Effect of Dietary α-Linolenic Acid Intake on Incorporation of Docosahexaenoic and Arachidonic Acids into Plasma Phospholipids of Term Infants

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ABSTRACT: The fractional conversion rates of plasma phospholipid α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) to docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6), respectively, and the fractional rates of incorporation of 22:6n-3 and 20:4n-6 into plasma phospholipids were determined in 27 healthy 3-wk-old term infants who had received formulas with ~16% of fat as 18:2n-6 and 0.4% (n = 6), 1.0% (n = 11), or 3.2% (n = 10) as 18:3n-3 from birth. The infants were given a single dose of both [U-^{13}C]18:2n-6 and [U-^{13}C]18:3n-3 with a feeding, and blood samples were collected 8, 12, and 24 h afterward for determination of the isotopic enrichments of the [M + 18] isotopomers of plasma phospholipid fatty acids by negative chemical ionization gas chromatography/mass spectrometry. A simple precursor/product compartmental model was used to estimate fractional rates of conversion and incorporation. All infants converted 18:3n-3 to 22:6n-3 and 18:2n-6 to 20:4n-6. Although the fractional rate of conversion of 18:3n-3 to 22:6n-3 did not differ among groups, the fractional rate of incorporation of 22:6n-3 into the plasma phospholipid fraction was greater in infants who received 3.2% vs. 0.4% or 1.0% 18:3n-3 (4.1 ± 2.2 vs. 1.6 ± 1.5 or 2.0 ± 1.0% of the plasma phospholipid 22:6n-3 pool daily). The fractional rate of conversion of 18:2n-6 to 20:4n-6 was less in infants who received the 3.2% 18:3n-3 intake (0.4 ± 0.3% of the plasma phospholipid 18:2n-6 pool daily vs. 1.1 ± 0.7% and 0.8 ± 0.5% in those who received 0.4 and 1.0% 18:3n-3, respectively). The fractional rate of incorporation of 20:4n-6 into plasma phospholipid also was less in the 3.2% vs. the 0.4 and 1.0% 18:3n-3 groups (2.7 ± 1.4% vs. 5.9 ± 2.6 and 4.4 ± 1.7%, respectively, of the plasma phospholipid 20:4n-6 pool daily).
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The consistent finding of higher docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) levels in plasma and tissue lipid fractions of infants fed either human milk, which contains these fatty acids, or formulas supplemented with these fatty acids vs. formulas containing only their precursors is frequently interpreted as evidence that infants either cannot convert the precursor essential fatty acids [α-linolenic (18:3n-3) and linoleic acid (18:2n-6)], respectively, to 22:6n-3 and 20:4n-6 or have limited capacities to do so. Recent studies have shown that both term and preterm infants can convert precursors to products (1,2), but whether conversion is adequate to meet the considerable needs of 22:6n-3 and 20:4n-6 by developing tissues remains unknown. Further, while a high intake of either 18:3n-3 or 18:2n-6 relative to the other is known to inhibit desaturation and elongation of the other (3,4), little quantitative information about this issue is available. Recently, we studied effects of 18:3n-3 intake, or the intake ratio of 18:2n-6/18:3n-3, on plasma and erythrocyte fatty acid patterns, growth and visual development of term (5) and preterm infants (6). In a subset of these infants, we also attempted to quantitate fractional rates of conversion of 18:3n-3 to 22:6n-3 and 18:2n-6 to 20:4n-6 as well as fractional rates of incorporation of 22:6n-3 and 20:4n-6 into the plasma phospholipid fraction. Calculated fractional rates of conversion and incorporation of this subset of 3-wk-old term infants who had received 18:2n-6 intakes of 16% of total fat and 18:3n-3 intakes of 0.4, 1, or 3.2% of total fat from birth are reported here.

MATERIALS AND METHODS

Subjects. Twenty-seven term neonates participating in the previously mentioned study (5) and assigned at birth to receive a formula with ~16% of fat as 18:2n-6 and 0.4% (group A, n = 6), 1.0% (group B, n = 11), or 3.2% as 18:3n-3 (group C, n = 10) were selected randomly at the time of initial enrollment to participate in the studies reported here. The three groups were comparable with respect to gestational age, birth weight, head circumference, gender, and ethnic background, as well as age and weight at the time of study. Except for 18:3n-3 content, nutrient contents of the formulas were identical and similar to those of Enfamil® (Mead-Johnson Nutritionals, Evansville, IN), i.e., 9% of energy as protein, 41% as lactose, and 50% as fat. The study was approved by the Institutional Review Boards of Baylor College of Medicine (Houston, TX) and Harris County Hospital District (Houston, TX). Written, informed parental consent was obtained for all infants enrolled.

At 3 wk of age, each infant was given from 25–40 mg/kg of [U-^{13}C]18:2n-6 and 20–25 mg/kg of [U-^{13}C]18:3n-3

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methyl esters (95–99% chemical purity, 99% isotopic purity; Martek Biosciences Corp., Columbia, MD). Labeled fatty acids were added to a small aliquot of formula, the mixture was sonicated for 60 min, and the sonicated mixture with labeled fatty acids intact (based on periodic assessments) was fed to the infant immediately by nipple. To minimize loss of labeled fatty acids, additional aliquots of formula were added to the sonication vessel before being fed to the infant.

Blood samples were collected in EDTA tubes 8, 12, and 24 h after administration of the labeled fatty acids; plasma and erythrocytes were separated immediately and stored at −70°C until processed further. Plasma phospholipids were separated as described by Hamilton and Comai (7), and fatty acids of this fraction were derivatized to pentafluorobenzyl esters (8) using 1 mL of 0.1M tetrabutylammonium phosphate as buffer and 400 μL of 0.13 M α-bromo-2,3,4,5,6-pentafluorotoluene in methylene chloride as reagent (80°C for 1 h).

Enrichment of the [M + 18] isotopomers of the pentaflurobenzyl esters was determined by negative chemical ionization gas chromatography/mass spectrometry (Hewlett-Packard 5989 mass spectrometer equipped with a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA), using selected ion monitoring. Samples were eluted on capillary columns, and chromatographic peaks were identified both by comparison of retention times to those of fatty acid standards (Sigma Chemical Co., St. Louis, MO) and by mass determination. Isotopic enrichments (precision, 1–5%) were expressed as tracer/tracee ratios.

A simple precursor/product compartmental model was used to quantitate fractional rates of conversion of plasma phospholipid 18:3n-3 and 18:2n-6, respectively, to 22:6n-3 and 20:4n-6 and fractional rates of incorporation of 22:6n-3 and 20:4n-6 into the plasma phospholipid fraction. Parameters of the compartmental model were computed with the Simulation Analysis and Modeling program (SAAM; University of Washington, Seattle, WA).

In this model (Fig. 1), compartments M_1 and M_2 represent relative concentrations of precursor and product fatty acids in the plasma phospholipid fraction as determined by conventional gas chromatography (9). The decline in precursor enrichment (Fig. 2) secondary to some combination of exchange, oxidation, and/or storage is expressed by the rate constant $k_{0,1}$; that secondary to conversion of the precursor to the longer-chain, more unsaturated product(s) is expressed by the rate constant $k_{2,1}$. Thus, the fractional rate of conversion of the precursor can be calculated as: $k_{2,1}/(k_{0,1} + k_{2,1}) \times 100$. Since the phospholipid content of neither the precursor nor product changes appreciably over the period of study, it is assumed that the fractional rate of incorporation of the product into the plasma phospholipid fraction is equal to its fractional catabolic rate, expressed by the rate constant $k_{0,2}$. The fractional rate of incorporation, of course, is related to the fractional rate of synthesis of the product. All rate constants can be derived from the exponential curves of the decline in precursor enrichment and the increase in product enrichment observed in each infant over the 24-h period of study (Fig. 2). Because behavior of tracer is assumed to be the same as for tracee, the rate constants calculated from changes in enrichment of the labeled precursors and products are assumed to be the same for the total precursor and product pools.

Statistically significant differences among groups ($P < 0.05$) in fractional rates of conversion of precursor to product and incorporation of product into the plasma phospholipid fraction were detected by analysis of variance followed, if indi-

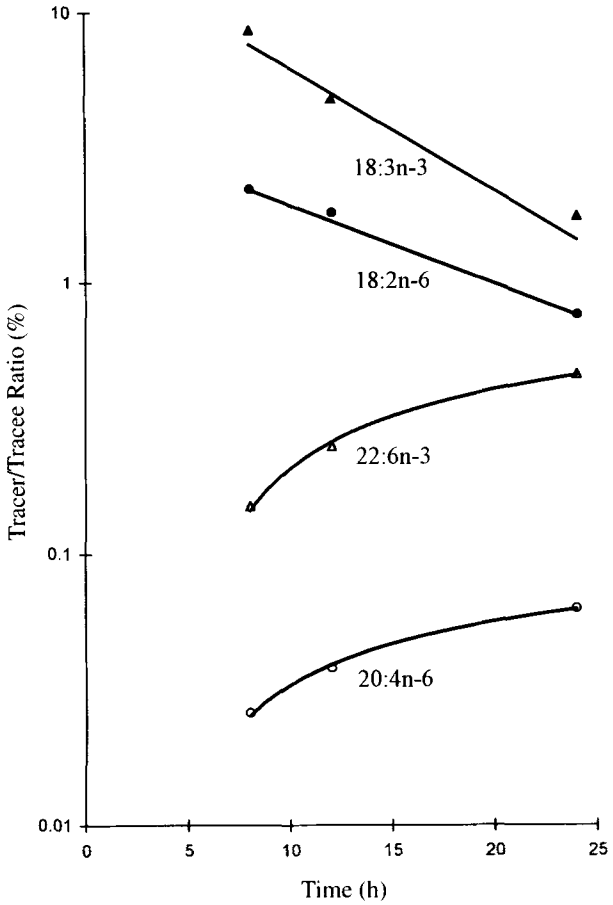

FIG. 2. Tracer/tracee ratios (specific activity) of precursors (18:3n-3 and 18:2n-6) and products (22:6n-3 and 20:4n-6) in plasma phospholipids of a representative three-week-old infant 8, 12, and 24 h after administration of [U-^{13}C]18:2n-6 and [U-^{13}C]18:3n-3 methyl esters (note semilogarithmic scale).

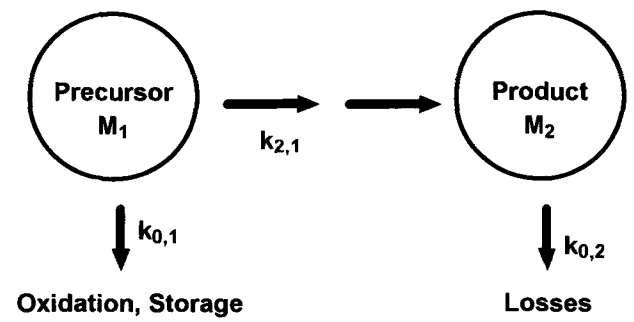

FIG. 1. Precursor/product compartmental model. M_1 and M_2 represent the plasma phospholipid precursor and product pools, respectively. Fractional catabolic rate of the precursor = $k_{0,1} + k_{2,1}$; fractional catabolic rate of the product = fractional rate of incorporation of the product = $k_{0,2}$; fractional conversion rate of the precursor = $k_{2,1}/(k_{0,1} + k_{2,1}) \times 100$.

cated, by a multiple comparison procedure (Fisher's Least Significant Difference).

RESULTS

Figure 2 shows the decline in enrichment of [M + 18] isotopomers of precursors, 18:3n-3 and 18:2n-6, and the increase in enrichment of [M + 18] isotopomers of 22:6n-3 and 20:4n-6 observed in a representative infant. Enrichments of [M + 18] isotopomers of n-3 fatty acids observed in the plasma phospholipid fraction of all infants 8, 12, and 24 h after administration of the labeled precursor are shown in Table 1. Those of the n-6 fatty acids at the same times are shown in Table 2. From these data, it is clear that all infants converted 18:3n-3 to 22:6n-3 and 18:2n-6 to 20:4n-6; however, there was considerable variability within as well as among groups. This variability is not surprising; the precursor enrichment is a function of the dose of precursor administered, the rate and extent of its absorption, as well as the rate and extent of its metabolism and incorporation into the plasma phospholipid fraction, all of which, in turn, affect enrichment of the products. Thus, to quantitate rates of conversion and incorporation, it is necessary to consider, simultaneously, enrichments of both precursors and products, including rates of change in enrichments of each.

TABLE 1
Isotopic Enrichments (% tracer/tracee) of n-3 Fatty Acids

	18:3n-3			22:6n-3		
	8 h	12 h	24 h	8 h	12 h	24 h
Group A						
1	28.18	19.88	4.41	0.09	0.15	0.27
2	4.95	9.95	3.43	0.02	0.05	0.10
3	18.61	12.26	3.34	0.03	0.06	0.14
4	23.48	15.75	4.20	0.29	0.43	0.96
5	50.23	22.37	6.80	0.06	0.12	0.24
6	39.31	26.49	6.51	0.09	0.18	0.40
Group B						
1	53.85	29.87	12.73	0.05	0.14	0.20
2	41.98	26.49	5.98	0.07	0.11	0.36
3	24.57	23.81	7.82	0.05	0.11	0.24
4	19.34	18.58	6.56	n.d.[a]	0.29	0.72
5	26.41	17.03	n.d.	0.16	0.36	n.d.
6	29.65	12.24	2.76	0.10	0.19	0.43
7	13.55	12.51	2.58	0.04	0.13	0.23
8	14.97	10.42	9.62	0.15	0.34	0.28
9	13.19	52.87	3.70	0.17	0.28	0.40
10	26.90	22.91	5.95	0.06	0.16	0.29
11	11.29	9.47	1.99	0.06	0.13	0.25
Group C						
1	6.80	4.83	2.36	0.05	0.14	0.24
2	1.15	0.95	0.39	0.003	0.009	0.019
3	8.58	4.78	1.76	0.15	0.25	0.46
4	4.13	2.93	1.26	0.01	0.03	0.09
5	9.47	6.27	2.25	0.05	0.10	0.23
6	7.27	4.61	1.25	0.03	0.06	0.10
7	3.71	2.83	0.81	0.01	0.03	0.08
8	1.83	1.07	0.33	0.03	0.06	0.10
9	1.84	0.94	0.31	0.01	0.02	0.04
10	1.73	1.25	0.47	0.02	0.04	0.10

[a]n.d. = Not detected.

TABLE 2
Isotopic Enrichments (% tracer/tracee) of n-6 Fatty Acids

	18:3n-3			22:6n-3		
	8 h	12 h	24 h	8 h	12 h	24 h
Group A						
1	0.58	0.46	0.20	0.005	0.010	0.015
2	0.21	0.15	0.07	0.002	0.003	0.008
3	0.46	0.48	0.19	0.005	0.014	0.019
4	1.90	1.58	0.75	0.063	0.086	0.108
5	1.80	1.43	0.62	0.038	0.046	0.075
6	0.84	0.91	0.42	0.028	0.049	0.069
Group B						
1	2.66	1.67	1.09	0.017	0.031	0.037
2	3.41	1.63	0.72	0.022	0.030	0.070
3	1.74	1.43	0.63	0.042	n.d.[a]	n.d.
4	2.04	1.61	0.72	0.032	0.070	0.091
5	1.65	1.20	0.56	0.055	0.048	0.105
6	1.60	0.73	0.27	0.016	0.025	0.034
7	0.83	0.68	0.32	n.d.	0.018	0.030
8	0.84	0.98	0.69	0.012	0.042	0.025
9	2.01	1.38	0.62	0.041	0.051	0.057
10	2.00	2.54	1.37	0.033	0.071	0.097
11	1.44	1.32	0.57	0.028	0.046	0.071
Group C						
1	2.04	1.24	0.83	0.012	0.022	0.035
2	1.82	0.25	0.21	0.001	0.003	0.010
3	2.22	1.82	0.76	0.026	0.038	0.063
4	1.57	1.36	0.74	0.005	0.010	0.023
5	3.48	2.64	1.17	0.024	0.039	0.062
6	1.38	1.24	0.57	0.009	0.014	0.020
7	0.45	0.47	0.21	0.002	0.004	0.010
8	0.63	0.47	0.19	0.013	0.021	0.028
9	0.45	0.43	0.17	0.003	0.007	0.012
10	0.34	0.35	0.17	n.d.	0.005	0.008

[a]n.d. = Not detected.

Mean rate constants and fractional rates of conversion and incorporation of the three groups, as calculated from the model using the data summarized in Tables 1 and 2, are shown in Table 3. Overall, the data from all infants fit the model with a residual error of 10% or less. The mean fractional rate of conversion of 18:3n-3 to 22:6n-3 did not differ significantly among groups. However, since the 18:3n-3 content of the plasma phospholipids (M_1) of group C was 3.7-fold larger than that of group A and 3.3-fold larger than that of group B, the fractional rate of incorporation of 22:6n-3 into the plasma phospholipid fraction was significantly higher in group C vs. groups A and B.

The mean fractional rate of conversion of 18:2n-6 to 20:4n-6 was lowest in group C, which received the highest 18:3n-3 intake. The mean fractional rate of incorporation of 20:4n-6 into the plasma phospholipid fraction also was lowest in group C.

DISCUSSION

The data reported above confirm that 3-wk-old term infants can convert 18:3n-3 and 18:2n-6, respectively, to 22:6n-3 and 20:4n-6. They also provide quantitative estimates of the effect of the dietary intake of 18:3n-3 on fractional rates of conversion of 18:3n-3 to 22:6n-3 and 18:2n-6 to 20:4n-6, as well as the fractional rates of incorporation of 22:6n-3 and 20:4n-6

TABLE 3
Parameters (mean ± SD) of the Precursor/Product Compartmental Model

	Group A 0.4% 18:3n-3 (n = 6)	Group B 1.0% 18:3n-3 (n = 11)	Group C 3.2% 18:3n-3 (n = 10)
n-3 Fatty acids			
M_1 (nmol/mL)	3.7 ± 1.2[a]	4.1 ± 1.4[a]	13.7 ± 1.8[b]
M_2 (nmol/mL)	41.5 ± 18.0[a]	64.7 ± 24.6[b]	78.9 ± 35.2[b]
$k_{0,1}$	2.48 ± 0.40[a]	2.33 ± 0.64[a,b]	1.96 ± 0.39[b]
$k_{2,1}$	0.18 ± 0.13[a]	0.32 ± 0.18[b]	0.21 ± 0.10[a,b]
FRC (% M_1/d)[c]	6.92 ± 5.18	12.13 ± 6.91	9.65 ± 3.86
FRI (% M_2/d)[d]	1.57 ± 1.54[a]	1.95 ± 1.01[a]	4.10 ± 2.18[b]
n-6 Fatty acids			
M_1 (nmol/mL)	770.7 ± 134.9[a]	1070.4 ± 90.9[b]	911.4 ± 76.6[a]
M_2 (nmol/mL)	205.4 ± 90.7	281.1 ± 55.8	219.0 ± 96.5
$k_{0,1}$	1.49 ± 0.09	1.63 ± 0.56	1.64 ± 0.60
$k_{2,1}$	0.017 ± 0.010[a]	0.012 ± 0.005[a]	0.006 ± 0.004[b]
FRC (% M_1/d)	1.13 ± 0.71[a]	0.82 ± 0.46[a]	0.41 ± 0.26[b]
FRI (% M_2/d)	5.94 ± 2.12[a]	4.42 ± 1.71[a]	2.66 ± 1.40[b]

[a,b]Values with different superscripts are significantly different ($P < 0.05$) from other values.
[c]FRC = Fractional rate of conversion $(k_{2,1}/(k_{0,1} + k_{2,1}) \times 100$.
[d]FRI = Fractional rate of incorporation $(k_{0,2} \times 100)$.

into the plasma phospholipid fraction. Dietary 18:3n-3 intake did not result in a statistically significant difference in fractional rates of conversion of 18:3n-3 to 22:6n-3. Because the 18:3n-3 content of the plasma phospholipid fraction was highest in the group that received the 3.2% 18:3n-3 intake, however, this group's fractional rate of incorporation of 22:6n-3 into the plasma phospholipid fraction was 2.5-fold greater than that of the group that received only 0.4% of total fat as 18:3n-3. In contrast, the highest vs. the lowest 18:3n-3 intake resulted in a 64% lower fractional rate of conversion of 18:2n-6 to 20:4n-6 and a more than 50% lower fractional rate of incorporation of 20:4n-6 into the plasma phospholipid fraction.

The validity of these conclusions, of course, depends on the validity of the model used to calculate fractional rates of conversion of precursor fatty acids and incorporation of the products into the plasma phospholipid fraction. In addition to the assumptions of steady state and equivalence of labeled and unlabeled pools, the model assumes that mass flow from precursor to product is unidirectional. While none of these assumptions is entirely valid, all are reasonable. More problematic with respect to the physiological relevance of the model are the assumptions that the only source of precursor converted to product is the precursor content of the plasma phospholipid fraction and that the only source of product incorporated into the plasma phospholipid fraction is that converted from the precursor content of this fraction. In addition, since it must be assumed that the plasma phospholipid fraction is part of a central, rapidly miscible pool, it also is assumed that the fatty acid composition of the plasma fraction is representative of all fractions of the central pool. Despite the number of assumptions inherent in the model, all apply equally to each dietary group. Thus, any differences among groups in calculated fractional rates of conversion of precursors to products and/or incorporation of products into the plasma phospholipid fraction are likely to reflect relative differences in fractional rates of conversion and incorporation incident to 18:3n-3 intake.

While the data reported apply strictly to only the plasma phospholipid fraction, it is likely that they are applicable to other fractions as well. For example, in rats, a modest increase in 18:3n-3 intake resulted in a lower 20:4n-6 content in all plasma and tissue lipid fractions (10). Further, in young human adult volunteers, a high intake of 18:2n-6 suppressed incorporation of long-chain polyunsaturated n-3 fatty acids into all plasma lipid fractions (11). Thus, it is very likely that the higher rate of incorporation of 22:6n-3 and the lower rate of incorporation of 20:4n-6 into plasma phospholipids observed in infants who received the highest dietary 18:3n-3 intake occurs also in the plasma triglyceride and cholesterol ester fractions. Interestingly, the mean fractional rate of incorporation of 20:4n-6 into the plasma phospholipid fraction observed in the group of infants who received the lowest 18:3n-3 intake (i.e., 5.9 ± 2.1% SD of the plasma phospholipid 20:4n-6 pool daily) is very similar to "the fractional rate of synthesis" of serum total lipid 20:4n-6 reported by Demmelmair *et al.* (1) in younger infants fed a low 18:3n-3 formula, i.e., 6.2 ± 1.0% SEM of the 20:4n-6 content of the serum total lipid fraction daily.

Unfortunately, neither the data reported here nor elsewhere are sufficient to calculate, or estimate, the amounts of long-chain polyunsaturated fatty acids synthesized daily. With respect to the data reported here, this is largely because there are no data concerning the magnitude of the pool of precursor and product fatty acids of any lipid fraction. This lack of information with respect to the plasma pool size can be remedied, but doing so will not clarify the relationship between plasma and tissue pools. Conversely, it is clear from the data reported here that a dietary 18:3n-3 intake of 3.2 vs. 0.4% of total fat results in a roughly 2.5-fold greater rate of incorporation of 22:6n-3 into the plasma phospholipid fraction and, concurrently, depresses both the rate of conversion of 18:2n-6 to 20:4n-6 and the rate of incorporation of 20:4n-6 into the plasma phospholipid fraction by about the same percentage. These are the first reported data concerning relative rates of conversion of 18:3n-3 and 18:2n-6 to 22:6n-3 and 20:4n-6, respectively, and the relative rates of incorporation of 22:6n-3 and 20:4n-6 into any lipid fraction. Whether these relative rates apply to fractions other than the plasma phospholipid fraction remains to be determined.

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