Significant utilization of dietary arachidonic acid is for brain adrenic acid in baboon neonates

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Abstract Dietary arachidonic acid (20:4n-6) utilization in-vivo for carbon recycling into de-novo lipogenesis and conversion to n-6 long chain polyunsaturates was investigated in baboon neonates using [U-^{13}C]20:4n-6. Neonates consuming a formula typical of human milk received a single oral dose of [^{13}C]arachidonic acid in *sn*-2 position of either triglyceride or phosphatidylcholine at 18–19 days of postnatal life. Neonate brain, retina, liver, and plasma were obtained 10 days later (28–29 days of life). Low isotopic enrichment (0.27–1.0%Total label) was detected in dihomo-γ-linolenic acid (20:3n-6) in all tissues, but label incorporation into saturates or monounsaturates was not detected. In neonate brain and retina, 16% and 11% of total label was recovered in 22:4n-6, respectively. The relative contribution of dietary fatty acids to postnatal brain 22:4n-6 accretion can be estimated for dietary 20:4n-6 and preformed 22:4n-6 as 17% and 8%, respectively, corresponding to efficiencies of 0.48% and 0.54% of dietary levels, respectively. These results demonstrate in term baboon neonates that in vivo *1*) 20:4n-6 was retroconverted to 20:3n-6, *2*) 20:4n-6 did not contribute significantly to de novo lipogenesis of saturates and monounsaturates, and *3*) the preformed 20:4n-6 contribution to brain 22:4n-6 accumulation was quantitatively a significant metabolic fate for dietary 20:4n-6.—Wijendran, V., P. Lawrence, G-Y. Diau, G. Boehm, P. W. Nathanielsz, and J. T. Brenna. **Significant utilization of dietary arachidonic acid is for brain adrenic acid in baboon neonates. *J. Lipid Res.* 2002.** 43: **762–767.**

Supplementary key words infant • primate • stable isotope tracer • carbon recycling • polyunsaturated fatty acids • dihomo-gamma linolenic acid

Arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) are well known to be essential constituents of the central nervous system (CNS) (1, 2). 20:4n-6 also serves as a precursor for series two eicosanoids, which play important roles in the regulation of several physiological processes. 20:4n-6 and 22:6n-3 rapidly accumulate in the CNS during human brain growth spurt, which starts at the beginning of the third trimester of pregnancy and imposes high demands until about 2 years of age in humans (3). Prominent dietary sources of these long chain PUFAs (LCP, C ⩾ 20) for the newborn are preformed 20:4n-6 and 22:6n-3 and their precursor fatty acids linoleic acid (18:2n-6) and linolenic acid (18:3n-3), all of which are present in human milk.

There are at least two other major fates for the dietary essential fatty acids 18:2n-6 and 18:3n-3. They can be oxidized exhaustively as energy substrates (4–6); at least 80% of 18:3n-3 intake was oxidized to CO_2 in young animals (5). They also are subject to partial oxidation to acetate that is subsequently used for de novo synthesis of saturated (SFA) and monounsaturated fatty acids (MUFA), referred to as carbon recycling (7–10). Recent studies have clearly established that carbon recycling into de novo lipogenesis is quantitatively a major pathway for 18:2n-6 and 18:3n-3 utilization in pregnant, fetal, and newborn animals (9, 10).

Similar metabolic fates are in principle available to 20:4n-6. Quantitative information on dietary 20:4n-6 partitioning between total oxidation, oxidative chain shortening, and carbon recycling, or chain elongation/desaturation to 22-carbon n-6 LCP are currently lacking. An early study by Sinclair et al. (11) using radiolabeled 20:4n-6 in rats showed that the major fraction (91%) of the label was in tissue lipid 20:4n-6 pool and a small amount of 20:4n-6 label was incorporated into SFA and MUFA in developing brain lipids at 1–2 days post-dose. In vitro studies using isolated rat hepatocytes and peroxisomes (12–14) showed that 20:4n-6 was β oxidized to shorter chain products and to acetate, which could be recycled. Further, significant retroconversion of 20:4n-6 to dihomo-γ-linolenic acid (20:3n-6) and 18:2n-6 was demonstrated in vitro in skin fibroblast cells in culture (15, 16) and isolated rat hepato-

Abbreviations: CNS, central nervous system; FAME, fatty acid methyl ester; LCP, long chain PUFA; MUFA, monounsaturated fatty acids; PC-20:4*, phosphatidylcholine with 20:4* in the *sn*-2 position and palmitate in the *sn*-1 position; SFA, saturated fatty acids; TG-20:4*, triglyceride with 20:4* in the *sn*-2 position and palmitate in the *sn*-1, 3 positions; 20:4*, [^{13}C]arachidonic acid.

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cytes (17). Quantitative information on 22:6n-3 retroconversion (18) and carbon recycling into de novo lipogenesis (9) in vivo has been better characterized.

A potentially important aspect of 20:4n-6 metabolism in vivo is its function as the immediate precursor for adrenic acid (22:4n-6). 22:4n-6 is the third most abundant PUFA (C ⩾ 18) in the brain and is particularly enriched in myelin lipids (1, 19, 20). Rapid accumulation of 22:4n-6 in brain, comparable to 20:4n-6 accretion levels, occurs during the early post-natal period of brain growth spurt in human infants (1). Human milk contains a small amount (~3 mg/dl) of 22:4n-6 (21). While the specific functions of 22:4n-6 are not yet clear, it is suggested to play an important role in myelination in neural tissues (20, 22), its abnormality is implicated in the pathogenesis of Alzheimer's disease (23), and in vitro evidence indicates that it serves as a substrate for dihomoeicosanoid formation (24, 25). Conversion of 20:4n-6 to 22:4n-6 proceeds via a single chain elongation step, whereas the other n-6 PUFAs (18:2n-6, 18:3n-6, and 20:3n-6) must undergo desaturation and elongation with 20:4n-6 as an intermediate product, hence 20:4n-6 may be an efficient dietary source for 22:4n-6 compared with other n-6 PUFA precursors in vivo. The conversion of 20:4n-6 to 22:4n-6 may represent quantitatively an important pathway of 20:4n-6 utilization in infants to meet the rapid increase of 22:4n-6 in neural tissues during the early post-natal period. To our knowledge, 20:4n-6 contribution to 22:4n-6 in developing CNS has not been reported in any species.

The objective of this study was to investigate the quantitative significance of 20:4n-6 utilization in vivo for carbon recycling into products of de novo lipogenesis, retroconversion to n-6 PUFA precursors, and conversion to n-6 LCP compared with 20:4n-6 accumulation in baboon neonate tissues. Previously, we reported tissue accretion of preformed 20:4n-6 originating as dietary triglyceride or phospholipids in these baboon neonates (26). In the present report we show metabolic partitioning of 20:4n-6 utilization among available fates using a single oral dose of stable isotope tracer [U-^{13}C]20:4n-6 and high precision gas chromatography combustion isotope ratio mass spectrometry (GCC-IRMS).

METHODS

Animals and diet

Pregnant baboons were bred at the Southwest Foundation for Biomedical Research (San Antonio, Texas). After confirmation of pregnancy they were transported to Cornell University (Ithaca, NY). The Cornell Institutional Animal Care and Use Committee approved the care of animals and study protocol. Details on the care of pregnant baboons have been described previously (27). Neonates were delivered by Cesarean-section under halothane anesthesia at estimated conceptual ages of 176 to 178 dGa. Neonate details are summarized in **Table 1**. They were housed initially in enclosed incubators and transferred after 5 days of age to individual stainless steel cages in a controlled-access nursery where the temperature (28°C) and humidity (50%) were controlled.

TABLE 1. Characteristics of neonate baboons

Gestational age at CS[a] (d)	176 ± 0.75
Birth Weight (g)	894 ± 98
Body weight at sacrifice (g)	1107 ± 131
Weight gain (g)	213 ± 62
Gender	5M, 2 F
Age at dosing (d)	18.5 ± 0.5
Age at sacrifice (d)	28.5 ± 0.5
Time between dosing and sacrifice (d)	10
Dose amount[b] (mg)	9.9 ± 2.5
Organ Weights (g)	
Brain	99.5 ± 11.4
Liver	30.4 ± 5.6

Data expressed as mean ± SD (n = 7).
[a] CS = Cesarean section.
[b] 20:4n-6 concentration (mg) analyzed in the dose orally administered to the neonates.

Neonate baboons consumed a formula containing 20:4n-6 and 22:6n-3, provided by Numico Research Group (Friedrichsdorf, Germany). Formula LCP were derived from microalgal and fungal sources, and the fatty acid composition of the formula has been reported in detail elsewhere (26). Formula fatty acid composition was similar to human milk and contained 0.4 g/dl of 18:2n-6 (14.0% wt), 15.8 mg/dl of 20:4n-6 (0.55% wt), 7 mg/dl of 22:4n-6 (0.25% wt), 46mg/dl of 18:3n-3 (1.6% wt), and 8.6mg/dl of 22:6n-3 (0.30% wt).

Doses and sampling

Labeled dose containing [U-^{13}C]20:4n-6 (20:4*) in triglyceride (TG) or phosphatidylcholine (PC) was chemically synthesized and provided by Roche (Switzerland). The TG tracer (TG-20:4*) contained 20:4* in the *sn*-2 position with unlabeled 16:0 in the remaining positions. PC tracer (PC-20:4*) contained 20:4* in *sn*-2 position with 16:0 in the *sn*-1 position. The TG or the PC dose did not contain any other fatty acids in detectable levels. Either TG-20:4* or PC-20:4* was weighed and sonicated into 2.5 ml warm reconstituted formula with unlabeled PC (soy lecithin, Sigma Chemical, St Louis, MO) and olive oil carriers. A 50 μl sample of the dose was saved for fatty acid analysis. Neonates were randomized to receive either a single bolus dose of TG-20:4* (n = 3) or PC-20:4* (n = 4). Doses along with three rinses were administered orally to the neonates at 18–19 days of age. Neonates were fed their normal formula as usual immediately after oral administration of the labeled dose.

Neonates were euthanized 10 days post dose (28–29 days of age) by exsanguination under halothane anesthesia. Neonate brain, retina, liver, and plasma were collected. Blood was collected with heparin as anticoagulant, and plasma was separated immediately by centrifugation at 4°C and frozen in liquid N_2. The brain and liver tissues were quickly removed, weighed, aliquoted, and immediately frozen in liquid N_2. Retina was collected in saline and immediately frozen in liquid N_2. All samples were stored at −80°C until analysis.

Lipid extraction and analysis

Total lipids were extracted from samples of brain occipital lobe including the visual cortex, right liver lobe, whole retina, and plasma by the Bligh and Dyer method (28). Butylated hydroxytoluene was used as an antioxidant. Fatty acid methyl esters (FAME) were prepared using 14% BF_3 in methanol (29). FAME were analyzed using a Hewlett Packard 5890 series II gas chromatograph-flame ionization detector (GC-FID) with a BPX 70 column (60 m × 0.32 mm I.D. × 0.25 μm film) and H_2 as carrier gas. Quantitative profiles were calculated using the internal standard (17:0) and an equal weight FAME mixture to derive re-

sponse factors for each FA. GC-FID conditions and calibration details have been reported previously (29).

Tracer enrichment analysis was performed using high precision GC-combustion isotope ratio mass spectrometry (GCC-IRMS) described in detail previously (30). Briefly, high precision data are presented as the relative deviation of the sample isotope ratio from the standard Pee Dee Belemnite (PDB) with a $^{13}C/^{12}C$ isotope ratio = R_{PDB} = 0.0112372, as:

$$\delta^{13}C_{PDB} = \left(\frac{R_X - R_{PDB}}{R_{PDB}}\right) \times 1000 = \left(\frac{R_X}{R_{PDB}} - 1\right) \times 1000$$

where $\delta^{13}C_{PDB}$ is referred to in permil (‰) units and X refers to the sample. R_X derived from the above equation was directly converted to atom percent (AP) ^{13}C, which is a measure of the percent of ^{13}C in each fatty acid peak. Atom percent excess (APE), describing the tracer/tracee ratio in each fatty acid (30), was determined by subtracting baseline isotope levels. Baseline isotope ratios were obtained from two undosed control animals. The absolute mass (g or mol) of 20:4* tracer in any sampled pool was calculated by multiplying APE by the concentration of 20:4n-6 determined by GC-FID. Percent of total labeled fatty acids (percent total) was calculated for each labeled fatty acid which reflects the distribution of 20:4* label into fatty acids in a particular pool, after correcting for the ratio of carbon in the analyte fatty acid to that in 20:4*.

We previously reported differences in absolute tissue accretion of 20:4n-6 in these animals from TG or PL doses (26). Distribution of label into tissue fatty acids expressed as percent total label (%Total) is calculated as the percent ratio of the label found in one fatty divided by the sum of all label found in all fatty acids per unit (g, ml) pool. Initial statistical analysis by student's *t*-test ($P < 0.05$) showed no differences in the distribution of label into tissue fatty acids (as percentage of total) between TG- and PC-dosed animals. Therefore, results from TG-20:4n-6* and PC-20:4n-6* dosed neonates are presented here as pooled data (n = 7).

RESULTS

Table 2 presents the results of brain ^{13}C label enrichment of fatty acids in $\delta^{13}C$ and APE units. Tracer incorporation into 16:0, 18:0, 18:1, or 22:6n-3 was not different between baseline control and dosed neonates. In contrast, n-6 LCP were all enriched. The dose fatty acid, 20:4n-6, showed the highest labeling, as expected, while its elongation product 22:4n-6 showed about one third of the APE. Labeling was also found in 20:3n-6 and 22:5n-6. Labeling in retina was similar, as shown in **Table 3**. Labeling was undetectable in the major SFA and MUFA; however, APE was greater in n-6 PUFA. Unlike brain, 22:6n-3 showed slight labeling.

Tables 4 and **5** show labeling data for liver and plasma, respectively. No excess ^{13}C was detected in SFA and MUFA, and label was detected in n-6 PUFA with the same general trend. As in brain, no labeling was found in 22:6n-3. 18:2n-6 was not labeled in any of these tissues. Label distribution into 20:3n-6 was 0.28 ± 0.15%, 1 ± 0.46%, 0.79 ± 0.36%, and 0.27 ± 0.19%Total label in the neonate brain, retina, liver, and plasma respectively, 10 days post-dose.

TABLE 2. Brain ^{13}C label enrichment of fatty acids in neonates after an oral dose of [U-^{13}C]-20:4n-6

	$\delta^{13}C$ (‰)		
	Baseline Controls (n = 2)	Dosed Neonates (n = 7)	APE[a]
SFA-MUFA			
16:0	−20.1 ± 0.7	−20.7 ± 0.8	ND[b]
18:0	−21.8 ± 0.4	−21.7 ± 0.8	ND
18:1	−23.9 ± 0.4	−24.5 ± 0.4	ND
n-6 PUFA			
18:2	−25.0 ± 0.6	−25.4 ± 0.8	ND
20:3	−21.0 ± 0.06	−15.7 ± 3.3	0.0049 ± 0.0037
20:4	−23.4 ± 0.3	138.0 ± 46.6	0.182 ± 0.051
22:4	−21.2 ± 0.1	34.1 ± 17.5	0.066 ± 0.019
22:5	−23.3 ± 0.5	14.3 ± 9.7	0.043 ± 0.012
n-3 PUFA			
22:6n-3	−24.3 ± 0.3	−24.5 ± 0.9	ND

Data presented as mean ± SD.
[a] APE, atom percent excess.
[b] ND, not detectable.

TABLE 3. Retinal ^{13}C label enrichment of fatty acids in neonates after an oral dose of [U-^{13}C]-20:4n-6

	$\delta^{13}C$ (‰)		
	Baseline Controls (n = 2)	Dosed Neonates (n = 7)	APE[a]
SFA-MUFA			
16:0	−19.3 ± 1.4	−17.5 ± 1.9	ND[b]
18:0	−21.4 ± 0.8	−21.1 ± 1.4	ND
18:1	−22.6 ± 0.2	−23.6 ± 3.2	ND
n-6 PUFA			
18:2	−24.6 ± 0.7	−23.8 ± 1.8	ND
20:3	−22.5 ± 1.5	−9.9 ± 7.3	0.016 ± 0.001
20:4	−21.3 ± 0.04	286 ± 76.5	0.343 ± 0.084
22:4	−20.9 ± 1.5	153 ± 33.9	0.197 ± 0.037
22:5	−20.3 ± 0.7	91.8 ± 22.1	0.130 ± 0.024
n-3 PUFA			
22:6n-3	−24.9 ± 0.7	−18.9 ± 4.0	0.0060 ± 0.0039

Data presented as mean ± SD.
[a] APE, atom percent excess.
[b] ND, not detectable.

TABLE 4. Liver ^{13}C label enrichment of fatty acids in neonates after an oral dose of [U-^{13}C]-20:4n-6

	$\delta^{13}C$ (‰)		
	Baseline Controls (n = 2)	Dosed Neonates (n = 7)	APE[a]
SFA-MUFA			
16:0	−20.8 ± 0.9	−19.8 ± 0.8	ND[b]
18:0	−25.4 ± 0.9	−24.6 ± 0.4	ND
18:1	−28.4 ± 0.5	−29.1 ± 0.7	ND
n-6 PUFA			
18:2	−28.1 ± 0.7	−27.5 ± 0.9	ND
20:3	−23.9 ± 0.1	−10.0 ± 2.0	0.015 ± 0.003
20:4	−22.3 ± 0.2	488 ± 88.2	0.464 ± 0.100
22:4	−20.8 ± 0.3	269 ± 62.9	0.327 ± 0.071
22:5	−22.5 ± 0.4	95.3 ± 21.9	0.141 ± 0.014
n-3 PUFA			
22:6n-3	−26.90 ± 0.86	−27.72 ± 1.42	ND

Data presented as mean ± SD.
[a] APE, atom percent excess.
[b] ND, not detectable.

TABLE 5. Plasma ^{13}C label enrichment of fatty acids in neonates after an oral dose of [U-^{13}C]-20:4n-6

	$\delta^{13}C$ (‰)		
	Baseline Controls (n = 2)	Dosed Neonates (n = 7)	APE[a]
SFA-MUFA			
16:0	−21.1 ± 1.2	−20.5 ± 1.1	ND[b]
18:0	−26.0 ± 0.7	−25.6 ± 1.9	ND
18:1	−28.6 ± 0.4	−27.7 ± 1.1	ND
n-6 PUFA			
18:2	−28.3 ± 0.8	−27.3 ± 0.5	ND
20:3	−23.0 ± 0.6	−16.7 ± 1.4	0.0074 ± 0.0027
20:4	−22.7 ± 0.9	449 ± 138	0.515 ± 0.15
22:4	−25.6 ± 0.3	65.6 ± 37.6	0.100 ± 0.049
n-3 PUFA			
22:6n-3	−25.4 ± 0.7	−25.8 ± 0.38	ND

Data presented as mean ± SD.
[a] APE, atom percent excess.
[b] ND, not detectable.

Figure 1 shows the distribution of label in major n-6 LCP in neonate brain, retina, liver, and plasma as a %Total detected in each respective tissue 10 days post-dose. The distribution in the brain and retina are similar to one another but differ from liver. In brain, about 79% of the label appeared in 20:4n-6, with 16% present in 22:4n-6; similarly, in retina 79% of label appeared in 20:4, with 11% recovered as 22:4n-6. In contrast, liver 20:4n-6 was 92%Total, and only 5% in 22:4n-6. Plasma ^{13}C collected at 10 days post-dose was even richer in 20:4n-6 at 97%Total and only 2%Total in 22:4n-6. Label appearance in 22:5n-6 ranged from 2–4%Total label in the neonate tissues.

DISCUSSION

The present study investigated preformed 20:4n-6 utilization and its metabolism in vivo in baboon neonates using a single oral dose of U-^{13}C-labeled 20:4n-6. The baboon neonate, with brain growth pattern comparable to humans (31), is a highly relevant model for 20:4n-6 utilization during human brain growth spurt.

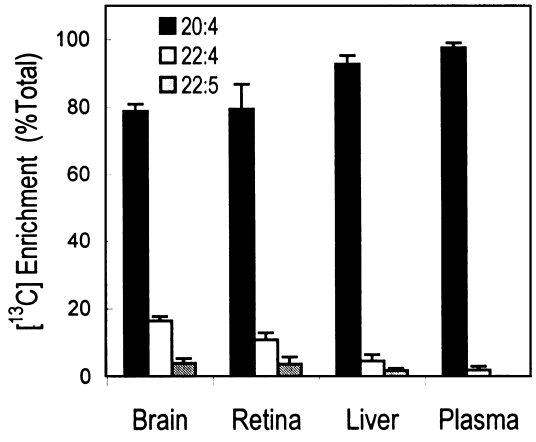

Fig. 1. Distribution of [U-^{13}C]20:4n-6 label into n-6 long chain PUFA (LCP) in brain, retina, liver, and plasma, expressed as %Total, in 4 weeks old term baboon neonates at 10 days post-dose. In neonate brain and retina 16% and 11% of total label was recovered in 22:4n-6, respectively.

Detection of label incorporation into 20:3n-6 in all tissues investigated indicates that chain shortening and retro-conversion of 20:4n-6 occurred in vivo. Retroconversion of 20:4n-6 to 20:3n-6 has been demonstrated in-vitro in rat hepatocytes (17) and human skin fibroblast cells (15, 16). However, in baboon neonates in vivo, 20:4n-6 contribution to the 20:3n-6 pool was relatively small, as evidenced by ⩽1% of total label recovered in 20:3n-6. Retroconversion of 20:4n-6 to 18:2n-6, which was reported in rats (32), was below detectable limits in these primate neonates.

Evidence from previous studies suggest that peroxisome-dependent β-oxidation and chain shortening of 20:4n-6 is one process involved in retroconversion to 20:3n-6 (15, 16). Gordon et al. (15) have shown that human skin fibroblasts cells in culture converted 20:4n-6 to 16:3n-6 through a peroxisome-dependent oxidative process. Further, Spector et al. (16) provided evidence that peroxisomal oxidation of 20:4n-6 was a main pathway in retroconversion of 20:4n-6 to 20:3n-6 in human skin fibroblasts. In baboon neonates, a similar process involving 2 cycles of peroxisomal β-oxidation of 20:4n-6 would yield 16:3n-6, which could have been chain elongated to 20:3n-6.

Label incorporation into 22:6n-3 in retina indicates that [^{13}C]acetate from [U-^{13}C]20:4n-6 was recycled for elongation of unlabeled n-3 PUFA precursors to form labeled C20 products. Again, utilization of 20:4n-6 carbon for formation of n-3 LCP was minor, as evidenced by ⩽2% of total labeled recovered as 22:6n-3.

Our data shows that preformed 20:4n-6 did not contribute significantly to SFA or MUFA synthesized de novo in the primate neonate. The dilution of label into large endogenous SFA/MUFA pools must to be considered with respect to carbon recycling of 20:4n-6. Under the conditions of these measurements, 0.003 APE could have been detected corresponding to label enrichment as low as 0.8% total label in brain or liver 16:0 and 18:1. Hence, as 20:4n-6 label distribution into SFA or MFA were <0.8% total label in all tissues investigated, we conclude that 20:4n-6 contribution to neonate tissue SFA or MFA is minor in term baboon infants.

In contrast, substantial carbon recycling of 18:2n-6, 18:3n-3, and 22:6n-3 into SFA and MUFA has been demonstrated in newborn animals (7–10). In fetal and neonate rhesus monkeys dosed with [U-^{13}C]22:6n-3, about 16% of total label in the brain was detected in SFA and MUFA, indicating substantial utilization of 22:6n-3 carbon for SFA and MUFA synthesis (9). Thus, 20:4n-6 appears to be unique in that it is the only functionally major PUFA studied to date that is not utilized to a substantial extent for de novo synthesis of SFA or MUFA in primate infants. The physiologic significance of this observation is yet to be investigated.

A major fraction of 20:4n-6 consumed (79–93% of total label) accumulated as 20:4n-6 in tissue lipids, consistent with its primary function as a predominant structural con-

stituent in membrane lipids. However, substantial conversion of 20:4n-6 to 22:4n-6 occurred in-vivo in the primate neonate as evidenced by 5–16% total label recovered in 22:4n-6 in neonate tissues 10 days after dosing. Label enrichment in 22:5n-6 was ≤4%total label in all neonate tissues studied, indicating that AA utilization for 22:5n-6 was relatively low compared with its conversion to 22:4n-6.

Based on the tracer data, we can quantitatively estimate the contribution of dietary preformed 20:4n-6 to brain 22:4n-6 accumulation. Our calculations based on tracer enrichment measurements indicate that overall 0.48% of preformed 20:4n-6 consumed in formula appeared as brain 22:4n-6 at 10 days post-dose (percentage dose of AA* recovered as brain 22:4n-6*). The neonates consumed an average of 44 mg 20:4n-6/day. Thus, 0.2 mg (44 × 0.48/100) of formula 20:4n-6/day was utilized for conversion to brain 22:4n-6. The baboon brain weight at term gestation of 182 days is about 80 g (33). Brain 22:4n-6 concentration in the fetal baboon was about 0.675 mg/g wet weight (27), which was not correlated to gestational age. The mean total 22:4n-6 content of neonate brain at 4 weeks of age in these neonates was 90 mg. Combining these factors, net accretion of 22:4n-6 during the 4 week postnatal period is about 36 mg. About 6 mg (0.2 × 28) of formula 20:4n-6 appeared as brain 22:4n-6 over the 4 weeks period in these neonates. Thus, about 17% (6/36) of brain 22:4n-6 accrued postnatally was derived from dietary preformed 20:4n-6 in term baboon neonates at 4 weeks of age.

Neonate diets also contained 18:2n-6, which is a major contributor to brain 20:4n-6 (27) and probably 22:4n-6. The relative efficacy or absolute contributions of dietary 20:4n-6 compared with 18:2n-6 as a source for brain 22:4n-6 in primates is not known. Using data from a recent study conducted in our laboratory of baboon neonates consuming breastmilk of similar composition to the formula of the present study, 18:2n-6 conversion to brain 22:4n-6 can be estimated as 0.087% of 18:2n-6 consumed (unpublished data). The relative conversion efficiency of dietary 20:4n-6 versus 18:2n-6 to brain 22:4n-6, on a molar basis, can then be estimated as about 5.5-fold (0.48/0.087).

Estimates of absolute contributions to brain 22:4n-6 must take into account the considerably greater concentration of 18:2n-6 compared with 20:4n-6 in formula and breastmilk. The average 18:2n-6 intake per day in our neonates was about 1.1 g/day; thus 27 mg or about 75% (27/36) of brain 22:4n-6 that accrued postnatally was derived from dietary 18:2n-6.

Dietary 18:2n-6 and 20:4n-6 account for 75% + 17% = 92% of brain 22:4n-6 accumulation. The likely source for the remaining estimated 8% or 3 mg (0.08 × 36) of brain 22:4n-6 is from the 7 mg/dl of preformed 22:4n-6 found in formula. Neonates consumed an average of 19.5 mg 22:4n-6/day. Thus, over the course of the study (4 weeks), neonates consumed 553 mg 22:4n-6; an efficiency of 3/553 or 0.54% of dietary preformed 22:4n-6 would account for the remaining brain 22:4n-6.

It should be noted that these estimates apply strictly to diets similar to the formula used in this study. This calculation also assumes that brain 22:4n-6 turnover is negligible compared with input, an assumption that is supported by data for other major LCP 22:6n-3 (34) and 20:4n-6 (27) in rapidly growing baboon brain. Nevertheless, the data indicates that 20:4n-6 conversion to brain 22:4n-6 is quantitatively a substantial pathway for 20:4n-6 utilization in primate neonates.

In conclusion, in this study of baboon neonates consuming a formula with fatty acid and particularly LCP concentrations similar to primate breast milk, we have found: *1*) 20:4n-6 was retroconverted to 20:3n-6 in vivo in primate neonates at a very low level compared with 20:4n-6 accumulation in tissue lipids; *2*) 20:4n-6 did not contribute significantly to de novo synthesis of SFA and MUFA; *3*) contribution to brain 22:4n-6 accumulation was quantitatively a significant metabolic fate for dietary 20:4n-6; and *4*) the relative contributions of dietary 18:2n-6, 20:4n-6, and preformed 22:4n-6 to brain 22:4n-6 can be approximately estimated to be 75%, 17%, and 8% respectively, though efficiency of accretion was approximately equal for dietary 20:4n-6 and 22:4n-6 (0.48–0.54%).

This work was supported by NIH grant EY10208 and Numico Research, Germany.

Manuscript received 16 November 2001 and in revised form 13 February 2002.

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