Linoleate, α-linolenate, and docosahexaenoate recycling into saturated and monounsaturated fatty acids is a major pathway in pregnant or lactating adults and fetal or infant rhesus monkeys

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Abstract Carbon recycling and desaturation and elongation of linoleate, α-linolenate and docosahexaenoate in ten fetuses and two nursing infants of chow-fed rhesus monkey mothers were studied in vivo using uniformly labeled tracer molecules and high precision mass spectrometry. Doses of [U-^{13}C]-18:2n–6, [U-^{13}C]-18:3n–3 or [U-^{13}C]-22:6n–3 free fatty acids were infused intravenously to the adults, and milk, maternal plasma, fetal plasma and tissues, and infant plasma were analyzed for enrichment in fatty acids of length C_{14} to C_{22}. Conversion of tracer fatty acids to palmitic, stearic, oleic, and long chain polyunsaturated fatty acids was observed in fetal liver, brain, and retina ca. 5 days after dosing, and in milk and infant plasma 1 and 7 days after dosing. Animals dosed with [U-^{13}C]-22:6n–3 accumulated more label in the fetal organs compared to the animals dosed with [U-^{13}C]-18:3n–3 or [U-^{13}C]-18:2n–6. The greatest fractions of doses were found in the fetal brains at levels of 0.21%, 0.24%, and 1.7% for the [U-^{13}C]-18:2n–6, [U-^{13}C]-18:3n–3, and [U-^{13}C]-22:6n–3-dosed mothers, respectively. Label was found in saturated and monounsaturated fatty acids in liver, brain and retina (0.05–1.5 ppm dose/mg lipid) for all doses. These results demonstrate that *1*) recycling of carbon from 18:2n–6, 18:3n–3, and 22:6n–3 into saturates and monounsaturates is a major metabolic pathway in chow-fed primates in the perinatal period; *2*) less than 2% of the n–3 doses are found in brain fatty acids of developing fetuses from chow-fed mothers; and *3*) [^{13}C]-22:6n–3 accumulates in retina and brain at an order of magnitude higher level when provided as preformed [^{13}C]-22:6 n–3 compared to [^{13}C]-18:3n–3.—**Sheaff Greiner, R. C., Q. Zhang, K. J. Goodman, D. A. Giussani, P. W. Nathanielsz, and J. T. Brenna.** Linoleate, α-linolenate, and docosahexaenoate recycling into saturated and monounsaturated fatty acids is a major pathway in pregnant or lactating adults and fetal or infant rhesus monkeys. *J. Lipid Res.* 1996. **37:** 2675–2686.

Supplementary key words linoleic acid • α-linolenic acid • docosahexaenoic acid • rhesus monkeys • fatty acid recycling • isotope ratio mass spectrometry • ^{13}C labeling

Omega–3 (n–3) and omega–6 (n–6) fatty acids are well known to be important constituents of the central nervous system (CNS) (1, 2). The parent n–3 fatty acid, α-linolenic acid (18:3n–3) and the parent n–6 fatty acid, linoleic acid (18:2n–6), cannot be synthesized in amounts required by mammals and serve as substrates for desaturation and elongation to long-chain n–3 and n–6 metabolites such as docosahexaenoic acid (22:6 n–3) or arachidonic acid (20:4n–6), which are necessary for appropriate CNS growth and function (3–6). Previous studies have focused primarily on the efficacy of 18:3n–3 and 18:2n–6 as substrates for conversion to their respective long-chain polyunsaturated fatty acids (LC-PUFA) and the relative importance of preformed dietary 22:6n–3 or 20:4n–6 under conditions such as premature birth (7, 8). Studies in animals and humans have also examined the relative efficiency of 22:6n–3 or 20:4n–6 accumulation in the developing CNS when dietary 22:6n–3 or 20:4n–6 is available or when only 18:3n–3 or 18:2n–6 is present in the diet (9–20).

In addition to desaturation and elongation, ingested 18:3n–3 and 18:2n–6 can be oxidized exhaustively to CO_2 and used for energy. In rats, the oxidation rate of 18:3n–3 is similar to that of lauric acid (12:0), which is the most efficiently used fatty acid for energy produc-

Abbreviations: CNS, central nervous system; LC-PUFA, long-chain polyunsaturated fatty acids; TCA, tricarboxylic acid; SFA, saturated fatty acids; MFA, monounsaturated fatty acids; PL, phospholipid; GC, gas chromatography; FAME, fatty acid methyl esters; HPLC, high performance liquid chromatography; FA, fatty acid; dGA, days of gestation; CS, Caesarean section; FID, flame ionization detector; BHT, butylated hydroxytoluene; AP, atom percent; APE, atom percent excess; GCC–IRMS, gas chromatography combustion–isotope ratio mass spectrometry.

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tion. In contrast, longer, more unsaturated fatty acids such as arachidonic acid (20:4n–6), have slower oxidation rates (21). In the brain, 22:6n–3 is the most abundant polyunsaturate, and 18:3n–3 exists at very low levels. It is generally thought that the brain prefers uptake of preformed 22:6n–3 to elongation and desaturation of 18:3n–3, as the oxidation level of 18:3n–3 is greater than that of 22:6n–3 (22, 23).

In addition to serving as a precursor for LC-PUFA synthesis and for complete oxidation, 18:3n–3 and 18:2 n–6 can be oxidized partially with substantial levels of C recycling (24, 25). Recycled C may arise from the export to the cytosol of citrate labeled by the β-oxidation product acetyl-CoA in the tricarboxylic acid (TCA) cycle. In the cytosol, citrate is cleaved to acetyl-CoA and oxaloacetate, after which acetyl-CoA can be used for fatty acid synthesis. The C from 18:3n–3 and 18:2n–6 may be recycled and used for the synthesis of saturated fatty acids (SFA) and monounsaturated fatty acids (MFA), particularly 16:0, 16:1, 18:0, and 18:1. Several studies have reported the detection of label in SFA and MFA of rat liver phospholipids (PL) and brain lipids after a dose of labeled 18:3n–3 or 22:6n–3 (22, 26, 27). Dwyer and Bernsohn (9) detected extensive labeling in 16:0, 18:0 and 18:1n–9 3 days after an intracranial dose of [^{14}C]-18:3n–3 to 21-day-old rats. Likewise, Li, Wetzel, and O'Brien (28) detected a peak in the specific activity of 16:0 in the rod outer segments of the retina 24 h after a dose of [^{14}C]-18:3n–3 to male weanling rats. These studies show the appearance of label in SFA-MFA, but did not examine the recycling of the carbons from 18:3n–3 and 22:6n–3 in detail. Cunnane et al. (29) have also shown that a mixture of C_{16} and C_{18} n–3 fatty acids is used extensively for synthesis of cholesterol appearing in the brain of newborn rats.

There are substantial differences in desaturation among species with a trend toward high rates of desaturation in small species, particularly the rat (30). The growth of the primate brain is uniquely rapid compared to other mammals, placing a high demand for its most abundant structural fatty acids, 22:6n–3 and 20:4n–6 (31–33). We therefore undertook a study of 18:3n–3, 18:2n–6, and 22:6n–3 metabolism in the primate during the perinatal period to detect relative levels of desaturation and elongation compared to recycling.

In recent years, we (20, 34) and others (29, 35–37), have developed a novel, high sensitivity approach to stable isotope-based biotracer studies and applied it to questions of lipid metabolism. Gas chromatography (GC) coupled directly to a high precision mass spectrometer is used in concert with uniformly labeled tracers to detect and quantify at high precision the $^{13}C/^{12}C$ ratio of all peaks eluting from the GC column (38).

In this study, we examine the absolute and relative accumulation of labeled n–3 LC-PUFA (20:5n–3, 22:5n–3, and 22:6n–3), n–6 LC-PUFA (20:3n–6, 20:4 n–6, 22:4n–6, and 22:5n–6) and labeled SFA–MFA (16:0, 16:1n–7, 18:0 and 18:1n–9) in fetal liver, brain, and retina after a physiological dose of [U-^{13}C]-18:2 n–6 (n = 4), [U-^{13}C]-18:3n–3 (n = 3) or [U-^{13}C]-22:6 n–3 (n = 3) to chow-fed pregnant rhesus monkeys. We also show the fate in milk and infant plasma of a [U-^{13}C]-18:3n–3 dose to lactating rhesus monkeys (n = 3). Some of these data have been reported in abstract form (39, 40).

MATERIALS AND METHODS

Animals

Thirteen female rhesus monkeys (*Macaca mullata*) were bred at the Southwest Foundation for Biomedical Research (San Antonio, TX) and transported to the Laboratory for Pregnancy and Newborn Research soon after confirmation of pregnancy. All procedures were approved by the Cornell Institutional Animal Care and Use Committee. All facilities were approved by the American Association for Laboratory Animal Care. A thorough veterinary examination was performed upon arrival, and animals were housed in individual cages within view of at least two other monkeys. They were acclimated to a 14-h light and 10-h dark cycle, and were jacketed and connected to a flexible tether and swivel. After a minimum of 30 days of acclimation, the monkeys were provided under halothane general anesthesia with chronic indwelling catheters in the maternal femoral artery and vein, and with electrodes used to monitor myometrial activity. These methods have previously been described in detail (41–43). Pregnant females were fed a typical chow-based non-human primate diet and were given treats consisting of fruits, potatoes, and nuts each day. Lipid analysis showed the diet to contain 0.45% and 0.51% of 20:4n–6 and 22:6n–3, respectively (Table 2).

At an average of 147 ± 6 days of gestation dGA (term = 165 dGA), the pregnant animals received an intravenous dose of [U-^{13}C]linoleic acid, [U-^{13}C]α-linolenic acid, or [U-^{13}C]-docosahexaenoic acid over 1 or 4 h. **Table 1** indicates the ages and weights of the pregnant animals as well as the doses administered to these animals. Caesarean section (CS) was performed 5.3 ± 0.8 days after the dose was administered. Fetuses were killed by exsanguination while still under halothane general anesthesia. Fetal liver, brain, and retina were weighed, flash frozen, and stored at −80°C until analysis. Finally, three nursing mothers each received an intravenous dose of approximately 10 mg [U-^{13}C]-18:3n–3 when

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TABLE 1. Characteristics of pregnant animals

Rhesus	Dose	Dose[a] *mg*	DGA at Dosing[b]	DGA at CS[c]	Days between Dose and CS	Maternal Weight[d] kg	Fetal Weight[e] gm	Fetal Sex
18:2A	18:2n–6	12.1	150	155	5	7.3	506	F
18:2B	18:2n–6	13.2	149	155	6	6.8	518	F
18:2C	18:2n–6	23.1	152	157	5	7.3	502	F
18:2D	18:2n–6	17.0	149	154	5	6.4	371	M
18:3A	18:3n–3	4.2	138	143	5	5.9	388	M
18:3B	18:3n–3	2.1	138	143	5	6.8	402	M
18:3C	18:3n–3	16.8	152	156	4	5.5	403	M
22:6A	22:6n–3	4.7	138	143	5	5.9	380	F
22:6B	22:6n–3	6.5	149	156	7	7.3	618	M
22:6C	22:6n–3	2.1	152	158	6	9	416	M

[a]Dose was infused as free fatty acid form and was uniformally labeled with ^{13}C.
[b]Days of gestation (age of fetus) when the dose was administered.
[c]Days of gestation (age of fetus) when the cesarean section occurred.
[d]Maternal weight was recorded near time of dosing or at maternal catheterization.
[e]Fetal weight was recorded at time of cesarean section.

their male infants were 32 days of age. Maternal plasma was collected before the dose for use as a baseline sample, and milk and fetal plasma from two infants were collected at 1 and 7 days after the dose. These samples were prepared immediately for analysis.

Dose

The physiological dose of [U-^{13}C]-18:2n–6 (18:2 n–6*), [U-^{13}C]-18:3n–3 (18:3n–3*) or [U-^{13}C]-22:6n–3 (22:6n–3*) was purified from a U-^{13}C-labeled algal oil (Martek Biosciences, Columbia, MD) in a two-step method described previously (20). Briefly, fatty acid methyl esters (FAME) were separated by degree of unsaturation on Ag-loaded solid phase extraction columns (Varian, Harbor City, CA). This step is sufficient to purify the 22:6n–3* to 98% and it is used without further purification. The algal oil from which 18:3n–3* is derived contains 16:3n–3*, therefore a second step using high-performance liquid chromatography (HPLC) was used to separate FAME based on chain length. FAME purity was >99%, verified by capillary GC. The Me-18:2n–6*, Me-18:3n–3*, and Me-22:6n–3* were hydrolyzed to the free fatty acids and sonicated into 5.0 mL 10% Intralipid diluted with 5.0 mL sterile saline. In the pregnant animals, 9.5 mL of the final solution was infused i.v. over 1 to 4 h after an overnight fast. The rest of the dose, 0.5 mL, was given as a bolus 1 h prior to the CS. In the lactating animals, the entire dose was given as a bolus after an overnight fast.

Compositional analysis

Total lipids were extracted from liver, brain, and retina samples by the method of Bligh and Dyer (44), modified as discussed previously. Heptadecanoate (17:0) in hexane was added as an internal standard. Samples were saponified by adding 1.0 mL 0.5 N methanolic NaOH and heating at 60°C for 10 min. FAME derivatives were prepared by adding 1.0 mL 14% BF_3 in methanol and heating at 100°C for 2 min. Hexane (1.0 mL) was added before the tubes were vortexed and placed in a boiling water bath for an additional minute. After 1.0 mL saturated NaCl solution was added, tubes were centrifuged at 3000 rpm and the top hexane layer containing the FAME was transferred to another tube and the hexane was evaporated by a stream of N_2. The samples were resuspended in a metered amount of hexane for quantitative analysis by GC with a flame ionization detector (GC-FID) and for isotopic enrichment by gas chromatography combustion–isotope ratio mass spectrometry (GCC-IRMS). Butylated hydroxytoluene (BHT) was added to solvents as an antioxidant. FAME were analyzed using a Hewlett-Packard 5890 GC with a DB23 (J&W Scientific) fused silica capillary column (30 m, 0.32 mm id., 0.25 film thickness) with H_2 carrier gas and N_2 auxiliary gas. Response factors were determined daily using a standard mixture and final values are expressed and % total fatty acids, of chain length C_{14}, by weight.

Tracer analysis

Isotopic analysis was performed using GCC-IRMS, discussed in detail elsewhere (32, 36). Briefly, the effluent of Varian 3400 capillary GC was swept by He carrier into a furnace held at 850°C and loaded with a solid source of O_2. Organic compounds entering the furnace were quantitatively converted to CO_2 and H_2O, and swept through a water trap that maintains chromatographic resolution. Dried CO_2 was then admitted to the ion source of a Finnigan 252 high sensitivity, high precision IRMS instrument, with dedicated detectors/electronics for each of the major masses of CO_2 m/z = 44, 45, and 46, and an absolute sensitivity of about 10^3 molecules/

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ion detected. Chromatograms from the three channels were acquired continuously during the GC run. Peaks were identified in the $m/z = 44$ channel, extrapolated to the other channels, and isotope ratios corresponding to $R = [^{13}C]/[^{12}C]$ were calculated using the m/z 46 signal to adjust for the contribution of the ^{17}O-substituted CO_2 appearing at $m/z = 45$. The average analytical precision of these measurements was about CV = 0.05% (500 ppm).

Notation, nomenclature, and calculations

High precision IRMS data is usually expressed as the relative deviation in parts per thousand from the international standard Pee Dee Belemnite (PDB), relatively rich in ^{13}C with an isotope ratio defined as $R_{PDB} = 0.0112372$, using:

$$\delta^{13}C_{PDB}(‰) = \left(\frac{R_{SPL} - R_{PDB}}{R_{PDB}}\right) \times 1000 \qquad Eq.\ 1)$$

$\delta^{13}C_{PDB}$ is expressed in "permil" (‰) units and "SPL" refers to the sample. This notation is less useful for tracer studies where atom fraction, or more commonly, atom percent (AP), is more convenient than ratio-based expressions. Here, we convert data to AP or atom percent excess (APE), calculated by subtracting baseline isotope levels, as the index of enrichment level, and use this notation for further calculations. This procedure also has the effect of correcting for the contribution to isotope ratio made by the methyl C added in derivatization, so no separate correction is necessary.

The overall concentration of tracer per unit weight of tissue can be found by multiplying the APE by the concentration of tracer per unit tissue. This quantity, designated "total label", defines the overall conversion of labeled tracer to a particular metabolite in a tissue, on a molar basis. As GCC-IRMS detects overall enrichment, no information as to number of ^{13}C per molecule is directly available from these analyses. That is, for a particular intermediate enrichment, GCC-IRMS does not distinguish between pools with relatively few, highly enriched molecules, and those with relatively many molecules at low enrichment. Thus, it is most correct to refer to moles of total label appearing in a particular pool as "dose equivalents", as the distribution of labeled carbon among the particular molecular species is not determined.

For these specific studies, 20 or 22 C fatty acids, at most only 18 C atoms derived from the 18:3n–3* or 18:2n–6* are labeled. A correction is applied to express conversion in terms of 18:3n–3* or 18:2n–6* equivalents to adjust for the endogenous C added during chain elongation and to enable direct comparison of 18:3n–3* and 18:2n–6* conversion to each fatty acid metabolite on a molar basis. The equation takes the form:

$$D^* = APE_F \times Q_F \times \frac{[C]/[D^*]}{[C]/[F]} \qquad Eq.\ 2)$$

where D* is dose-equivalents, F is the analyte fatty acid, Q_F is the quantity of F derived from GC-FID measurements, and "[C]/[x]" designates the moles of C per mole of either D* or F. For the particular case of 22:6n–3 (=F) derived from a dose of 18:3n–3 (=D*), we have:

$$18{:}3^* = APE_{22:6} \times Q_{22:6} \times \frac{18}{22} \qquad Eq.\ 3)$$

The concept of dose equivalents is particularly relevant to resynthesis of saturated and monounsaturated fatty acids from C derived from 18:3n–3*, 18:2n–6*, and 22:6n–3*, as the dose must be partially oxidized to acetate units with fatty acids assembled by de novo synthesis.

Label is reported as fraction (parts per million ppm) of dose found in a particular pool. Because the dose sizes administered to the pregnant animals were not equivalent, the size of the dose is accounted for by reporting the data as ppm of the dose/mg lipid. This also corrects for any differences in amount of lipid in the tissues. The "sum of total label" represents the sum total of all label found per mg lipid and is found by adding the label found in all fatty acids in a particular tissue. A one-way ANOVA was calculated to compare the three pregnant groups, using $P < 0.05$ as a criteria of significance.

RESULTS

Tissue fatty acid compositions

The fatty acid compositions of the chow and the fetal liver, brain, and retina from the 18:2n–6*, 18:3n–3*, and 22:6n–3*-dosed groups (total n = 10) are presented in **Table 2.** Increasing concentration of 20:4 n–6 and 22:6n–3 from fetal liver, to brain, to retina is evident, and is consistent with previous observations, termed "biomagnification" (45). Overall, n–3 FA also increase from liver to brain to retina, while n–6 FA decrease slightly from liver to brain, with retina indistinguishable from brain. The combined trend causes the n–6/n–3 ratio to decline from the chow value of about 7.0 to the organ values of 3.6 (liver) to 2.0 (brain) to 1.1 (retina), reflecting the relative importance of n–3 fatty acids in each tissue.

The infant plasma and milk compositions for the

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TABLE 2. Fatty acid composition (wt % of total fatty acids) of non-human primate chow (for the pregnant animals), fetal liver, brain and retina presented as mean ± standard deviation (n = 10)

Fatty Acid	Chow	Fetal Liver	Fetal Brain	Fetal Retina
14:0	1.48	0.48 ± 0.2	1.71 ± 0.21	1.10 ± 0.63
16:0	23.03	27.02 ± 2.6	30.02 ± 3.33	24.17 ± 3.1
18:0	6.83	19.12 ± 10.5	20.99 ± 4.0	18.46 ± 2.02
20:0	0.12	0.19 ± .09	0.07 ± 0.04	0.23 ± 0.13
22:0	ND[a]	0.20 ± 0.1	0.11 ± 0.03	0.33 ± 0.30
24:0	ND	0.32 ± 0.2	0.25 ± 0.15	0.22 ± 0.23
Σ SFA	31.46	47.24 ± 12.3	53.0 ± 6.86	44.45 ± 2.72
14:1	0.15	0.14 ± 0.14	0.14 ± 0.08	0.34 ± 0.45
16:1n−7	1.74	1.86 ± 0.8	2.53 ± 2.05	1.25 ± 0.6
18:1n−9	18.85	10.96 ± 3.9	10.11 ± 1.4	12.16 ± 1.27
18:1n−7	1.74	2.12 ± 1.4	3.84 ± 1.27	2.82 ± 1.05
20:1	0.27	0.16 ± 0.05	0.32 ± 0.12	0.87 ± 0.28
22:1	ND	0.18 ± 0.09	0.21 ± 0.16	0.35 ± 0.29
24:1	ND	0.17 ± 0.09	0.09 ± 0.06	0.25 ± 0.20
Σ MFA	22.75	15.6 ± 5.5	16.46 ± 3.80	17.94 ± 1.65
18:2n−6	39.14	15.47 ± 4.7[b]	0.70 ± 0.15[c]	2.00 ± 0.60[d]
20:2n−6	0.21	0.33 ± 0.3	0.51 ± 0.49	0.19 ± 0.21
20:3n−6	0.13	2.11 ± 0.8	1.23 ± 0.29	2.55 ± 0.34
20:4n−6	0.45	9.72 ± 2.3	10.88 ± 1.67	11.20 ± 0.61
22:4n−6	0.11	0.36 ± 0.1	4.58 ± 0.76	1.83 ± 0.23
22:5n−6	ND	0.58 ± 0.24	1.99 ± 0.33	1.32 ± 0.38
Σ n−6	40.14	28.45 ± 6.64[b]	19.85 ± 2.69[c]	19.09 ± 1.33[c]
18:3n−3	4.28	0.34 ± 0.14	0.05 ± 0.04	0.26 ± 0.56
20:5n−3	0.62	0.25 ± 0.13	0.18 ± 0.10	0.28 ± 0.11
22:5n−3	0.33	0.39 ± 0.19	0.29 ± 0.08	0.52 ± 0.19
22:6n−3	0.51	7.01 ± 1.9[b]	9.51 ± 1.25[b]	16.61 ± 1.64[c]
Σ n−3	5.74	8.12 ± 2.1[b]	10.09 ± 1.23[c]	18.15 ± 1.3[d]
n−6/n−6	6.98	3.6 ± 0.9[b]	1.98 ± 0.25[c]	1.06 ± 0.10[d]

[a]Not detected.
[b,c,d]Different superscripts in a row indicate significantly different values ($P < 0.05$).

postnatal animals are presented in **Table 3.** At both time points, n−6/n−3 ratios are greater in milk and infant plasma than fetal tissue levels. Milk and infant plasma at day 1 are greater than the values for the chow diet of the pregnant animals. This may simply reflect a greater relative concentration of n−6 compounds in the chow of the lactating animals as they were fed a different chow lot than the pregnant animals. The infant plasma is richer in 18:2n−6, 20:4n−6, and 22:6n−3 than the milk, further suggesting concentration of polyunsaturated fatty acids and LC-PUFA in particular.

Tracer results

The tracer data permit comparison of 18:2n−6*, 18:3n−3*, and 22:6n−3* incorporation into tissues during a period of high demand for these lipids. The tracer results only depict label incorporation into tissues and distribution among fatty acids at one time point along a curve and do not imply rates of conversion of these fatty acids. **Figure 1** shows that the 22:6n−3*-dosed pregnant animals accumulated an average of 16-, 6-, and 7-fold more total label in the fetal liver, brain, and retina, respectively, compared to the 18:3n−3*-dosed pregnant animals. The level of incorporation of 18:2

TABLE 3. Fatty acid composition (wt % of total fatty acids) in milk and infant plasma presented as mean ± standard deviation

Fatty Acid	Milk[a] (n = 3)	Infant Plasma (day 1) (n = 2)	Infant Plasma (day 7) (n = 2)
14:0	2.10 ± 0.47	2.94 ± 1.87	0.30 ± 0.21
14:1	0.09 ± 0.01	ND[b]	ND
16:0	28.28 ± 2.77	25.53 ± 5.46	22.24 ± 0.72
16:1n−7	3.49 ± 2.72	0.37 ± 0.53	ND
18:0	6.12 ± 0.56	8.79 ± 0.86	9.29 ± 1.33
18:1n−9	31.88 ± 2.16	15.09 ± 0.91	20.02 ± 3.84
20:0	0.02 ± 0.03	0.23 ± 0.08	ND
20:1	0.23 ± 0.14	ND	ND
24:0	0.31 ± 0.01	0.81 ± 0.25	1.68 ± 0.66
Σ SFA-MFA	75.52 ± 3.65	53.74 ± 1.61	53.53 ± 4.89
18:2n−6	24.56 ± 3.78	36.62 ± 3.60	36.29 ± 3.18
20:2n−6	0.39 ± 0.11	0.38 ± 0.28	0.16 ± 0.12
20:3n−6	0.57 ± 0.33	1.33 ± 0.41	0.42 ± 0.29
20:4n−6	0.28 ± 0.03	4.32 ± 0.02	3.12 ± 2.21
Σ n−6	25.81 ± 3.45	42.66 ± 2.93	39.91 ± 5.8
18:3n−3	0.99 ± 0.29	0.53 ± 0.43	0.93 ± 0.21
20:3n−3	0.15 ± 0.12	ND	ND
22:6n−3	0.53 ± 0.05	3.04 ± 0.89	5.54 ± 0.71
Σ n−3	1.67 ± 0.26	3.57 ± 1.32	6.48 ± 0.92
n−6/n−3	15.52 ± 1.5	12.97 ± 5.62	6.69 ± 1.84

[a]Sample taken 7 days after the dose of [U-^{13}C]-18:3n−3.
[b]Not detected.

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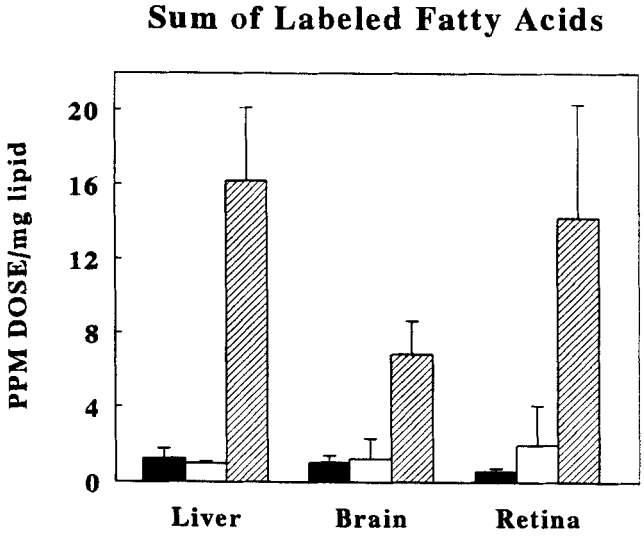

Fig. 1. Accumulation of total labeled fatty acids in the fetal liver, brain and retina (PPM dose/mg lipid) approximately 5 days after a dose of [U-^{13}C]-18:2n–6* (■), [U-^{13}C]-18:3n–3* (□), or [U-^{13}C]-22:6n–3* (▨) was administered to the mother. Error bars reflect standard deviations. Animals dosed with [U-^{13}C]-22:6n–3 incorporated significantly greater amounts of label in all tissues compared to the other two groups ($P < 0.05$).

n–6* into fetal liver, brain and retina was similar to that of 18:3n–3*.

As shown in **Figs. 2A–C,** the sums of total label found in SFA-MFA were similar among the three groups in all three fetal tissues, indicating that carbon from 18:2 n–6*, 18:3n–3*, and 22:6n–3* was recycled into these fatty acids. Labeled 18:3n–3 was converted to 22:6 n–3* in these animals, although the absolute total amount of 18:3n–3* converted to LC-PUFA(n–3)* was similar to the amount of C from 18:3n–3* that was recycled and used for the synthesis of SFA-MFA. Labeled 18:2n–6 was converted to 20:4n–6* and other LC-PUFA(n–6)* in these animals, but the total amount of 18:2n–6* converted to LC-PUFA(n–6)* was also similar to the amount of label detected in SFA-MFA. In the animals dosed with 22:6n–3*, more label was detected in the LC-PUFA(n–3) in all tissues compared to the animals dosed with 18:3n–3*, reflecting greater deposition of preformed 22:6n–3*.

Table 4, Table 5, and **Table 6** show that in all tissues, the animals dosed with 18:3n–3* exhibited a higher percentage of total label in SFA-MFA compared to the animals dosed with 18:2n–6* and 22:6n–3*. Further, the 22:6n–3* dosed animals incorporated a greater percentage of label in LC-PUFA(n–3) compared to the 18:2n–6* and 18:3n–3* dosed animals due to the incorporation of 22:6n–3* in these tissues. Greater than 55% of the label found in the fetal brains of the animals dosed with 18:2n–6* or 18:3n–3* was in SFA and MFA even though 20:4n–6 and 22:6n–3 are thought to be in high demand in this organ during this time of gestation.

There were trends in the distribution of label among n–3 or n–6 LC-PUFA in the tissues of all three groups. In all tissues of animals dosed with 18:3n–3* or 22:6 n–3*, the highest percentage of label was detected in 22:6n–3 compared to 20:5n–3 and 22:5n–3, which accounted for <12% of the label in all tissues. In the animals dosed with 18:2n–6*, 20:4n–6 had the highest percentage of label in all tissues, but 22:4n–6 and 22:5n–6 were also labeled. A very small percentage of total label, <2%, was detected in LC-PUFA(n–3) in the tissues of animals dosed with 18:2n–6* indicating that some of the carbon from 18:2n–6 was used for the elongation of n–3 fatty acids. Similarly, <4% of total label was detected in the LC-PUFA(n–6) in tissues of animals dosed with 18:3n–3* and 22:6n–3* indicating that some of the carbon from these fatty acids was recycled and used for elongation of n–6 fatty acids.

Table 7 presents the total accumulation of label in each whole tissue. Overall, less than 2% of the dose was incorporated into the whole brains of animals dosed with 22:6n–3* and was several-fold lower for the 18:3 n–3* and 18:2n–6*-dosed animals. The brain incorporated the most label in all three groups due to the relative size difference between the liver, brain, and retina.

The distribution of label in the maternal and fetal plasma at the CS is shown in **Table 8** and **Table 9.** The data are quite variable, but the majority of label in the maternal and fetal plasma was found in the dosed fatty acid. For example, in the animals dosed with 18:3n–3*, 67% of the label in the maternal plasma was still present as 18:3n–3*. SFA-MFA and LC-PUFA were also enriched in all groups.

Results of tracer doses at days 1 and 7 in milk and neonatal plasma are presented in **Fig. 3** and **Fig. 4,** respectively. Baseline maternal plasma isotope ratios were used as background levels for neonatal plasma APE calculations as neonatal baseline samples were not available. LC-PUFA isotope enrichments were not different from baseline levels in either biological fluid at either time point. In contrast, label was detected in SFA-MFA in all samples. In day 1 milk, 18:3n–3* is the most prominent labeled FA, with lower levels of label in SFA-MFA. Oleate contains the highest level of label in the SFA-MFA, and is not significantly different from the 18:3 n–3* level. At 7 days after the dose, 18:1n–9 contains the highest level of label and is statistically greater than 18:3n–3* which has dropped significantly since 1 day after the dose. Nonsignificant increases in both 16:0 and 14:0 are also detected in milk. In contrast, labeling in infant plasma increases significantly from day 1 to day 7 for both 18:1n–9 and 18:3n–3, the latter of which

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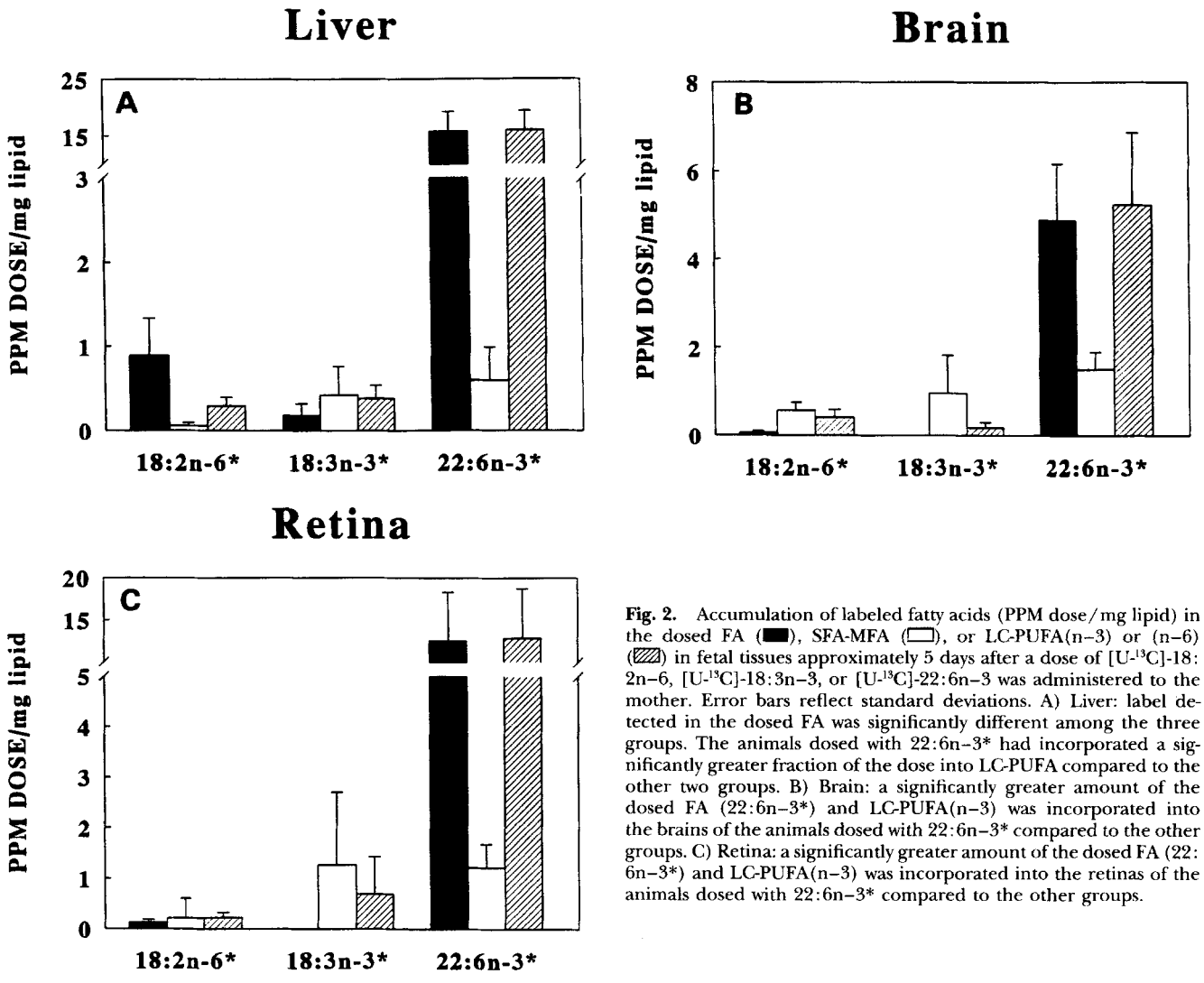

Fig. 2. Accumulation of labeled fatty acids (PPM dose/mg lipid) in the dosed FA (■), SFA-MFA (□), or LC-PUFA(n–3) or (n–6) (▨) in fetal tissues approximately 5 days after a dose of [U-^{13}C]-18:2n–6, [U-^{13}C]-18:3n–3, or [U-^{13}C]-22:6n–3 was administered to the mother. Error bars reflect standard deviations. A) Liver: label detected in the dosed FA was significantly different among the three groups. The animals dosed with 22:6n–3* had incorporated a significantly greater fraction of the dose into LC-PUFA compared to the other two groups. B) Brain: a significantly greater amount of the dosed FA (22:6n–3*) and LC-PUFA(n–3) was incorporated into the brains of the animals dosed with 22:6n–3* compared to the other groups. C) Retina: a significantly greater amount of the dosed FA (22:6n–3*) and LC-PUFA(n–3) was incorporated into the retinas of the animals dosed with 22:6n–3* compared to the other groups.

is the most enriched peak at 7 days postdose, as shown in Fig. 4. Significant enrichment was also detected in 14:0, 16:0, 18:0, and 18:1 at both time points.

DISCUSSION

The brain growth spurt in the rhesus monkey takes place in utero, during which time the CNS acquires much of the necessary LC-PUFA(n–3 and n–6) (31). Fetuses in the present study were at the peak of their brain growth spurt while neonates were past the peak but within the period where the absolute requirements for LC-PUFA are very high. These animals can therefore be considered a model for n–3 fatty acid metabolism during the human brain growth spurt, timed at the third trimester of term pregnancy and continuing until 2 years of age (46). The variability in the data may be explained by the differences in gestation ages of the animals during the experiment (Table 1), differences in the length of the experiments, and biological variability.

Detection of 18:2n–6*, 18:3n–3*, and 22:6n–3* in fetal tissues approximately 5 days after a physiological dose to the pregnant animal clearly shows that these three fatty acids are transferred intact from the mother to the fetus, with metabolites appearing in the liver, brain, and retina. The transfer of [1-^{14}C]linoleic and palmitic acids from mother to fetus has been demonstrated in the *Macaca irus* (47). The 22:6n–3* dosed group accumulated about an order of magnitude more total label in the tissues compared to the 18:3n–3* and 18:2n–6* dosed group, indicating that 22:6n–3* is more efficiently incorporated into fetal liver, brain, and retina compared to 18:3n–3* and 18:2n–6*. Similarly, Sinclair and Crawford (48) showed that 16- to 17-day-

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TABLE 4. Distribution of total label appearing in fetal liver fatty acids approximately 5 days after a dose of [U-^{13}C]-18:2n-6, [U-^{13}C]-18:3n-3, or [U-^{13}C]-22:6n-3 was administered to the mother

Fatty Acid	[U-^{13}C]-18:2n-6	[U-^{13}C]-18:3n-3	[U-^{13}C]-22:6n-3
16:0	3.79 ± 3.68	27.31 ± 24.3	2.16 ± 2.09
16:1n-7	0.07 ± 0.14	0.15 ± 0.25	0.01 ± 0.02
18:0	0.56 ± 1.11	1.72 ± 1.92	0.12 ± 0.06
18:1n-9	0.43 ± 0.59	11.26 ± 10.8	1.69 ± 1.52
Σ SFA-MFA	4.84 ± 4.08	40.45 ± 31.5	3.98 ± 3.35
18:2n-6	70.57 ± 4.30	ND	ND
20:3n-6	8.01 ± 1.63	ND	ND
20:4n-6	10.71 ± 3.26	0.75 ± 1.3	0.52 ± 0.9
22:4n-6	0.70 ± 0.34	0.05 ± 0.9	0.004 ± 0.01
22:5n-6	1.84 ± 0.85	0.13 ± 0.23	0.01 ± 0.02
Σ n-6 LC-PUFA	21.25 ± 2.50	0.93 ± 1.6	0.54 ± 0.94
18:3n-3	ND	19.06 ± 17.3	0.10 ± 0.09
20:5n-3	ND	4.73 ± 4.11	0.51 ± 0.45
22:5n-3	0.75 ± 0.60	3.93 ± 4.5	0.73 ± 0.45
22:6n-3	0.04 ± 0.08	30.03 ± 9.27	92.66 ± 3.26
Σ n-3 LC-PUFA	0.79 ± 0.65	38.68 ± 16.7	93.96 ± 2.65

Data presented as mean % of total label detected in liver fatty acids ± standard deviation; ND, not detected.

TABLE 6. Distribution of total label appearing in fetal retina fatty acids approximately 5 days after a dose of [U-^{13}C]-18:2n-6, [U-^{13}C]-18:3n-3, or [U-^{13}C]-22:6n-3 was administered to the mother

Fatty Acid	[U-^{13}C]-18:2n-6	[U-^{13}C]-18:3n-3	[U-^{13}C]-22:6n-3
16:0	32.38 ± 4.9	42.67 ± 18.6	4.43 ± 3.62
16:1n-7	0.47 ± 0.27	ND	0.12 ± 0.21
18:0	3.80 ± 2.05	1.29 ± 1.33	0.29 ± 0.50
18:1n-9	2.36 ± 2.06	7.71 ± 6.76	3.54 ± 3.06
Σ SFA-MFA	39.01 ± 8.9	51.67 ± 24.1	8.39 ± 1.58
18:2n-6	20.67 ± 4.49	ND	ND
20:3n-6	14.35 ± 2.76	ND	ND
20:4n-6	11.93 ± 1.98	1.90 ± 3.3	0.44 ± 0.77
22:4n-6	3.64 ± 1.60	0.48 ± 0.83	0.72 ± 1.2
22:5n-6	6.33 ± 1.10	0.72 ± 1.25	0.59 ± 1.03
Σ n-6 LC-PUFA	36.25 ± 3.43	3.12 ± 5.38	1.75 ± 3.04
18:3n-3	ND	ND	0.34 ± 0.48
20:5n-3	ND	2.88 ± 2.98	1.43 ± 0.58
22:5n-3	1.60 ± 0.79	8.42 ± 10.2	0.92 ± 0.45
22:6n-3	ND	33.91 ± 5.59	87.13 ± 3.44
Σ n-3 LC-PUFA	1.60 ± 0.79	45.21 ± 18.7	89.71 ± 2.84

Data presented as mean % of total label detected in retina fatty acids ± standard deviation; ND, not detected.

old rats dosed with ^{14}C-22:6n-3 incorporated significantly more radioactivity in their livers than rats dosed with ^{14}C-18:3n-3, while brains accumulated similar amounts of radioactivity (48) in one experiment but significantly different amounts in another study (22). Anderson and Connor (49) showed similar, but less dramatic, results in 15-day-old hepatectomized rats.

Conversion of 18:3n-3* and 18:2n-6* to LC-PUFA(n-3)* and LC-PUFA(n-6)*, respectively, was observed in the animals. The efficiency of conversion of 18:3n-3* to 22:6n-3* and subsequent incorporation into tissue was much lower than the tissue incorporation when preformed 22:6n-3* was infused. The 22:6n-3*-dosed group had 53-, 35-, and 21-fold more label in liver, brain, and retina 22:6n-3, respectively, compared to the 18:3n-3* dosed animals. This indicates that in utero, the primate fetus more readily incorporated preformed 22:6n-3* into tissues compared to 18:3n-3*, when the LC-PUFA is present in the diet. Retroconversion of 22:6n-3* was also indicated by the presence of small amounts of 20:5n-3* and 22:5n-3* in the 22:6n-3*-dosed animals, as shown previously in rats (22). Anderson, Connor, and Corliss (23) reported retro-conversion in serum, liver, and retina, but not in brain of chicks. Wang and Anderson (50) also reported retroconversion of 22:5n-3 in the retina and retinal pigment epithelium of frogs.

TABLE 5. Distribution of total label appearing in fetal brain fatty acids approximately 5 days after a dose of [U-^{13}C]-18:2n-6, [U-^{13}C]-18:3n-3, or [U-^{13}C]-22:6n-3 was administered to the mother

Fatty Acid	[U-^{13}C]-18:2n-6	[U-^{13}C]-18:3n-3	[U-^{13}C]-22:6n-3
16:0	47.92 ± 6.97	53.24 ± 22.8	11.5 ± 5.9
16:1n-7	ND	1.6 ± 2.77	ND
18:0	7.72 ± 3.33	14.10 ± 8.16	1.37 ± 1.58
18:1n-9	0.68 ± 0.88	8.92 ± 8.01	2.63 ± 1.49
Σ SFA-MFA	56.32 ± 9.45	77.88 ± 19.1	15.5 ± 7.69
18:2n-6	5.91 ± 1.81	ND	ND
20:3n-6	6.25 ± 5.07	ND	ND
20:4n-6	12.41 ± 4.79	0.8 ± 1.3	1.8 ± 3.1
22:4n-6	8.94 ± 0.61	0.42 ± 0.7	ND
22:5n-6	7.91 ± 1.35	0.01 ± 0.01	ND
Σ n-6 LC-PUFA	35.5 ± 8.61	1.20 ± 2.14	1.8 ± 3.1
18:3n-3	ND	ND	ND
20:5n-3	ND	1.94 ± 0.5	2.75 ± 3.25
22:5n-3	0.26 ± 0.3	3.73 ± 4.8	3.91 ± 3.76
22:6n-3	ND	14.17 ± 10.4	69.23 ± 4.07
Σ n-3 LC-PUFA	0.26 ± 0.3	19.84 ± 15.2	75.90 ± 2.91

Data presented as mean % of total label detected in brain fatty acids ± standard deviation; ND, not detected.

Recycled acetate can be used to elongate unlabeled 18:2n-6 and 18:3n-3 to yield labeled LC-PUFA not derived from labeled C_{18} precursors. Very minor amounts of label were found to cross over from n-3 doses into n-6 fatty acids and vice versa. The levels observed were generally below 4% of the total label in fetal tissues and accounted for only a small fraction of LC-PUFA. We conclude that labeled LC-PUFA results predominately from elongation of labeled C_{18} precursors rather than elongation of unlabeled C_{18} with labeled acetate.

SFA-MFA were labeled in all tissues, presumably by partial oxidation of the infused fatty acid to 2C units and synthesis of fatty acids de novo. The absolute amount of label detected in the SFA-MFA in all tissues was similar among the groups. However, the relative

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TABLE 7. Accumulation of label in whole fetal liver, brain, and retina approximately 5 days after a dose of [U-^{13}C]-18:2n–6, [U-^{13}C]-18:3n–3, or [U-^{13}C]-22:6n–3 was administered to the mother

Tissue	[U-^{13}C]-18:2n–6	[U-^{13}C]-18:3n–3	[U-^{13}C]-22:6n–3
Liver	0.075 ± 0.04	0.035 ± 0.005	0.74 ± 0.3
Brain	0.21 ± 0.06^{a}	0.24 ± 0.21^{a,b}	1.7 ± 0.7^{b}
Retina	$1.5 \times 10^{-4} \pm 5.4 \times 10^{-5}$	$2.4 \times 10^{-4} \pm 1.8 \times 10^{-4}$	$6.2 \times 10^{-4} \pm 2.5 \times 10^{-4}$

Data presented as % of administered dose ± standard deviation.
^{a,b}Different superscripts in a row indicate significantly different values ($P < 0.05$).

TABLE 8. Distribution of total label detected in maternal plasma approximately 5 days after a dose of [U-^{13}C]-18:2n–6, [U-^{13}C]-18:3n–3, or [U-^{13}C]-22:6n–3 was administered

Fatty Acid	[U-^{13}C]-18:2n–6	[U-^{13}C]-18:3n–3	[U-^{13}C]-22:6n–3
16:0	ND	7.99 ± 3.47	0.80 ± 1.3
16:1n–7	ND	ND	0.14 ± 0.16
18:0	ND	6.18 ± 7.9	0.84 ± 1.45
18:1n–9	18.22 ± 19.6	0.79 ± 1.38	0.49 ± 0.86
Σ SFA-MFA	18.22 ± 19.6	14.9 ± 10.9	2.27 ± 2.5
18:2n–6	72.15 ± 17.4	ND	ND
20:3n–6	7.08 ± 0.9	ND	ND
20:4n–6	2.06 ± 1.9	3.23 ± 3.7	0.59 ± 0.73
22:4n–6	0.15 ± 0.3	0.33 ± 0.26	0.20 ± 0.23
22:5n–6	0.02 ± 0.05	1.14 ± 0.85	0.23 ± 0.20
Σ n–6 LC-PUFA	81.46 ± 3.2	5.03 ± 4.32	1.02 ± 0.87
18:3n–3	ND	67.39 ± 22.5	0.01 ± 0.02
20:5n–3	ND	3.31 ± 2.8	0.90 ± 0.42
22:5n–3	ND	5.32 ± 3.21	1.33 ± 0.45
22:6n–3	0.09 ± 0.15	3.97 ± 3.9	94.4 ± 2.43
Σ n–3 LC-PUFA	0.09 ± 0.15	12.6 ± 8.2	96.7 ± 3.3

Data presented as mean % of total label ± standard deviation; n = 3 for each group (one animal dosed with 18:2n–6* was not catheterized); ND, not detected.

TABLE 9. Distribution of total label detected in fetal plasma approximately 5 days after a dose of [U-^{13}C]-18:2n–6, [U-^{13}C]-18:3n–3, or [U-^{13}C]-22:6n–3 was administered to the mother

Fatty Acid	[U-^{13}C]-18:2n–6	[U-^{13}C]-18:3n–3	[U-^{13}C]-22:6n–3
16:0	16.19 ± 13.3	14.01 ± 3.42	2.33 ± 2.54
16:1n–7	4.11 ± 6.63	0.67 ± 1.16	0.18 ± 0.26
18:0	0.66 ± 0.69	1.35 ± 2.34	0.23 ± 0.40
18:1n–9	4.85 ± 6.00	ND	ND
Σ SFA-MFA	25.82 ± 18.01	16.04 ± 5.63	2.74 ± 2.69
18:2n–6	61.06 ± 13.4	ND	ND
20:3n–6	4.99 ± 2.88	ND	ND
20:4n–6	7.47 ± 3.57	7.49 ± 7.09	0.55 ± 0.48
22:4n–6	0.08 ± 0.15	0.08 ± 0.14	ND
22:5n–6	0.22 ± 0.45	0.15 ± 0.26	ND
Σ n–6 LC-PUFA	12.92 ± 5.98	7.72 ± 6.72	0.55 ± 0.48
18:3n–3	ND	44.05 ± 22.9	0.05 ± 0.06
20:5n–3	ND	3.61 ± 1.77	ND
22:5n–3	0.11 ± 0.22	7.67 ± 2.37	1.28 ± 1.44
22:6n–3	0.09 ± 0.17	20.90 ± 13.07	95.39 ± 4.26
Σ n–3 LC-PUFA	0.19 ± 0.23	32.18 ± 13.70	96.66 ± 2.83

Data presented as mean % of total fatty acids ± standard deviation; n= 3 for each group (one animal dosed with 18:2n–6* was not catheterized); ND, not detected.

amount of label in SFA-MFA compared to the LC-PUFA(n–3)* or (n–6)* varied among the groups because of the greater incorporation of total label in tissues of the 22:6n–3*-dosed animals. It is interesting that carbon from all three fatty acids was detected in SFA-MFA at similar levels while the fraction of 22:6 n–3* incorporated into tissues was much higher compared to the incorporation of 18:2n–6* and 18:3n–3* as shown in Fig. 1 and Table 7. It is not clear whether there is a physiological advantage for pregnant and lactating non-human primates to expend the energy required to recycle the carbon of n–3 and n–6 fatty acids as SFA-MFA, rather than using them as consumed. One explanation is the presence of LC-PUFA in the diet, which may cause excess LC-PUFA to be converted to less oxidizable SFA-MFA prior to storage.

The dilution of the labeled fatty acids into active endogenous pools must be considered before drawing conclusions from the data. For example, in the liver, the relative concentration of 18:2n–6 is 46-fold higher than the concentration of 18:3n–3. Therefore, the re-

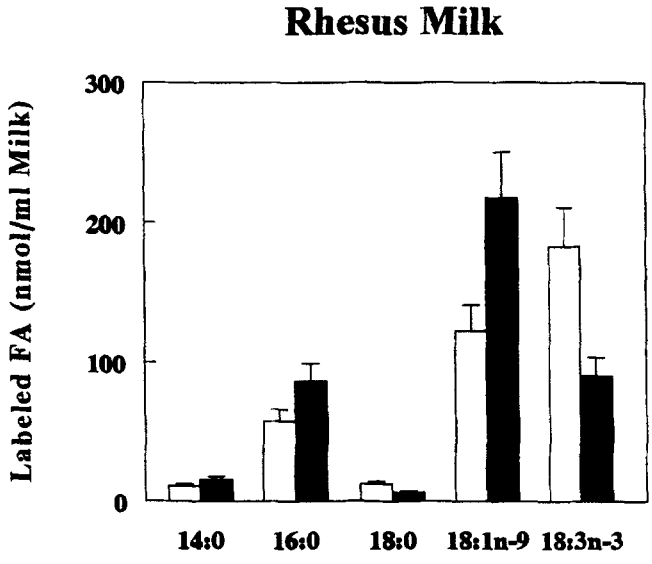

Fig. 3. Level of [U-^{13}C]-18:3n–3 and other ^{13}C-labeled fatty acids in the milk of lactating rhesus monkeys 1 (□) and 7 (■) days after they were administered a dose of [U-^{13}C]-18:3n–3. Error bars reflect standard deviations (n = 3).

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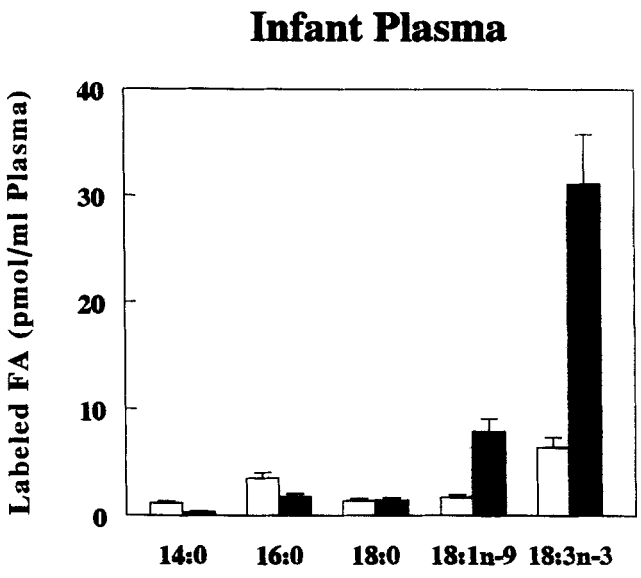

Fig. 4. Level of [U-^{13}C]-18:3n–3 and other ^{13}C-labeled fatty acids in the plasma of suckling infants 1 (▭) and 7 (■) days after their mothers were dosed with [U-^{13}C]-18:3n–3. Error bars reflect standard deviations (n = 2).

cycling of C from 18:2n–6 must be greater than the recycling of C from 18:3n–3 to result in similar levels of labeled SFA-MFA. Similarly, the data suggest that 18:2n–6* and 18:3n–3* were converted to their respective LC-PUFA metabolites at comparable levels in the fetal tissues. However, when the dilution of these fatty acids into the total endogenous tissue pools is taken into consideration, the conversion of 18:2n–6* to LC-PUFA(n–6) is greater than the conversion of 18:3n–3* to LC-PUFA(n–3). Measurement of the active pools is required to evaluate rates of recycling.

The distribution of label in the maternal plasma at CS indicates how the mother metabolized the infused fatty acid and which fatty acids she makes available to the fetus. The presence of labeled SFA-MFA and LC-PUFA in the maternal plasma of all three groups suggests recycling of carbons from 18:2n–6*, 18:3n–3* and 22:6n–3* and conversion of 18:2n–6* and 18:3 n–3* to their respective metabolites is occurring in the mother. It is not known whether the small amount of labeled 20:4n–6 and 22:6n–3 could supply the fetus with adequate amounts of these fatty acids. In this study, dietary LC-PUFA or conversion of essential fatty acids to LC-PUFA by the fetus could also contribute to the incorporation of these molecules in the fetal CNS.

The data from the lactating animals and their infants shows no evidence of enrichment in the LC-PUFA, although label is clearly found in the SFA-MFA. The infant's brain continues to grow at a rapid absolute rate during this period although it is beginning to slow as its body weight growth rate catches up. It is possible that low enrichment levels of LC-PUFA were present in the plasma but were not detected because of the lower dose to the mother compared to the pregnant animals, as well as low levels of conversion. We did not have access to infant tissue other than plasma and therefore cannot determine whether label appeared in the liver, brain, or retina.

In conclusion, this work demonstrates that in chow-fed rhesus monkeys *1*) recycled carbon from 18:2n–6*, 18:3n–3*, and 22:6n–3* appear in SFA-MFA at similar levels in the fetal or infant animal, and at substantial levels in the milk of the lactating animal; *2*) the recycling of C from 18:3n–3* and 18:2n–6* to SFA-MFA* is comparable to the level of 18:2n–6* and 18:3n–3* desaturation/elongation to 20:4n–6* and 22:6n–3*, respectively, in the pregnant animal, and may be greater in the lactating animal and infant; and *3*) in utero, 18:3n–3* is not as efficiently converted to 22:6 n–3* and incorporated into fetal tissues as preformed 22:6n–3*. The elucidation of the physiological significance of the extensive recycling of carbon of n–3 and n–6 fatty acids in the chow-fed animal awaits further investigation.

The authors are grateful to Leah Houghton for dose preparation and to James Winter for careful technical assistance throughout this study. This work was supported by NIH grant EY10208. RCS and KJG acknowledge predoctoral support from NIH training grant DK07158.

Manuscript received 5 January 1996, in revised form 14 August 1996, and in re-revised form 27 September 1996.

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