n-6 fatty acid metabolism in the newborn infant: is linoleic acid sufficient to meet the demand for arachidonic acid?

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Abstract: *Two compartmental models were developed to assess the contributions of linoleic acid, 18:2n-6, and di-homo-g-linoelic acid, 20:3n-6, toward maintaining plasma homeostasis concentrations of arachidonic acid, 20:4n-6, in newborn infants. 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^{-1}, respectively). Rate constant coefficients of n-6 FAs were determined from the time-course concentrations of labeled-FAs and endogenous plasma n-6 FA values were used to approximate steady state concentrations. Eight percent (range: 2-21%) of plasma ^{13}C-U-18:2n-6 was utilized for synthesis of ^{13}C -18:3n-6, -20:2n-6 and -20:3n-6 and 70% 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 only 26%. Turnover of 18:2n-6 in subjects and of 20:4n-6 in plasma was 4.2 g kg^{-1} d^{-1} (CV: 0.58) and 4.3 mg kg^{-1} d^{-1} (CV: 0.81), respectively. Intake of 18:2n-6 and 20:4n-6 were estimated to be 3.0 g kg^{-1} d^{-1} (± 1.7) and 2.8 mg kg^{-1} d^{-1} (± 2.2), respectively. Infants required additional 18:2n-6 (1.2 g kg^{-1} d^{-1}) above predicted intake amounts to maintain plasma concentrations of 18:2n-6. The percent conversion of 18:2n-6 to 20:4n-6 was incapable of sustaining plasma 20:4n-6 concentrations in nearly all subjects necessitating a supplemental intake of ~ 4 mg kg^{-1} d^{-1} of 20:4n-6.*

Key words: *infants, fatty acid metabolism, linoleic acid, compartmental model, kinetics, arachidonic acid, isotope tracer*

Introduction

As it has become increasingly accepted by nutritionists that formulas containing the long chain polyunsaturated fatty acids (PUFA), 20:4n-6 and 22:6n-3, benefit early development in infants then guidelines are needed for determining what quantities of these fatty acids are required in the diet daily to meet the metabolic demands of newborns [1, 2]. To this end, two compartmental models were developed using isotope tracer data to assess the contributions of both 18:2n-6 and 20:3n-6 toward maintaining plasma concentrations of 20:4n-6 in gestational age-appropriate newborn infants during the first week of life.

During the early postnatal period placental transfer of nutrients ceases and infants rapidly develop an increasing capacity to nurse. Body weight decreases (typically, infants loose 5-10% body weight during the first week of life) and body fat reserves are recruited to meet the demand for energy. Consequently, the balance of energy equilibrium shifts during the early postnatal period which is likely to have an impact on lipid metabolism until intake volumes become established.

Two independent compartmental models were developed from the plasma masses of endogenous n-6 FAs 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) FAs using the WinSAAM (Windows Simulation, and Analysis Modeling) program. The n-6 FA kinetic parameters were determined for each subject and mean values were calculated for the cohort. 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

Subject characteristics and clinical procedures

A description of the subject characteristics and clinical procedures may be found elsewhere [3] but are briefly outlined here. *Table 1* gives a brief description of subject data and feeding regimen. Infants (n = 10), with gestational ages greater than 34 wk were accepted into the protocol after receiving informed consent from the mothers and admitted to the Hospital Sótero del Rio and Clínica Presbiteriana Madre-Hijo in Santiago, Chile. 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^{-1}) but devoid of 20:3n-6 and 20:4n-6. Infants were nursed and/or fed expressed breast milk when available, and/or Similac® infant formula upon demand. The quantity of expressed milk and the amount of formula consumed were determined for each subject. Subjects received a mixture of ^{13}C-U-18:2n-6 (100 mg kg^{-1}) and deuterated $^{2}H_5$-20:3n-6 (2 mg kg^{-1}). 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 the 4th and 7th d after dosing. Plasma was separated by centrifugation and frozen at – 80 °C.

Stable isotopes

Carbon-13-uniformly-labeled linoleate (^{13}C-U-18:2n-6, ^{13}C > 95%) and deuterium labeled di-homo-γ-linolenate (19, 19, 20, 20, 20-$^{2}H_5$-20:3n-6, ^{2}H > 95%) ethyl esters were greater than 95% chemical purity (Cambridge Isotope Laboratories, Andover, MA).

Lipid extraction and analytical procedures

A complete description of the lipid extraction procedures and gas chromatography (GC) and GC-mass spectrometry (MS) conditions may be found elsewhere [4]. Plasma lipids were extracted using a modified Folch procedure [5]. Plasma lipids were analyzed as their methyl esters by GC analysis on a polar capillary column with flame ionization detection. Fatty acids were also derivatized to their Pentafluorobenzyl esters and analyzed using negative chemical ionization GC-MS analysis.

doi: 10.1684/ocl.2007.0115

Table 1. *Subject description and feedind intake data.*

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

Abbreviations. ID (patient ID): BW (body weight): GA (gestional age).

Compartmental models

The compartmental models were developed based on the existing knowledge of fat absorption, n-6 FA metabolism, and circulation of lipids in blood. Two independent compartmental models of n-6 FA metabolism *(figures 1* and *2)* were developed using the concentration time-courses of the labeled-FAs and concentrations of endogenous FA in plasma 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. 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 ($R_{I,J}$) *(table 2)* from substrate-compartment J to product-compartment I is obtained by multiplying the mass (M_J) *(table 3)* of endogenous FA in compartment J by $L_{I,J}$ and is given in μg h^{-1}. The percentage of isotope transferred from J to I is given as $P_{I,J}$ *(table 4)* and is a percent 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 compartmental model for 18:2n-6 consisted of six compartments *(figure 1)*. 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. Arrows connecting the six compartments indicate flow along the path. 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.

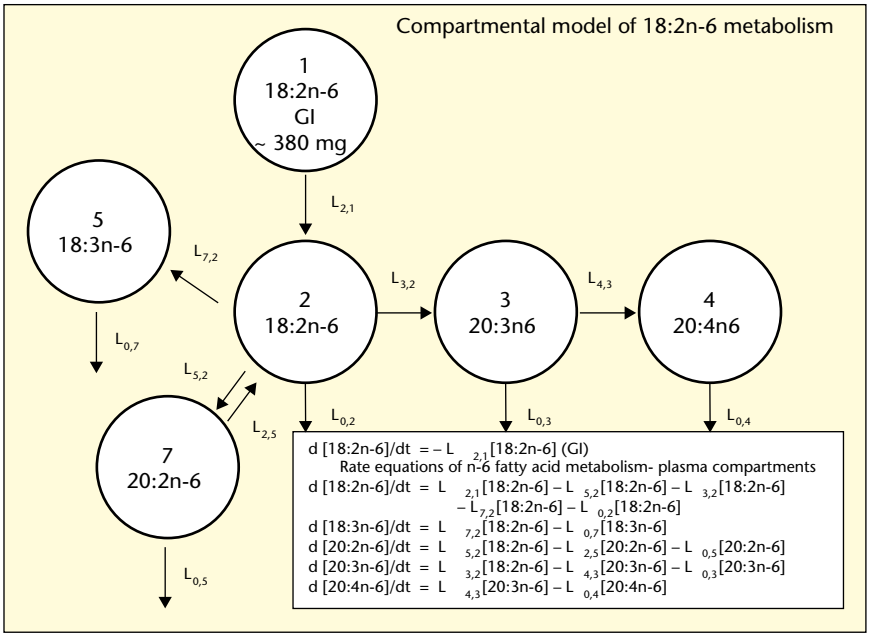

Figure 1. *Diagram of compartmental model for 18:2n-6 metabolism. Open circles represent plasma and gastrointestinal (GI) compartments for the n-6 fatty acids. L(j) values represent kinetic constants that were determined from the model calculations. $L_{0,j}$ values indicate loss of isotope from the pathway. In the boxed area are the differential equations which pertain to appearance and disappearance of isotope in the various compartments.*

Constraints and limits

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 3)* and these values were held constant. For purposes of estimating a daily n-6 FA intake for each subject, the FA content of the infant formula, availability of breast milk, and frequency of feeding were entered into the model *(table 1)*. To determine differences between the efficacy of the two precursors (18:2n-6 and 20:3n-6) toward synthesis of 20:4n-6, a paired t-test analysis was performed on values of the rate parameters using each subject as its own control. A p-value of .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 non-linear least squares routine. The error model included

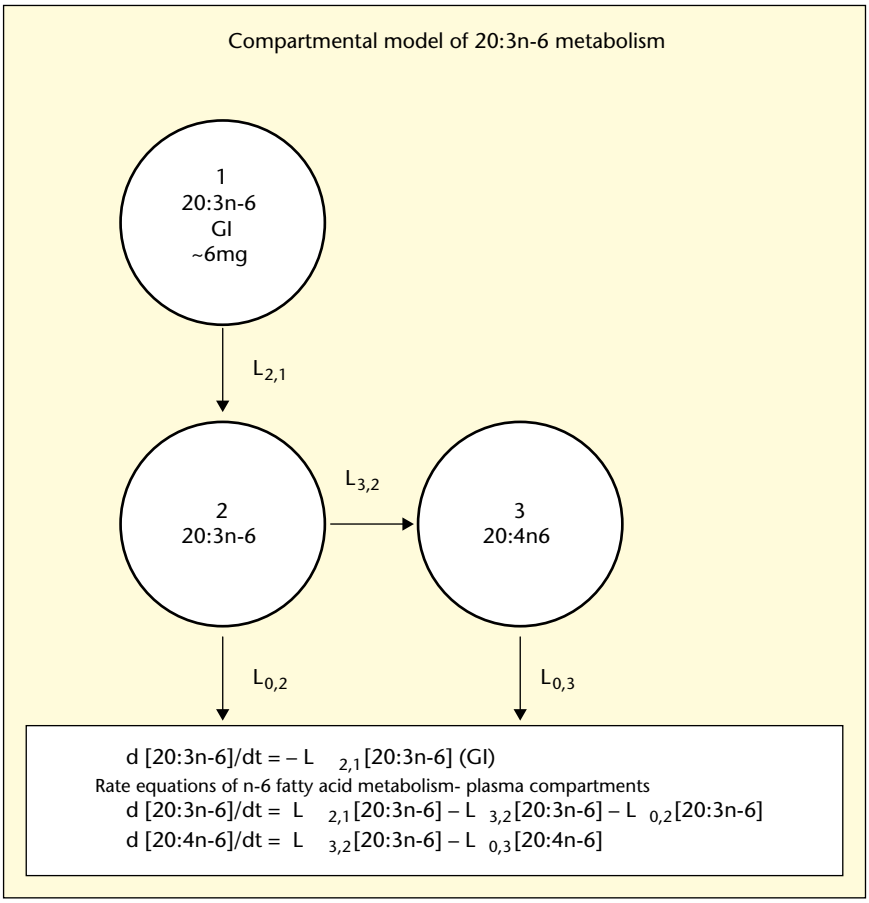

Figure 2. *Diagram of compartmental model for 20:3n-6 metabolism. Open circles represent plasma and gastrointestinal (GI) compartments for the n-6 fatty acids. L(j) values represent kinetic constants that were determined from the model calculations. In the boxed area are the differential equations which pertain to appearance and disappearance of isotope in the various compartments.*

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 standard deviation of 0.1 to each measurement. Daily dietary n-6 FA intake values (U_j) *(table 5)* 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.

Results and discussion

Ten (8 male and 2 female) infants completed the protocol. Most received supplemental feeding with breast milk and/or infant formula in increasing volume during the study. Two 18:2n-6 compartments, one for the isotope administration (GI) and the second for the appearance of the FA in the plasma were incorporated into the model *(figure 1)*. Approximately 94% of labeled-18:2n-6 ethyl ester was absorbed (range: 89-99%) based on the amount of isotope recovered from the feces over 48 h. Using the area under the curve calculation (AUC), the mean value of ^{13}C-U-18:2n-6 (± SD) appearing in the plasma was 254.5 ± 58.5 nmol·mL^{-1} h. The mean AUC value for $^{2}H_5$-20:3n-6 (AUC ± SD) appearing in the plasma was 8.5 ± 3.9 nmol·mL^{-1} h.

The synthetic and utilization rates, $R_{X,J}$, *(table 2)* represent the total mass of each n-6 FA that exit the substrate compartment J and is either transferred to product compartment I or leaves the pathway (0) (but not necessarily the system). The mean value for turnover of 18:2n-6 through the system was 4.2 g kg^{-1} d^{-1} (CV: 0.58) and the mean turnover of 18:2n-6 in the plasma ($R_{0,2}$) was 43 mg d^{-1} (CV 0.65) for the group. The high turnover rate may be associated with the very early postnatal period and as the intake of breast milk and/or formula increases this value may moderate reflecting the change in the lipid composition of the diet [6]. However, consistent with the present findings a high fractional turnover of 18:2n-6 (mean value 93.7% d^{-1}) was also observed in adult male subjects [7]. The mean daily turnover in mg d^{-1} of the other n-6 FA in the 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^{-1} or 0.94 mg d^{-1}

Table 2. *Synthetic and disappearance rates for n-6 fatty acids in plasma.*

Disappearances and synthetic rates	µg hr^{-1}												
	82	**83**	**84**	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**Mean**	**SD**	**cv**
$R_{0,1}$	399340	1010500	194200	268610	71826	149890	274010	117800	1432300	27594	394607	229863	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		22	0.73
$R_{0,4}$	99.3	212	210	2120	507	68.4	662	0	146	211		315	0.74

Ri,j values are disappearance and synthetic rates using each subject's n-6 fatty acid kinetic constants and steady state masses (M_i). For example, $R_{0,2}$ represents the amount of 18:2n-6 that exits the plasma and $R_{0,1}$ is the amount of 18:2n-6 passing trough the system.

Table 3. *Total plasma fatty acids.*

Plasma fatty acids (μg)												
compartment/n-6 fatty acid	**Subject ID**											
	82	**83**	**84**	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**Mean**	**SD**
M_2/18:2	16136	29212	19902	11381	17700	9768	20493	10025	29439	28913	19287	3899
M_3/2O:3	3536	3424	2733	3666	1891	2057	3205	2393	5243	5369	3359	599
M_4/2O:4	16829	16664	9122	14285	14283	8367	17419	10957	29521	24377	16182	3303
M_7/20:2	548	393	752	nd	747	1625	645	936	590	1617	873	325
M_5/18:3	485	309	212	288	241	399	517	141	757	310	366	90

Plasma fatty acids determined by gas chromatography analysis. Values expressed in micrograms of total plasma volume.

Table 4. *Percent of labeled fatty acids transferred through compartments.*

value *100%													
% flux	**Subject ID**												
	82	**83**	**84**	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**Mean**	**SD**	**cv**
$P_{2,1}$ *18:2n-6*	0.002	0.001	0.004	0.002	0.002	0.002	0.022	0.014	0.004	0.001	0.005	0.002	0.38
$P_{3,2}$ *LNA ->20:3n-6*	0.008	0.003	0.007	0.029	0.133	0.011	0.003	0.010	0.001	0.177	0.038	0.033	0.85
$P_{7,2}$ *LNA ->20:2n-6*	0.014	0.004	0.005	0.009	0.055	0.007	0.008	0.002	0.003	0.018	0.012	0,008	0.68
$P_{5,2}$ *LNA ->1B:3n-6*	0.019	0.003	0.010	0.120	0.072	0.015	0.009	0.002	0.007	0.016	0.027	0.020	0.74
$P_{4,3}$ *20:3n-6 ->20:4n-6*	0.943	0.761	0.278	0.016	0.824	0.400	0.910	1.010	0.968	1.017	0.713	0.184	.26

Percent transfer is defined as the percentage of labeled fatty acid that remains in the system and is transferred between two compartments. Thus $P_{(3,2)}$ represents the percent of labeled 18:2n-6 transfered to 20:3n-6 in the scheme given in *figure 1*.

Table 5. *Predicted daily fatty acid intake amounts.*

	μg hr^{-1}												
Compartment/ Fatty acid	**82**	**83**	**84**	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**Mean**	**SD**	**cv**
U_2/18:2	285686	722857	139271	192214	51394	107307	200129	85336	1027571	151129	296289	160108	0.57
U_3/20:3	6	11	54	98	12	16	34	32	33	68	36	15	0.40
U_4/20:3	53	137	133	1513	338	41	410	311	50	111	310	221	0.91
U_5/20:2	6	1	16	145	52	102	19	159	3	65	57	30	0.83
U_7/18:3	0	1	13	17	2	5	1	4	1	5	5	3	0.57

Predicted daily values were determined from each subject's feeding regimen as describted in *table 1* trought the 168 hr period. Values are expressed in micrograms per hour.

(CV 0.36) from the ^{13}C-FA and 53 μg h^{-1} (CV 0.48) from the ^{2}H-FA.

The proportion of the plasma n-6 FA $P_{I,J}$ directed towards biosynthesis was determined and these values are given in *table 4*. On average about 0.5% of the administered dose of ^{13}C-18:2n-6 and 0.3% of $^{2}H_5$-20:3n-6 appeared in the plasma ($P_{2,1}$). 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 of ^{13}C-20:4n-6 was 71% (CV 0.26) *(table 4)*. This contrasts with a much smaller value (26%, $p < .02$) of $^{2}H_5$-20:3n-6 destined for the synthesis of $^{2}H_5$-20:4n-6 (CV 0.56) (data not shown). This suggests that that the preferred substrate for 20:4n-6 biosynthesis is 20:3n-6 arising from 18:2n-6. However, when taking into consideration the percentage of each labeled substrate appearing in the plasma, and the overall percent conversion of each precursor to 20:4n-6, then dietary 20:3n-6, as measured by $^{2}H_5$-20:3n-6 affords approximately a 6-fold greater delivery of 20:4n-6 compared to 18:2n-6 as measured by ^{13}C-18:2n-6. Sauerwald *et al.*, estimated that the fractional rate of conversion (FRC) of 18:2n-6 to 20:4n-6 (FRC is identical to the P-value used here) was between 0.4-1.1% in 3 wk-old infants and these values depended on the α-linolenic acid content of the formula [8]. In the present study, the net mean FRC for conversion of 18:2n-6 to 20:4n-6 was 2.7% in these newborns.

Using the appropriate feeding regimen for each subject *(table 1)*, intake values for 18:2n-6, 20:2n-6, 18:3n-6, 20:3n-6 and 20:4n-6 were calculated *(table 5)* that were consistent with each FA's synthetic and disappearance rates and total plasma concentration *(table 2)*. The daily mean (± SD) intake of

18:2n-6 and 20:4n-6 were calculated to be 3.0 (± 1.8) g kg^{-1} d^{-1} and 2.8 (± 2.4) mg $kg^{-1}d^{-1}$, respectively.

The compartmental model for 18:2n-6 predicted an 18:2n-6 intake amount of 3.0 g kg^{-1} d^{-1} (CV 0.42) with a turnover rate through the system of 4.2 g kg^{-1} d^{-1} (CV 0.58) for these subjects which is consistent with the plasma concentration of 18:2n-6. This is significant since results arising from this study form a basis on which to determine the effects of feeding a particular infant formulation on maintenance of plasma fatty acid homeostasis. The study also has the unique capability of isolating and comparing values of intermediate steps, such as in the conversion of 20:3n-6 to 20:4n-6. However, certain precautions should be considered before the current compartmental model can be successfully adapted for the determination of dietary requirements of 18:2n-6 in infants. The high rate of turnover of 18:2n-6 observed here may only be relevant to the very early postnatal period reflecting a high demand for 18:2n-6 as an energy resource. Since, during the first few days after birth intake volumes were low, then it is likely that body lipid stores supplied the remainder of the 18:2n-6 as the steady state plasma concentrations did not decrease. As infants become adjusted to nursing with increased availability of energy-rich lipids (including medium chain triglycerides) this value may decrease. The values determined for the percent conversion of 18:2n-6 to 20:4n-6 in the current model were of a similar magnitude to those observed in 3 wk old infants. Yet these rates of 20:4n-6 synthesis are incapable of sustaining plasma 20:4n-6 concentrations and an intake of approximately 4 mg kg^{-1} d^{-1} is needed to meet this demand, an amount that is only a fraction of that which is available from human milk.

Acknowledgements. *The author acknowledges several collaborators involved in this study. Ricardo Uauy, Institute of Nutrition and Food Technology (INTA), Santiago, Chile, and Norman Salem Jr., Laboratory of Membrane Biochemistry and Biophysics, NIAAA, NIH were co-principal investigators and responsible for the study design. Adolfo Llanos and Patricia Mena were responsible for clinical exclusions, subject care and in specimen collection, Neonatology Unit, Hospital Sótero del Rio, Santiago, Chile. Yuhong Lin, Laboratory of Membrane Biochemistry and Biophysics, NIAAA, NIH had overall responsibility for quality control and preparation of isotopic labeled materials, analyses of plasma fatty acids and management of the data base.*

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