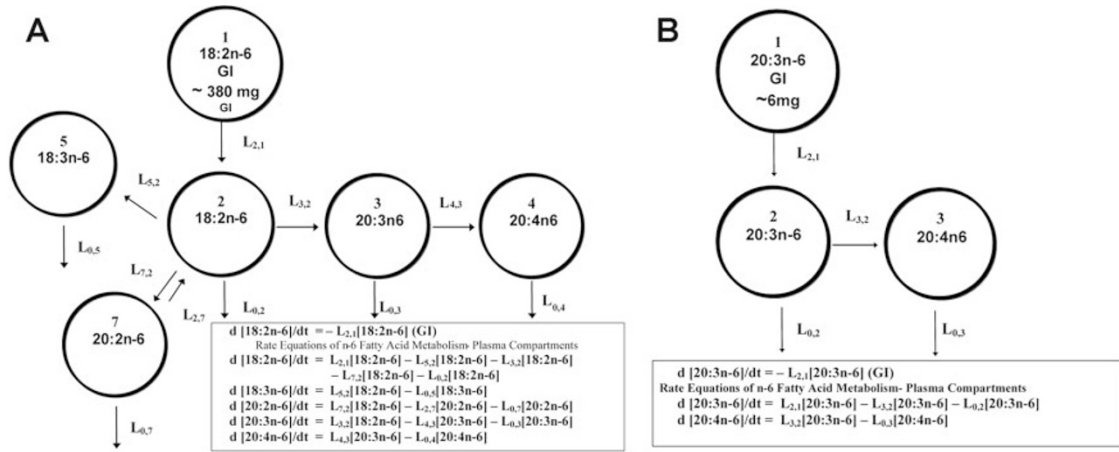
Figure 2. 18:2n-6 compartmental model (*A*) and 20:3n-6 compartmental model (*B*). Open circles represent plasma and gastrointestinal (GI) compartments for n-6 FA. $L_{X,J}$ values denote rate constant coefficients. $L_{0,J}$ values indicate loss of isotope from the pathway. Differential rate equations for determining flux of isotope through various compartments shown are in the boxed area.
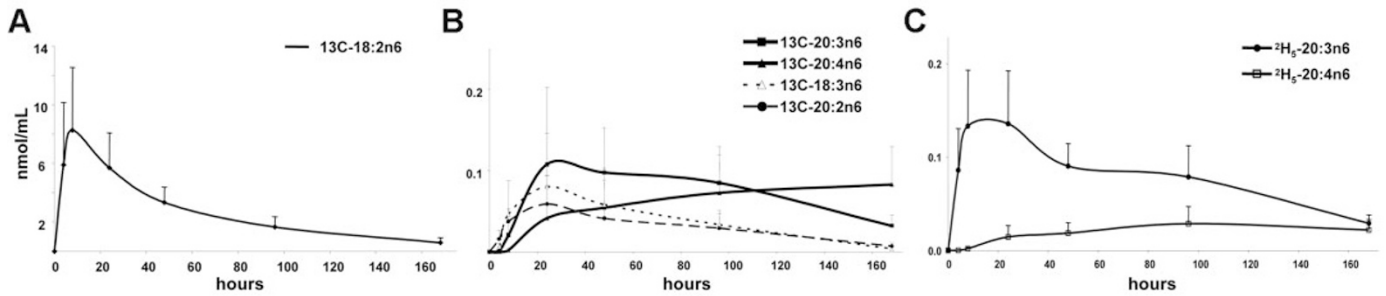
Figure 3. Plasma time course concentration curves for ^{13}C-U-18:2n-6 (*A*) and ^{13}C-18:3n-6, ^{13}C-20:2n-6, ^{13}C-20:3n-6, and ^{13}C-20:4n-6 (*B*) following an oral dose of ^{13}C-U-18:2n-6 (380 mg). (*C*) Time course concentration curves for $^{2}H_5$-20:3n-6 and $^{2}H_5$-20:4n-6 following an oral dose of $^{2}H_5$-20:3n-6 (6 mg) in 10 newborn infants. Error bars indicate SD of the mean.

Table 1. *Mean steady state mass values in micrograms for the fatty acid plasma compartments (M_J) 2, 3, 4, 5 and 7 (Fig. 2) from 10 newborn infants*

Compartment/ n-6 FA	Subject 82	83	84	86	87	88	89	90	91	92	Mean	SD	CV
M_2/18:2	16,136	29,212	19,902	11,381	17,700	9768	20,493	10,025	29,439	28,913	19,297	3899	0.10
M_3/20:3	3536	3424	2733	3666	1891	2057	3205	2393	5243	5369	3359	599	0.09
M_4/20:4	16,829	16,664	9122	14,285	14,283	8367	17,419	10,957	29,521	24,377	16,182	3303	0.10
M_7/20:2	548	393	752	nd	747	1625	645	936	590	1617	873	225	0.13
M_5/18:3	485	309	212	288	241	399	517	141	757	310	366	90	0.12

Amounts are given in micrograms of FA and are mean values determined over 7 d. Values are determined from mean concentration of individual FA over 168 h multiplied by the total plasma volume from each infant.

nd, the FA, 20:2n6, was not determined for subject 86 due to chromatographic peak interference.

a 15% decrease in 20:4n-6 concentration in subject 83 and 87 no other decreases were observed during the period.

Compartmental models and absorption of labeled FA. The compartmental model shown in Figure 1 simplifies the physiologic reality since the liver was not isolated as a separate compartment from the plasma. Both models presume that each plasma $L_{I,J}$ rate constant reflects several steps of metabolism that occur within the liver and imply that these values incorporate a function involving a FA transport step from the liver to plasma (dotted arrows) and that this transport function is similar for all FA measured in plasma.

There were two 18:2n-6 compartments, one for isotope administration (GI) and the second for the appearance of the FA in plasma (Fig. 2). In a preliminary study (Paulina Canales, unpublished thesis) with a separate group of newborn infants (*n* = 20), approximately 94% of labeled-18:2n-6 ethyl

Table 2. *Predicted mean and individual intake amounts (U_J) of n-6 FA in 10 infants during the first week of life given in micrograms per hour*

Intake/ n-6 FA	Subject 82	83	84	86	87	88	89	90	91	92	Mean	SD	CV
U_2/18:2	285,686	722,857	139,271	192,214	51,394	107,307	200,129	85,336	1,027,571	151,129	296,289	160,108	0.57
U_3/20:3	6	11	54	98	12	16	34	32	33	68	36	15	0.40
U_4/20:4	53	137	133	1513	338	41	410	311	50	111	310	221	0.71
U_5/20:2	6	1	16	145*	52	102	19	159	3	65	57	30	0.53
U_7/18:3	0	1	13	17	2	5	1	4	1	5	5	3	0.57

U_J values are calculated for each subject and adjusted for a reduced feeding schedule during the first 48 h.

*This value is estimated from turnover of labeled-20:2n-6 alone since steady state concentrations of the FA could not be determined due to chromatographic interference (see Table 1).

Table 3. *Subject characteristics*

ID	BW (g)	GA (wk)	Sex	Age at entrance (d)	Wt at entrance (g)	Wt at end (g)	Age at enteral feeding (d)	Formula intake mL/d	Breast milk intake mL/d
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

BW, birth weight; GA, gestational age.

ester was absorbed (range, 89–99%) based on the amount of isotope recovered from the feces over 48 h. This value, similar to findings in other infants (20), was used to estimate absorption of both isotope and dietary fat. It was presumed that maximum absorption of isotope into chylomicrons occurred between 4 and 6 h and that mean maximum plasma concentration of labeled 18:2n-6 at 8 h probably resulted from a mixture of chylomicron remnants and VLDL released from the liver (Fig. 3A). Sample collection began at 4 h to avoid complications arising from an under determination of labeled-precursors and subsequent labeled FA (21). Using the area under the curve calculation (AUC), mean values of ^{13}C-U-18:2n-6 and $^2H_5$-20:3n-6 ($\pm$ SD) appearing in plasma were 254.5 $\pm$ 58.5 and 8.5 $\pm$ 3.9 nmol/L/h, respectively.

Concentration time curves for N-6 FA. Figure 3, *a* and *b*, illustrates composite time curves for labeled ^{13}C-n-6 FA from all subjects. The graphs are for illustration purposes only since model-derived kinetic values were determined for each subject uniquely using the dosing regimen, plasma characteristics, and labeled n-6 FA time course data. Although masses of individual endogenous n-6 FA varied over a wide range (Table 1) quantitatively similar amounts of isotope appeared in each of the n-6 FA compartments (Fig. 3b). From composite time curves for $^2H_5$-20:3n-6 and $^2H_5$-20:4n-6 illustrated in Figure 3c it appears that a smaller amount of isotope was transferred from $^2H_5$-20:3n-6 to $^2H_5$-20:4n-6 in comparison to the time-course for ^{13}C-U-20:3n-6 to ^{13}C-20:4n-6 presented in Figure 3b.

Rate constant coefficients, R values, and p values. Individual fractional rate constant coefficient estimates ($L_{I,J}$) for

both 18:2n-6 and 20:3n-6 models were optimized for each subject before determining mean values for the group and final values are given in Tables 4 and 5, respectively. The synthetic and utilization rates, $R_{X,J}$, represent the total mass of individual n-6 FA that exit the substrate compartment J and is either transferred to product compartment I or leaves the pathway (0). These are given in micrograms per hour (Tables 5 and 6). In one case (subject 86), a value for $R_{0,5}$ could not be calculated because of a chromatographic interference for 20:2n-6 (Table 1). The mean value for turnover of 18:2n-6 through the system was calculated to be 4.2 g/kg/d (CV, 0.58) and mean turnover of 18:2n-6 in plasma ($R_{0,2}$) was 43 mg/d (CV, 0.65) for the group. The mean daily turnover in mg/d of other n-6 FA in plasma were: 0.41 (CV, 0.50), 2.4 (CV, 0.49), 0.73 (CV, 0.81), and 10.2 (CV, 0.74) for 18:3n-6, 20:2n-6, 20:3n-6, and 20:4n-6, respectively. The mean rate of synthesis of 20:4n-6 from 20:3n-6 ($R_{4,3}$) was 39.2 μg/h or 0.94 mg/d (CV, 0.36) from the ^{13}C-FA and 53 μg/h (CV, 0.48) from ^2H-20:3n-6 (Table 5). Other individual and mean values for synthetic and utilization rates for each n-6 FA are found in Tables 5 and 6.

The proportion of plasma n-6 FA, $P_{I,J}$, directed toward biosynthesis was determined and these values are given in Tables 5 and 7. Consistent with studies in adult humans (22,23), on average about 0.5% of the administered dose of ^{13}C-18:2n-6 and 0.3% of $^2H_5$-20:3n-6 appeared in plasma ($P_{2,1}$). Based on values in Table 7, the total mean percentage of plasma 18:2n-6 directed toward synthesis of all other n-6 FA was approximately 10.3% (range, 1.7–29%; CV 0.62). The mean percentage of plasma ^{13}C-20:3n-6 destined for synthesis

Table 4. *Individual and mean fractional transfer rate constant coefficients ($L_{I,J}$) of in vivo 18:2n-6 acid metabolism in 10 infants. ($L_{I,J}$) values are coefficients for transfer of fatty acid between and out of compartments 2–7*

						h^{-1}							
Fractional transfer rate constant coefficients													
	82	83	84	86	87	88	89	90	91	92	Mean	SD	CV
$L_{2,1}$	0.0017	0.0012	0.0080	0.00003	0.0001	0.0005	0.0014	0.0001	0.0001	0.0008	0.0014	0.0012	0.87
$L_{3,2}$	0.0005	0.0002	0.0002	0.0004	0.0009	0.0002	0.0024	0.0003	0.0005	0.0003	0.0006	0.0003	0.57
$L_{5,2}$	0.0003	0.0001	0.0003	0.0012	0.0022	0.0004	0.0008	0.0017	0.0003	0.0030	0.0010	0.0005	0.47
$L_{0,2}$	0.0364	0.0489	0.0378	0.0348	0.0124	0.0319	0.2941	0.1625	0.2111	0.0132	0.0883	0.0492	0.56
$L_{7,2}$	0.0007	0.0002	0.0004	0.0050	0.0012	0.0005	0.0027	0.0003	0.0014	0.0003	0.0013	0.0008	0.60
$L_{0,7}$	0.0121	0.0203	0.1223	1.0000	0.0743	0.0282	0.1000	0.0581	0.0334	0.1277	0.1576	0.1495	0.95
$L_{0,5}$	0.0342	0.0159	0.1071	0.3062	0.0000	0.0957	0.0900	0.2747	0.0253	0.3250	0.1274	0.0631	0.50
$L_{4,3}$	0.0070	0.0058	0.0084	0.0006	0.0357	0.0053	0.0273	0.0238	0.0145	0.0258	0.0154	0.0059	0.38
$L_{0,3}$	0.0004	0.0018	0.0218	0.0390	0.0076	0.0080	0.0027	0.0024	0.0005	0.0003	0.0085	0.0063	0.74
$L_{0,4}$	0.0060	0.0127	0.0230	0.1484	0.0240	0.0078	0.0380	0.0000	0.0050	0.0082	0.0273	0.0220	0.81

Table 5. *Individual and mean P_J, L_J, and R_J values in 10 newborn infants from an oral dose of $^2H_5$-20:3n-6*

p Value					Value * 100 (%)								
	89	92	91	90	88	87	86	84	83	82	Mean	SD	CV
$P_{2,1}$	0.0029	0.0009	0.0032	0.0038	0.0086	0.0050	0.0004	0.0019	0.0006	0.0014	0.0029	0.0013	0.47
$P_{3,2}$	0.2918	0.4214	0.1130	0.0911	0.0178	0.1586	0.9586	0.0421	0.2425	0.2697	0.2607	0.1459	0.56
Fractional transfer rate constant coefficients													
						hr^{-1}					Mean	SD	CV
$L_{2,1}$	0.00063	0.00030	0.00005	0.00003	0.00011	0.00063	0.00002	0.0014	0.0018	0.0014	0.0006	0.0004	0.55
$L_{0,2}$	0.0251	0.0063	0.0470	0.0359	0.0997	0.0000	0.0000	0.0252	0.0063	0.0058	0.0251	0.0163	0.65
$L_{3,2}$	0.0185	0.0046	0.0069	0.0036	0.0018	0.1397	0.0047	0.0011	0.0020	0.0021	0.0185	0.0227	1.23
$L_{0,3}$	0.0241	0.0166	0.0120	0.0016	0.0095	0.0300	0.1115	0.0106	0.0086	0.0166	0.0241	0.0168	0.70
Synthetic/utilization rates						μg/hr					Mean	SD	CV
$R_{3,2}$	53	158	114	26	17	55	49	12	21	22.2	53	25	0.48
$R_{0,2}$	330	218	898	258	913	293	0	262	66	60.2	330	171	0.52
$R_{0,3}$	180	97	1100	48	17	56	232	22	18	34.5	180	176	0.97

($P_{I,J}$), percentage transfer values, are determined from the ratio of the rate constant coefficients ($L_{I,J}$) and represent the percentage of FA transferred from two adjoining compartments. $R_{I,J}$ values represent the hourly disappearance and synthetic rates based on plasma n-6 FA kinetics and steady state masses.

N.B. Due to wide variability of the amount and type of nutrient intake (see Table 3), an accurate assessment of $R_{0,1}$ (20:3n-6) was not possible for all subjects.

Table 6. *Individual and mean synthetic and disappearance rates (R_J) for the n-6 FA in micrograms per hour in 10 infants*

Disappearances and synthetic rates						μg/hr							
	82	83	84	86	87	88	89	90	91	92	Mean	SD	CV
$R_{0,1}$	399,340	1,010,500	194,200	268,610	71,826	149,890	274,010	117,800	1,432,300	27,594	394,607	229,863	0.58
$R_{3,2}$	8.8	5.2	3.8	4.0	16.4	2.1	49.2	2.9	16.0	10.4	11.9	7.0	0.59
$R_{5,2}$	5.0	4.1	5.7	13.8	39.4	3.3	16.0	1.7	9.0	7.1	10.5	5.6	0.53
$R_{0,2}$	585	1427	753	396	219	331	6027	1629	6215	332	1791	1165	0.65
$R_{7,2}$	11.4	4.8	7.8	56.6	21.2	4.6	55.3	3.0	41.7	9.3	21.6	10.7	0.50
$R_{0,7}$	5.8	6.3	25.9	29	10.1	10.9	51.7	8.2	25.3	10.0	17.1	7.5	0.44
$R_{0,5}$	18.6	6.2	34.5	nd	131	150	58.1	257	14.9	96.1	100	45	0.45
$R_{4,3}$	25.3	20.0	23.0	2.3	33.7	11.1	87.5	56.9	76.2	56.0	39.2	14.2	0.36
$R_{0,3}$	0.5	6.3	59.7	143	19.0	15.4	8.7	1.0	2.5	49.0	30.5	22	0.73
$R_{0,4}$	99.3	212	210	2120	507	68.4	662	0	146	211	424	315	0.74

$R_{i,j}$ values represent disappearance and synthetic rates determined from each subject's n-6 FA kinetic constants and steady state masses. For example, $R_{0,2}$ represents the amount of 18:2n6 that exits the plasma and $R_{0,1}$ is the amount of 18:2n6 that moves through the entire system.

nd, this value could not be calculated as the mass of 20:n-6 for this subject could not be determined.

of ^{13}C-20:4n-6 was 71% (CV 0.26) (Table 7). This contrasts with a much smaller value (26%, $p < 0.02$) of $^2H_5$-20:3n-6 destined for synthesis of $^2H_5$-20:4n-6 (CV 0.56) (Table 5). This suggests that that the preferred substrate for 20:4n-6 biosynthesis is 20:3n-6 arising from 18:2n-6 and that this

difference may be related to a particular form of complex lipid (*e.g.* phospholipid class) in which each of the different isotopomers resides. However, when one takes into consideration the percentage of each labeled substrate appearing in plasma, and overall percentage conversion of each precursor to

Table 7. *Individual and mean p values (percent of fractional transfer) of labeled fatty acids between compartments 2 (18:2n-6),*
3 (20:3n-6), 4 (20:4n-6), 5 (18:3n-6), and 7 (20:2n-6)

p Values	Subject 82	83	84	86	87	88	89	90	91	92	Mean	SD	CV
$P_{2,1}$	0.0016	0.0014	0.0035	0.0018	0.0017	0.0022	0.0220	0.0140	0.0044	0.0012	0.0054	0.0020	0.38
$P_{3,2}$	0.0082	0.0029	0.0074	0.0294	0.1332	0.0107	0.0026	0.0104	0.0014	0.1767	0.0383	0.0327	0.85
$P_{7,2}$	0.0144	0.0036	0.0050	0.0086	0.0553	0.0067	0.0080	0.0017	0.0025	0.0180	0.0124	0.0084	0.68
$P_{5,2}$	0.0185	0.0034	0.0102	0.1203	0.0716	0.0148	0.0090	0.0018	0.0066	0.0161	0.0272	0.0201	0.74
$P_{4,3}$	0.9432	0.7612	0.2779	0.0160	0.8245	0.4000	0.9100	1.0100	0.9680	1.0167	0.7127	0.1839	0.26

Value *100 (%)

($P_{I,J}$) values represent a percentage of FA transferred from two adjoining compartments and determined from the ratio of the rate constant coefficients ($L_{I,J}$) multiplied by 100. Infants ($n = 10$) were given an oral dose of ^{13}C-U-18:2n-6.

20:4n-6, it appears that dietary 20:3n-6, as measured by ^{2}H$_5$-20:3n-6, affords approximately 6-fold greater delivery of 20:4n-6 compared with 18:2n-6 as measured by ^{13}C-18:2n-6.

Turnover. The $t_{1/2}$ of labeled n-6 FA in plasma are an indication of the frequency in which FA need to be replenished. The mean $t_{1/2}$ in h for each of the n-6 FA in plasma were calculated from the kinetic rate constant coefficients for the 10 subjects (Table 4). The mean values of $t_{1/2}$ ($\pm$ SD) for 18:2n-6, 20:2n-6, 18:3n-6, 20:3n-6, and 20:4n-6 were 18 ($\pm$7), 21 ($\pm$9), 18 ($\pm$8), 42 ($\pm$15) and 63 ($\pm$23) h, respectively. Notably, plasma $t_{1/2}$ for 20:3n-6 and 20:4n-6 were greater compared with the 18-carbon FA and this is somewhat similar to the turnover of long chain n-3 PUFA in adult humans (22). Interestingly, plasma turnover rates do not appear to be directly dependent on the mass of the endogenous FA since both 18:2n-6 and 20:4n-6, which together occupy a high percentage of the total mass of plasma n-6 FA (Table 1) have $t_{1/2}$ values that differ by a factor of three. Conversely, turnover rates for 20:2n-6 and 18:2n-6 were similar even though plasma concentrations of these two FA varied by almost an order of magnitude (Table 1).

n-6 FA intake. When taking into consideration the feeding regimen appropriate for each subject (Table 3), estimated n-6 FA intake were consistent with each FA's synthetic and disappearance rates (Table 6) and plasma concentration (Table 1). Subject feeding regimens take into consideration low intake volumes during the first 24–48 h. Intake values for 18:2n-6, 20:2n-6, 18:3n-6, 20:3n-6, and 20:4n-6 are given in μg/h (Table 2). The daily mean ($\pm$ SD) intake of 18:2n-6 and 20:4n-6 were calculated to be 3.0 ($\pm$1.8) g/kg/d and 2.8 ($\pm$2.4) mg/kg/d, respectively.

DISCUSSION

Two compartmental models were developed to assess the contributions of 18:2n-6 and 20:3n-6 toward maintaining plasma concentrations of 20:4n-6 in newborn infants. The present study has limitations peculiar to clinical investigations in newborns and our results must be interpreted in light of certain constraints. During the early postnatal period, infants cease receiving nourishment through placental transfer and rapidly develop an increasing capacity to nurse. During this transitional period, it is plausible that fat reserves, which are mobilized to meet energy demands, also increase turnover rate of FA in plasma. Additionally, six infants lost body weight

(mean, 5%; range, 0.25–11%) and four had an increase in body mass (2.8%; range, 2.1–4.4%). Also, our study population consists of infants who presented variable levels of postnatal metabolic stress and in some cases this basal pathologic condition affected onset of feeding and subsequent volume intake increments. Also, compartmental models based on plasma kinetics are somewhat limited in assessing true synthetic values of liver metabolism.

These limitations notwithstanding, the compartmental model for 18:2n-6 predicted an 18:2n-6 intake in amounts of 1.7–4.3 g/kg/d (mean, 3.0 g/kg/d; CV 0.42) with a turnover rate between 1.8 and 6.6 g/kg/d (mean, 4.2 g/kg/d; CV 0.58) in these subjects. It is likely that body lipid stores supplied the remainder of 18:2n-6 as plasma concentrations of 18:2n-6 did not decrease. The high turnover rate may be related to the early demand for energy and as intake volumes of breast milk and/or formula increase this value may moderate reflecting the composition of the diet (14). However, consistent with present findings a high fractional turnover of 18:2n-6 was observed in adult male subjects (mean value: 93.7%/d) (24).

The percentage of isotope transferred through the n-6 FA compartments was calculated for each FA and these values were used to determine the efficiency of the conversion of precursor to other n-6 FA. Approximately 10% of plasma 18:2n-6 was destined for biosynthesis of other n-6 FA values, which are substantially higher than those observed in adult males (~1.8%, combined percentage of 20:3n-6 and 20:4n-6) (24). Characteristic of early life, it is supposed that the much higher rate of synthesis may be related to the demand of the system for 20:4n-6 during development (13).

The mean daily rate of synthesis and turnover of 20:4n-6 in plasma of infants were estimated to be from 0.06 to 2.1 mg/d (mean, 0.95; CV 0.36) and from 0 to 51 mg/d (mean, 10.2; CV 0.74), respectively. Using a similar isotope procedure in 3-wk-old infants, Sauerwald *et al.* (14) estimated that the fractional rate of conversion (FRC) of 18:2n-6 to 20:4n-6 (FRC is identical to the p value) varied from 0.4 to 1.1% and this value depended on the α-linolenic acid content of the formula. In the present study, net mean FRC for conversion of 18:2n-6 to 20:4n-6 was 2.7%. This higher value may be due to several factors, including, the somewhat earlier postnatal stage of our subjects and perhaps also differences in each study's duration (24 *versus* 168 h). In adult males consuming their normal diets, the FRC of a bolus amount of labeled 18:2n-6 to 20:4n-6

was on the order of 0.3% (24). The compartmental analyses used in these two previous studies did not isolate 20:3n-6 as an intermediate in the synthesis of 20:4n-6 and when excluding this intermediate in the present model to conform to the two previous studies the net conversion would have risen to about 3.8%.

From rates of turnover and synthesis of 20:4n-6 for the entire group, the model predicted that an intake of ~4.0 mg/kg/d of 20:4n-6 is needed to sustain the infant's plasma concentration. The percent conversion of ^{2}H-20:3n-6 to ^{2}H-20:4n-6 was significantly less than that of the ^{13}C-labeled FA (from ^{13}C-18:2n-6), however, between the two models there were no differences in the rate constant coefficients for transfer of isotope from 20:3n-6 to 20:4n-6 or disappearance of 20:4n-6. Nevertheless, dietary 20:3n-6 would have been approximately six times more effective toward synthesis of 20:4n-6 than 18:2n-6 and this might make it a desirable substrate in the formulation of some infant diets.

The differences in the proposed compartmental models for n-6 FA metabolism in either infants (14) or adults (24) and the present model necessitate several comments. All three studies used plasma isotope data to calculate *in vivo* kinetic rate constants. Although, values obtained for conversion of 18:2n-6 to 20:4n-6 were of a similar magnitude in the three studies (range, 0.3–2.7%), the percentage conversion in the present study is more than twice the highest values calculated in the previous infant study (1.1%). The present study predicted n-6 FA intake and turnover values consistent with plasma concentrations of each FA and the two previous analyses did not attempt to address this point in their analyses. This is significant since results from the present study form a basis on which to hypothesize the effects of feeding a particular infant formulation on maintenance of plasma FA homeostasis. The current study also evaluated flux of each n-6 FA as an independent metabolite as well as a specific portion of the pathway, a technique that is valuable when evaluating various components of an entire system. The study also had the unique capability of isolating and comparing values of intermediate steps, such as in the conversion of 20:3n-6 to 20:4n-6.

In conclusion, the high rate of turnover of 18:2n-6 observed here may reflect low intake volumes during the very early postnatal period and as infants become adequately adjusted to nursing with the increased availability of energy-rich lipids this value may decrease. The percent conversion values of 18:2n-6 to 20:4n-6 in the current model and those in 3-wk-old infants were of a similar magnitude. However, such rates of 20:4n-6 synthesis are incapable of sustaining plasma 20:4n-6 concentrations in nearly all of these subjects necessitating an

intake of ~4 mg/kg/d from either human milk or a supplement.

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