Fetal baboons convert 18:3n-3 to 22:6n-3 in vivo: a stable isotope tracer study

Hui-Min Su,[1,*] Meng-Chuan Huang,[2,*] Nabil M. R. Saad,[*] Peter W. Nathanielsz,[†] and J. Thomas Brenna[3,*]

Division of Nutritional Sciences,* Savage Hall, and Laboratory for Pregnancy and Newborn Research,† Cornell University, Ithaca, NY 14853

Abstract **Using [^{13}C]-tracers and direct fetal doses, we show for the first time that the fetal primate converts α-linolenic acid (18:3) to docosahexaenoic acid (22:6) in vivo, and we estimate the relative bioefficacy of the two substrates for brain 22:6 accretion. Pregnant female baboons consumed a diet free of long chain polyunsaturates (LCP), with n-6/n-3 ratio of 10/1. In the third trimester of pregnancy (normal gestation = 182 days), they were instrumented with chronic indwelling catheters in the maternal femoral artery and the fetal jugular artery. Doses of either [U-^{13}C]-18:3 (18:3*, n = 3) or [U-^{13}C]-22:6 (22:6*, n = 2) were administered directly to the fetus. Blood was collected from fetus and mother, and the fetus was taken by cesarean section when electromyographic activity indicated that parturition was imminent. Fetal liver, brain, retina, and retinal pigment epithelium (RPE) were collected, and ^{13}C fatty acids determined. In 18:3*-dosed animals, labeled n-3 LCP were detected in fetal plasma at 1 day post-dose and peaked at 2–3 days; brain 22:6* was constant at 3, 5, and 9 days post-dose, at 0.57 ± 0.03 percent of dose (%Dose). In 22:6*-dosed animals, brain 22:6* was similar at 3 and 9 days post-dose (4.64 ± 0.43%Dose). From these data, we estimate that preformed 22:6 in the fetal bloodstream is 8-fold more efficacious for brain 22:6 accretion than is 18:3. Retina 22:6* was stable at about 0.0008%Dose from 3 to 9 days in 18:3-dosed animals, but RPE 22:6* dropped over the period; brain results were consistent with these observations. Liver showed about 0.5%Dose in 22:6* and in intermediary n-3 fatty acid metabolites 20:5* and 22:5* at 3 days post-dose, and declined afterward. Back-transfer of labeled fatty acids to the maternal bloodstream was measurable but not sufficient to compromise the quantitative conversion data in fetuses. We conclude 1) primate fetuses have the capacity to convert 18:3 to 22:6 in vivo; 2) fetal brain 22:6* as %Dose plateaus by 3 days post-dose; 3) fetal plasma 22:6 is about 8-fold more effective as a substrate for brain 22:6 accretion compared with 18:3; and 4) the fetal liver is likely to be an important site of 18:3 to 22:6 conversion.**—Su, H-M., M-C. Huang, N. M. R. Saad, P. W. Nathanielsz, and J. T. Brenna. **Fetal baboons convert 18:n-3 to 22:6n-3 in vivo: a stable isotope tracer study.** ***J. Lipid Res.*** **2000.** 42: **581–586.**

Supplementary key words primates • perinatal • lipid nutrition • polyunsaturated fatty acids • docosahexaenoic acid • linolenic acid • in vivo desaturation • stable isotopes • isotope ratio mass spectrometry • long chain polyunsaturated fatty acid metabolism

Docosahexaenoic acid (22:6n-3) is the major n-3 fatty acid of nervous tissues, particularly the brain and retina (1, 2). Most brain 22:6 accumulates during the period of rapid development (3), which runs from the last trimester of gestation and continues up to 2 years of age in humans (4). The importance of brain 22:6 is related to its significant roles in maintaining neurological and visual development. Decreases in brain and retina 22:6 result in altered visual acuity (5, 6), disturbance in electroretinographic measurements (7, 8) and learning impairment (9) in various species.

A source of n-3 fatty acids is indispensable for the human diet, as mammals do not have the Δ12 and Δ15 desaturases required for de novo synthesis of this family of fatty acids. α-Linolenic acid (18:3n-3) is the predominant precursor of 22:6 in most human diets.

Through a series of alternating desaturation and elongation reactions, 18:3 is converted to long chain polyunsaturates, particularly eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and 22:6. The relative efficiency with which this conversion is performed in part defines the dietary requirements. Human preterm and term infants fed formula with 18:3 as the only source of n-3 fatty acids display a drop in plasma 22:6 levels. Pre-

Abbreviations: AP, atom percent; APE, atom percent excess; CS, cesarean section; dGA, days of gestation age; 22:6, docosahexaenoic acid; FAME, fatty acid methyl esters; FID, flame ionization detection; GC, gas chromatography; GCC-IRMS, gas chromatography-combustion-isotope ratio mass spectrometry; 18:3, α-linolenic acid; IRMS, isotope ratio mass spectrometry; LCP, long chain polyunsaturated fatty acids; 18:3*, [^{13}C]linolenic acid; 20:5*, [^{13}C]eicosapentaenoic acid; 22:5*, [^{13}C]docosapentaenoic acid; 22:6*, [^{13}C]docosahexaenoic acid.

[1] Present address: Kennedy Krieger Institute, John Hopkins Medical Institutions, 707 N. Broadway, Baltimore, MD 21205.

[2] Present address: National Health Research Institutes, Taiwan.

[3] To whom correspondence should be addressed.
e-mail: jtb4@cornell.edu

term infants consistently show compromised visual function compared with those fed 22:6-supplemented formula or reference groups fed breast milk containing a moderate amount of 22:6. Visual function was measured by acuity cards, forced preferential looking, or visually evoked potentials (10–12). Infant rhesus monkey studies demonstrate that depletion of brain 22:6 in utero and neonatally causes functional deficiencies in vision that persist despite later biochemical repletion of brain and retinal 22:6 levels (5, 6).

The maternal circulation supplies n-3 fatty acids to the fetus in the form of 18:3 or preformed long chain polyunsaturates (LCP). Further metabolism by the fetal organs can convert 18:3 and intermediate LCP to 22:6 for incorporation into brain structural lipids, if the requisite enzymes are expressed in utero. Support for this possibility has been demonstrated by in vitro investigations showing significant Δ6 and Δ5 desaturase activities expressed in human fetal liver microsomes as early as 17–18 weeks of gestation (13, 14) and in prenatal rat liver and brain (15). Furthermore, recent stable isotope tracer studies unambiguously demonstrate that human preterm infants are capable of synthesizing 22:6 in vivo subsequent to enteral administration of ^{13}C-labeled 18:3 (16–18). However, there are no reports showing whether a primate fetus expresses the full pathway required for synthesis of 22:6 from 18:3 in vivo.

Recently, we have reported that the relative efficacy of 18:3 or 22:6 as a precursor for fetal brain 22:6 accumulation is about 20/1 following iv doses to the maternal bloodstream of pregnant baboons (19). These data do not directly demonstrate that the fetus is capable of converting 18:3 to 22:6, as reported in human neonates, because fetal 22:6 accretion can originate from maternal conversion prior to transport to the fetus. For this, direct doses of labeled 18:3 to the fetus are required.

The purpose of this study is to establish whether nonhuman fetal primates are capable of synthesizing 22:6 from 18:3 in vivo and, secondarily, to estimate the relative efficacy of 18:3 and 22:6 as substrate for 22:6 accumulation in fetal baboon brain and associated organs using ^{13}C stable isotope methodology, with analysis by high precision isotope ratio mass spectrometry (IRMS).

MATERIALS AND METHODS

Animals

Pregnant baboons (*Papio cynocephalus*) were bred at the Southwest Foundation for Biomedical Research (San Antonio, TX) or at the University of Illinois at Chicago (Chicago, IL) and transported to the Laboratory for Pregnant and Newborn Research at Cornell University (Ithaca, NY). The Cornell Institutional Animal Care and Use Committee approved the care of the animals, and the American Association for Laboratory Animal Care (AALAC) approved the facility. A complete veterinary examination was performed on all pregnant baboons upon arrival, and they were housed individually in cages in sight of at least one other baboon and a video showing other baboons. The room temperature and humidity were maintained at 24°C and 70%, respectively, with a 14-h light cycle and a 10-h dark cycle. After an acclimation period, animals were jacketed with a flexible tether and swivel. After at least 1 week of acclimation to the jacket, pregnant baboons and their fetuses were instrumented with catheters to permit continuous access to the bloodstream via a tether, and electromyography leads were attached to the uterus. These procedures have been described in detail elsewhere (20, 21).

Diets and dose

Substrate and product feedbacks are common phenomena in a wide variety of physiological processes. Uncontrolled tracer dilution by dietary tracee (n-3 fatty acids) or other fatty acids would lead to unreliable kinetics as well as uncertain effects on conversion, possibly due to competition for desaturation and elongation enzymes with n-3 fatty acids (22). To minimize possible effects on 18:3 to 22:6 conversion, mother baboons were fed, for at least the last 8 weeks of pregnancy, an LCP-free diet containing controlled levels of linoleic acid (18:2n-6) and 18:3. The diet had 2% of energy as 18:2, and 0.2% of energy as 18:3 (18:2/18:3 = 10) (Harlan Teklad, Madison, WI). The diet fatty acid composition was reported previously (19). Animals consumed this diet for at least 8 weeks before the administration of dose, and continued until cesarean section (CS).

A tracer dose of 2.24 ± 0.42 mg [U-^{13}C]-18:3 (18:3*) (n = 5) or 0.95 ± 0.11 mg [U-^{13}C]-22:6 (22:6*) (n = 2) was administered to the fetal jugular vein via a catheter in the third trimester. Experimental animal characteristics, including weights, and gestational ages, as well as dose administered, are presented in **Table 1**. The 18:3* or 22:6* was purified from an [U-^{13}C]algal oil (Martek Biosciences, Columbia, MD) as described previously

TABLE 1. Characteristics of animals used in study

	Baboon						
	1	2	3	4[f]	5[f]	6	7
Fatty acid dose	18:3n-3	18:3n-3	18:3n-3	18:3n-3	18:3n-3	22:6n-3	22:6n-3
Dose[a] (mg)	2.55	2.41	1.77	2.25	1.93	1.02	0.83
dGA at dosing[b]	139	136	137	135	146	130	131
dGA at CS[c]	142	141	146			133	140
Dosing period (d)	3	5	9			3	9
Maternal weight[d] (kg)	16	14	18	16	19	13	18
Samples collected[e]	T,F,M	T,M	T,F	F,M	F,M	T,M	T

[a] [U-^{13}C]-linolenic acid (18:3n-3*) was in a free fatty acid form, blended with Intralipid.
[b] Age of fetus (days of gestation) at dose administration.
[c] Age of fetus (days of gestation) at cesarean section.
[d] Maternal weight at the time of maternal catheterization near or at the cesarean section.
[e] T, fetal tissues; F, fetal plasma; M, maternal plasma.
[f] No tissue was available from these animals; plasma was collected from mother and fetus.

(23). The dose was sonicated into 0.5 ml of 20% Intralipid (KaviVitrum, Franklin, OH), an intravenous emulsion consisting primarily of LCP-free soybean oil with trace LCP added incidentally as a component of egg lecithin emulsifier, and was diluted with 1.5 ml of sterile saline approximately 12 h before dosing.

Sampling

For fetal plasma, baseline samples were drawn prior to dosing via a fetal carotid artery catheter, then after dosing once per day as available. Maternal baseline plasma was initially drawn from maternal femoral artery catheters, then drawn daily after dosing. Pregnancies were allowed to continue until electromyographic activity indicated that labor was imminent. At that time, CS was performed and the time recorded between dose administration and CS, as shown in Table 1. The fetus, continuously under halothane general anesthesia from the time of CS, was euthanized by exsanguination and fetal tissues collected immediately. The brain (occipital lobes) and liver were removed quickly, weighed, wrapped in aluminum foil, and frozen in liquid N_2. Retina and retinal pigment epithelium (RPE) were immediately dissected from the eyes, separated, collected, and stored in saline. All samples were kept in a freezer at −80°C until analysis.

Lipid extraction, fatty acid analysis, and tracer analysis

Total lipids were extracted from tissue homogenates by the method of Bligh and Dyer (24), and fatty acid methyl esters (FAME) prepared using 14% BF_3 in methanol. A known amount of fresh heptadecanoic acid (17:0; 99+% pure, Sigma Chemicals, St. Louis, MO) was added as an internal standard to the tissue homogenate prior to extraction. The purified FAME were dissolved in hexane with butylated hydroxytoluene (BHT) as an antioxidant, flushed with N_2, and stored in a −20°C freezer until analysis.

FAME were analyzed with a Hewlett Packard 5890 Series II gas chromatograph with flame ionization detector (GC-FID) using H_2 carrier gas. Quantitative profiles were calculated using the internal standard and an equal weight mixture to derive response factors for each fatty acid. GC conditions and calibration details are reported elsewhere (25). Tracer analysis was performed with a high precision gas chromatography-combustion-isotope ratio mass spectrometer (GCC-IRMS), described in detail previously (26, 27).

Calculations

The concentration of tracer in tissues was calculated from the isotopic enrichment measured by GCC-IRMS and the quantity of fatty acids determined by GC-FID, as has been described in detail elsewhere (26, 27). The molar amount of 18:3* present in any fatty acid pool is calculated as the product of the total fatty acid per unit of tissue and the atom fraction excess (AFE), corrected for the ratio of carbon in analyte fatty acid to that in 18:3* (28). For 18:3 this factor is 1; for C20 and C22 fatty acids it is 20/18 and 22/18, respectively. The final reported tracer concentration, therefore, refers to the mol of nascent (dose) 18:3* that have entered the particular pool.

Percent of dose (%Dose) was calculated from each labeled n-3 fatty acid (FA*) in each pool, divided by the total dose to each animal, multiplied by 100 and normalized to pool units (per liter plasma or per g whole organ). %Dose adjusts for different dose sizes among the animals and ensures that the differences are related to function rather than dose size. The fetal and maternal plasma kinetic curves were evaluated as mol %Dose per 100 ml of plasma at each sampling time point. Data are reported as %Dose found in whole fetal brain or liver, or per single retina, per single RPE. Least-squares fits through the data are presented to aid the eye, using Excel 2000 for Windows98 (Microsoft, Seattle, WA).

RESULTS

Fetal and maternal plasma

Fetal plasma kinetic curves for n-3 labeled fatty acids after a 18:3* dose to fetal baboons from 1 to 9 days post-dose are illustrated in **Fig. 1A** and **B.** Figure 1A shows that 18:3* dropped to 50% of its initial concentration at 2 days post-dose; 99% of 18:3* had disappeared by 8 days post-dose. About 10%Dose as 18:3* per deciliter fetal plasma was detected at 1 day post-dose. Figure 1B shows that 20:5* and 22:5* peaked at 2 days post-dose; in comparison, 22:6 appears slightly delayed, with its maximal mean level observed at 3 days post-dose.

Maternal plasma kinetic curves for n-3 labeled metabolites from 3 h to 4 days post-dose are presented in **Fig. 2.** 18:3* was detected as early as 3 h post-dose and increased at 5 h. Half the 18:3* disappeared by 1 day post-dose, and it continued to drop gradually. LCP* were detected at 3 h post-dose. By 1 day post-dose, 20:5* showed a mild peak and then gently dropped. 22:5* plateaued up to day 4 post-dose; 22:6* was not detected at 3 h but then gradually increased and had plateaued by 4 days.

Fetal liver

The time course of incorporation of n-3 fatty acids following an 18:3* or 22:6* iv dose to 3 fetuses is shown in **Fig. 3A** and **B**. Each point represents a single animal, for which the time point is plotted as the period between dosing and CS, at 3, 5, or 9 days. For 18:3*-dosed animals, 18:3* and its metabolites, 20:5*, 22:5*, and 22:6* were all detected at 3 days post-dose, and all labeled n-3 FA* gradually decreased at longer times post-dose.

A similar pattern was observed for two 22:6*-dosed groups. Three days after the fetal animal was injected with

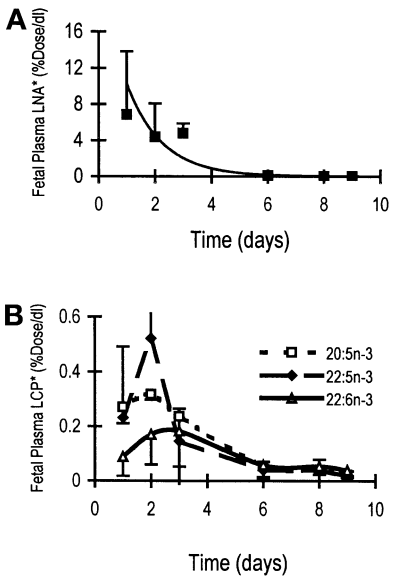

Fig. 1. A: Fetal plasma 18:3* kinetics after an 18:3* dose, expressed as %Dose per deciliter plasma. Data are presented as mean ± SD with n ≥ 2. An exponential least-squares fit is shown ($r^2 = 0.97$). B: Fetal plasma n-3 LCP* kinetics, expressed as %Dose. The lines connect the points.

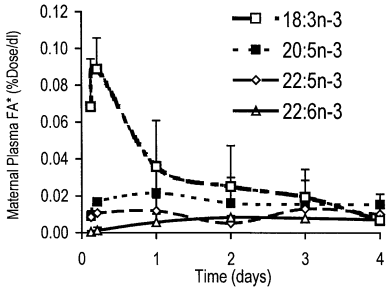

Fig. 2. Maternal plasma n-3 FA* kinetics after an 18:3* dose, expressed as %Dose per deciliter plasma. Data are presented as mean ± SD with n ⩾ 2.

22:6*, approximately 15%Dose was recovered as liver 22:6*. This amount dropped to 2.4% at 9 days post-dose. Liver 22:6* comprised 90–97% of all n-3 FA* measured in 22:6* dosed animals.

Fetal brain, retina, RPE

Neural tissue 22:6* resulting from 18:3* or 22:6* fetal dosing in the whole brain, retina, and RPE is presented in **Table 2**. The incorporation of 22:6* derived from preformed 22:6* was greater than that from the 18:3* dose. For both groups, brain 22:6* reached a plateau by the first time point investigated, 3 days post-dose. The average brain 22:6* plateau levels were 0.57 ± 0.03% and 4.64 ± 0.43% of the 18:3* and preformed 22:6* doses, respectively. From these data, we construct a ratio that indicates that preformed 22:6* was 8-fold more efficiently incorporated as brain lipids as compared with 18:3-derived 22:6*.

As observed in the brain, preformed 22:6* was preferentially incorporated as retina and RPE 22:6* as compared with 18:3-derived 22:6*. The range of 22:6* incorporation resulting from the 18:3* dose was 0.0007–0.0010%Dose per retina, whereas that from the 22:6* dose was 0.010–0.012%. No time-dependent changes can be discerned from these modest differences, therefore we pooled the data from the animals in each group to arrive at a relative efficacy of 13:1 in favor of 22:6 over 18:3 for retinal 22:6.

In contrast to retina, RPE 22:6* content declined sharply during the 3–9 days post-dose in both groups. For 18:3*-dosed animals, 18:3-derived* 22:6* dropped from 0.0006–0.0001%, whereas a drop from 0.005–0.002%Dose was observed for the two 22:6*-dosed animals. In animals dosed with 18:3*, 22:6* dropped for 3–5 days post-dose and then remained constant.

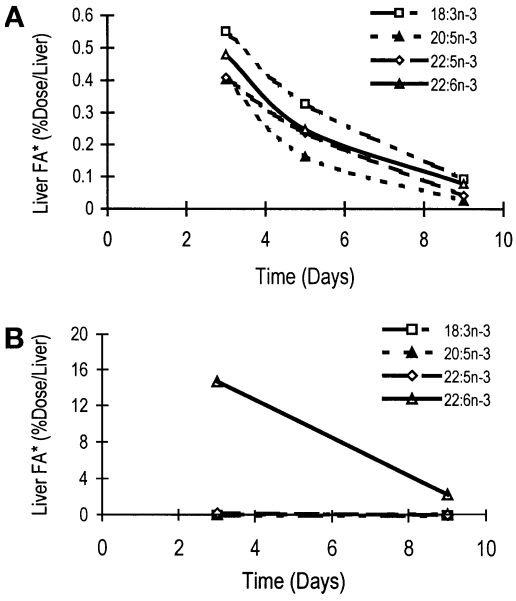

Fig. 3. Fetal liver n-3 FA* kinetics. Each point represent results from a single animal and is expressed as %Dose found in the whole organs. A: Fetal liver n-3 FA* kinetics resulting from 18:3* dose. B: Fetal liver n-3 FA concentrations at 2 times post-dose after a 22:6* dose.

TABLE 2. 22:6* accretion (%Dose) in fetal baboons following a fetal iv dose of 18:3* ([U-^{13}C]-18:3n-3) or 22:6* ([U-^{13}C]-22:6n-3)[a]

Organ	Time Day	%Dose as 22:6* *18:3 dosed[b]	%Dose as 22:6* *22:6 dosed[c]	Ratio[d]
Brain	3	0.56	4.94	8
	5	0.60	—	
	9	0.54	4.33	
Retina	3	0.0007	0.0098	13
	5	0.0007	—	
	9	0.0010	0.0112	
RPE	3	0.0006	0.005	
	5	0.0002	—	
	9	0.0001	0.002	

[a] Whole organ 22:6* (22:6n-3*) accretion due to doses, or either 18:3* ([U-^{13}C]-linolenic acid; 18:3n-3*) or 22:6* ([U-^{13}C]-docosahexaenoic acid; 22:6n-3*), expressed as percent of dose (%Dose). Each time point (day) represents separate animal; errors average 10%CV, and are associated only with the workup and chemical analyses.

[b] 18:3* dose was administered iv to fetal baboons; organs were collected at 3, 5, and 9 days post-dose.

[c] 22:6* dose was administered iv to fetal baboons; organs were collected at 3 and 9 days post-dose.

[d] Ratio: (18:3–derived 22:6)/(preformed 22:6–derived 22:6). In the brain, 22:6 derived from 18:3 (18:3* dose) was approximately 8-fold lower compared with that from preformed 22:6 (22:6*dose). Since brain 22:6* plateaus, we take this ratio as the bioequivalence. In the retina, 22:6 derived from 18:3 (18:3* dose) was approximately 13-fold lower compared with that from preformed 22:6 (22:6*dose). The ratio for the retinal pigment epithelium (RPE) is not calculated because 22:6* accretion did not plateau over the measurement period.

DISCUSSION

The goal of this study was to determine whether the fetal primate is capable of synthesizing 22:6 from its precursor, 18:3, in vivo. Back-transfer of the fetal dose to the maternal circulation was sufficient to induce detectable levels of labeled 18:3* in the maternal plasma for 1 day. Since the maternal liver is known to be capable of biosynthesizing 22:6 from 18:3, the possibility arises that the 18:3* entering the mother might have been taken up by her liver, converted to 22:6*, transferred back to the fetus, then incorporated into fetal organs. In this study, the data presented in Fig. 2 show that n-3 LCP* appear in the maternal bloodstream within 5 h of dosing, with total labeled n-3 metabolites at 0.12%Dose/dl. Compelling evidence shows that this is not the sole source of the fetal 22:6*. In previ-

ous work, 18:3* doses to the mother accumulated in fetal brain 22:6* at their maximal level of 0.075%Dose after about 10 days (19). This accretion level represents an upper bound for 18:3-derived-22:6 accretion in the present fetuses because, in the present experiment, the dose must be transferred from fetus to mother prior to maternal metabolism. Our measurements for fetal doses, shown in Table 2, indicate a plateau level of 0.6%Dose as 18:3-derived 22:6*. We conclude that maternal metabolism of the 18:3* dose accounts for a negligible fraction of the 22:6* found in the fetus, and that the primate fetus does have the full capacity to convert 18:3 to 22:6.

In this study, 18:3 or 22:6 as nonesterified fatty acid was administered to baboon fetuses via iv injection into the jugular artery as a component of a lipid emulsion. It is known that polyunsaturated fatty acids arrive at the maternal placenta either as albumin-bound nonesterified fatty acids or as triglycerides or phospholipids as components of lipoprotein particles (29). For the latter to be transported across the placenta, maternal lipoprotein lipase must liberate free fatty acids (30). In fetal circulation, they are carried via fetal plasma proteins such as albumin (30) or α-fetoprotein, which has been shown to bind 22:6 in the baboon fetus (31). These considerations suggest that intravenous administration of tracer into the fetal bloodstream as nonesterified fatty acids is very similar to the normal physiological form.

The sum of all labeled fatty acids found in liver and brain were similar at about 1.2–1.3%Dose at the first time point in the 18:3*-dosed group. These data are consistent with previous measurements showing that recovery of labeled fatty acids is less than about 3% in the major organs (19, 28). At 3 days post-dose, about the same amount of 18:3*-derived 22:6 was found in liver (0.4%Dose) and brain (0.6%Dose), whereas 15%Dose and 4.9%Dose were found as 22:6* for preformed 22:6*-dosed animals in liver and brain, respectively. Kinetics of the latter were markedly different, however, as brain 22:6* was stable across time, but the liver 18:3* and LCP* dropped dramatically to 10–15% of their original values by day 9. These findings are consistent with our hypothesis that brain 22:6 turnover is very slow, and also with the liver's known role as a major site of unsaturated fatty acid metabolism (28). The data do not permit an estimation of the relative amount of 22:6 synthesized from precursors in brain versus that imported from the plasma after synthesis in other organs.

These data clearly show that primate brain 22:6 accretion is more efficient with preformed 22:6 compared with 18:3, which is consistent with our previous studies (19, 28). In the present study, we observed that brain 22:6* accretion in fetal baboons dosed either with 18:3* or 22:6* reached a plateau level at day 9, starting from 3 days post-dose, suggesting the brain 22:6 turnover rate is small.

The 22:6 accretion from 18:3* was 0.6%Dose, and that from 22:6* was 4.6%Dose. Because the turnover of brain 22:6 is very low, we can take this as the difference in relative efficacy for these two sources of 22:6. The ratio of these figures, 8:1, is remarkably similar to the bioequivalence reported previously for primate neonates, which yielded a ratio of 7:1 (28). These data can also be compared with those from similar experiments with pregnant baboons in which the relative efficacy of iv doses to pregnant mothers yielded a ratio of 20:1 in favor of preformed 22:6 compared with 18:3-derived 22:6 (19). Apparently, once 18:3 or 22:6 enters the perinatal bloodstream, the two fatty acids are metabolized similarly from a relative, quantitative standpoint.

The 18:3 content of the maternal diet was a modest 0.2% of energy, whereas the ratio of 18:2 to 18:3 was 10:1 and is typical of Western diets. Low 18:3 intakes should optimize conversion, as efficiency should fall with increasing substrate availability, whereas the high ratio provides realistic competition for conversion from 18:2. Clearly, the efficacy ratio of 8:1 applies only to diets with fatty acid composition in this range, and cannot be applied to diets with very different total or relative fatty acid composition.

RPE 22:6* disappeared sharply in the 9-day animals, whereas retina 22:6* deposition was unchanged in both dosing groups. The accretion ratio for retina calculated from the pooled data yielded a ratio of 13:1, which, again, is remarkably similar to our measured ratio of 12:1 in neonates (28). Besides being a site for 22:6 synthesis in vitro (32), recent studies show that RPE is actively involved in the preferential uptake of C22 LCP from choriocapillaris, actively transporting them to photoreceptor cells; this is thought to be a mechanism for conservation of 22:6 in the retina (33). In the 18:3*-dosed animals, we observed that accretion of RPE 22:5* was greater than that of 22:6* (data not presented), a finding that is consistent with this former role. The 70–80% drop in RPE 22:6* in both dosing groups is consistent with active exchange of 22:6* between RPE and retina.

No 18:3n-3* was detected in brain, retina, and RPE. It has long been known that these organs are very low in 18:3, despite measurements that suggest that 18:3 traverses the blood-brain barrier. 18:3n-3* is apparently rapidly metabolized to n-3 LCP* once it passes the blood-brain barrier. Further studies are needed to clarify the transport mechanisms and initial metabolism of n-3 fatty acids in the brain and nervous system.

Previous studies have reported on the relative accretion of preformed LCP compared with their 18 carbon precursors. Using radio-labeled fatty acids, Sinclair (34) found that an oral dose of 18:3 and 22:6 to 2-week-old suckling rats led to the accumulation at 22 h of 59 times more labeled 22:6 in the brain from 22:6 than from 18:3. The large difference between these results and our data is probably due to the short post-dose sampling time in Sinclair's study. A similar advantage in favor of the long chain polyunsaturate was noted by Hassma and Crawford (35) for orally fed 20:4n-6 versus 18:2n-6. In nontracer studies, 1.1% dietary 22:6 was reported to be more efficient in supporting 22:6 deposition in the brain than 25% dietary 18:3 in rat pups. Even this extreme dietary level of 18:3 did not match brain 22:6 levels achieved with physiological levels of dietary 22:6 (36). This observation is consistent with a study conducted in newborn piglets showing that 0.4% kcal as C20 and C22 n-3 LCP were as effective as 1.7% of dietary kcal as 18:3 in supporting levels of 22:6 in growing piglet synaptic plasma membrane and retina (37), and

with a recent study showing that dietary 22:6 is preferred 10-fold compared with 18:3 in guinea pigs (38). It should be pointed out, however, that there is no clear evidence that the lower brain 22:6 levels achieved by 18:3-fed animals is not sufficient to fully optimize neural function.

In summary, the present data demonstrate for the first time 1) that the primate fetus has the capacity to synthesize 22:6 from 18:3 in vivo, 2) that both 18:3-derived 22:6* and preformed 22:6-derived 22:6* levels plateau in the fetal brain by 3 days at a ratio of about 8:1, and 3) that labeled n-3 LCP derived from an 18:3* or 22:6* dosing drop in fetal liver, consistent with an active transport mechanism that preferentially secretes these long chain metabolites for delivery to vital organs such as brain and retina.

This work was supported by NIH grant EY10208.

Manuscrips received 25 September and in revised form 30 November 2000.

REFERENCES

1. Innis, S. M. 1991. Essential fatty acids in growth and development. *Prog. Lipid Res.* **30:** 39–103.
2. Sastry, P. S. 1985. Lipids of nervous tissue: composition and metabolism. *Prog. Lipid Res.* **24:** 69–176.
3. Martinez, M. 1992. Tissue levels of polyunsaturated fatty acids during early human development. *J. Pediatr.* **120:** S129–S138.
4. Dobbing, J., and J. Sands. 1979. Comparative aspects of the brain growth spurts. *Early Hum. Dev.* **3:** 79–83.
5. Neuringer, M., W. E. Connor, C. Van Petten, and L. Barstad. 1984. Dietary omega-3-fatty acid deficiency and visual loss in infant rhesus monkeys. *J. Clin. Invest.* **73:** 272–276.
6. Neuringer, M., W. E. Connor, D. S. Lin, L. Barstad, and S. Luck. 1986. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc. Natl. Acad. Sci. USA.* **83:** 4021–4025.
7. Wheeler, T. G., R. M. Benolken, and R. E. Anderson. 1975. Visual membranes: specificity of fatty acid precursors of the electrical response to illumination. *Science.* **188:** 1312–1314.
8. Bourre, J. M., M. Francois, A. Youyou, O. Dumont, M. Piciotti, G. Pascal, and G. Durand. 1989. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic-activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning-tasks in rats. *J. Nutr.* **119:** 1880–1892.
9. Yamamoto, N., M. Saitoh, A. Moriuchi, M. Nomura, and H. Okuyama. 1987. Effect of dietary alpha-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid Res.* **28:** 144–151.
10. Uauy, R. D., D. G. Birch, E. E. Birch, J. E. Tyson, and D. R. Hoffman. 1990. Effect of dietary omega-3-fatty-acids on retinal function of very-low-birth-weight neonates. *Ped. Res.* **28:** 485–492.
11. Birch, E. E., D. G. Birch, D. R. Hoffman, and R. Uauy. 1992. Dietary essential fatty acid supply and visual acuity development. *Invest. Ophthamol. Vis. Sci.* **33:** 3242–3253.
12. SanGiovanni, J. P., S. Parra-Cabrera, G. A. Colditz, C. S. Berkey, and J. T. Dwyer. 2000. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics.* **105:** 1292–1298.
13. Chambaz, J., D. Ravel, M. C. Manier, D. Pepin, N. Mulliez, and G. Bereziat. 1985. Essential fatty acids interconversion in the human fetal liver. *Biol. Neonate.* **47:** 136–140.
14. Rodriguez, A., P. Sarda, C. Nessmann, P. Boulot, C. L. Leger, and B. Descomps. 1998. Delta6- and delta5-desaturase activities in the human fetal liver: kinetic aspects. *J. Lipid Res.* **39:** 1825–1832.
15. Bourre, J. M., M. Piciotti, and O. Dumont. 1990. Delta-6 desaturase in brain and liver during development and aging. *Lipids.* **25:** 354–356.
16. Carnielli, V. P., D. J. L. Wattimena, I. H. T. Luijendijk, A. Boerlage, H. J. Degenhart, and P. J. J. Sauer. 1996. The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. *Ped. Res.* **40:** 169–174.
17. Salem, J. N., B. Wegher, P. Mena, and R. Uauy. 1996. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc. Nat. Acad. Sci. USA.* **93:** 49–54.
18. Sauerwald, T. U., D. L. Hachey, C. L. Jensen, H. Chen, R. E. Anderson, and W. C. Heird. 1997. Intermediates in endogenous synthesis of C22:6 omega 3 and C20:4 omega 6 by term and preterm infants. *Ped. Res.* **41:** 183–187.
19. Greiner, R. C., J. Winter, P. W. Nathanielsz, and J. T. Brenna. 1997. Brain docosahexaenoate accretion in fetal baboons: bioequivalence of dietary alpha-linolenic and docosahexaenoic acids. *Ped. Res.* **42:** 826–834.
20. Nathanielsz, P. W., J. P. Figueroa, and E. R. Poore. 1984. Methods of investigation of the chronically instrumented pregnant maintain on a tether and swivel system. In Animal Models in Fetal Medicine. P. W. Nathanielsz, editor. Perinatology Press, Ithaca, NY. 110–160.
21. Morgan, M. A., S. L. Silavin, M. Randolph, G. G. Payne, R. E. Sheldon, J. I. Fishburne, R. A. Wentworth, and P. W. Nathanielsz. 1991. Effect of intravenous cocaine on uterine blood flow in the gravid baboon. *Am. J. Obstet. Gynecol.* **164:** 1021–1030.
22. Holman, R. T. 1998. The slow discovery of the importance of omega 3 essential fatty acids in human health [see comments]. *J. Nutr.* **128(2 Suppl):** 427S–433S.
23. Sheaff, R. C., H-M. Su, L. A. Keswick, and J. T. Brenna. 1995. Conversion of α-linolenate to docosahexaenoate is not depressed by high dietary levels of linoleate in young rats: tracer evidence using high precision mass spectrometry. *J. Lipid Res.* **36:** 998–1008.
24. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
25. Su, H-M., and J. T. Brenna. 1998. Simultaneous measurement of desaturase activities using stable isotope tracers or a non-tracer method. *Anal. Biochem.* **261:** 43–50.
26. Brenna, J. T., T. N. Corso, H. J. Tobias, and R. J. Caimi. 1997. High-precision continuous-flow isotope ratio mass spectrometry. *Mass Spectrom. Rev.* **16:** 227–258.
27. Goodman, K. J., and J. T. Brenna. 1992. High sensitivity tracer detection using high precision gas chromatography combustion isotope ratio mass spectrometry and highly enriched [U-13C]-labeled precursors. *Anal. Chem.* **64:** 1088–1095.
28. Su, H-M., L. Bernardo, M. Mirmiran, X. H. Ma, T. N. Corso, P. W. Nathanielsz, and J. T. Brenna. 1999. Bioequivalence of dietary alpha-linolenic and docosahexaenoic acids as sources of docosahexaenoate accretion in brain and associated organs of neonatal baboons. *Pediatr. Res.* **45:** 87–93.
29. Stammers, J., T. Stephenson, J. Colley, and D. Hull. 1995. Effect on placental transfer of exogenous lipid administered to the pregnant rabbit. *Pediatr. Res.* **38:** 1026–1031.
30. Dutta-Roy, A. K. 2000. Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. *Am. J. Clin. Nutr.* **71 (1 Suppl):** 315S–322S.
31. Naval, J., M. Calvo, J. Laborda, P. Dubouch, M. Frain, J. M. Sala-Trepat, and J. Uriel. 1992. Expression of mRNAs for alpha-fetoprotein (AFP) and albumin and incorporation of AFP and docosahexaenoic acid in baboon fetuses. *J. Biochem. (Tokyo).* **111:** 649–654.
32. Wang, N., and R. E. Anderson. 1993. Synthesis of docosahexaenoic acid by retina and retinal pigment epithelium. *Biochemistry.* **32:** 13703–13709.
33. Bazan, N. G., E. B. Rodriguez de Turco, and W. C. Gordon. 1994. Docosahexaenoic acid supply to the retina and its conservation in photoreceptor cells by active retinal pigment epithelium-mediated recycling. *World Rev. Nutr. Diet.* **75:** 120–123.
34. Sinclair, A. J. 1975. Incorporation of radioactive polyunsaturated fatty acids into liver and brain of developing rat. *Lipids.* **10:** 175–184.
35. Hassma, A. G., and M. A. Crawford. 1976. The differential incorporation of labelled linoleic, gamma-linolenic, dihomo-gamma-linolenic and arachidonic acids into the developing rat brain. *J. Neurochem.* **27:** 967–968.
36. Woods, J., G. Ward, and N. J. Salem. 1996. Is docosahexaenoic acid necessary in infant formula? Evaluation of high linolenate diets in the neonatal rat. *Pediatr. Res.* **40:** 687–694.
37. Arbuckle, L. D., and S. M. Innis. 1992. Docosahexaenoic acid in developing brain and retina of piglets fed high or low alpha-linolenate formula with and without fish oil. *Lipids.* **27:** 89–93.
38. Abedin, L., E. L. Lien, A. J. Vingrys, and A. J. Sinclair. 1999. The effects of dietary alpha-linolenic acid compared with docosahexaenoic acid on brain, retina, liver, and heart in the guinea pig. *Lipids.* **34:** 475–482.