Linoleic acid kinetics and conversion to arachidonic acid in the pregnant and fetal baboon

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Abstract **Linoleic acid plasma kinetics in pregnant baboons and its conversion to long chain polyunsaturates (LCP) in fetal organs is characterized over a 29-day period using stable isotope tracers. Pregnant baboons consumed an LCP-free diet and received [U-^{13}C]linoleic acid (18:2*) in their third trimester of gestation. In maternal plasma, 18:2* dropped to near baseline by 14 days post-dose, while labeled arachidonic acid (20:4*) plateaued at 10 days at about 70% of total labeled fatty acids. After 2–5 days, total tracer fatty acids decreased in visceral organs, but increased in the fetal brain. Maximal fetal incorporation of 18:2* was 1–2 days post-dose; thereafter it dropped while 20:4* increased reciprocally. Labeled 20:4 replaced 18:2* in neural tissues by 5 days post-dose. In liver, kidney, and lung, 20:4* became dominant by 12 days, but in heart the crossover was >29 days. Fetal brain 20:4* plateaued by 21 days at 0.025% of dose, while fetal liver 20:4* was constant from 1 to 29 days at 0.006% of dose. Under these dietary conditions we estimate that the fetus derives about 50% its 20:4 requirement from conversion of dietary 18:2, with the balance from maternal stores, and conclude that *1*) fetal organs accumulate 18:2 within a day of a maternal dose and convert much of it to 20:4 within weeks, *2*) modest dietary 18:2 levels may support fetal brain requirements for 20:4, and *3*) the brain retains n–6 fatty acids uniquely compared with major visceral organs.**—Su, H-M., T. N. Corso, P. W. Nathanielsz, and J. T. Brenna. **Linoleic acid kinetics and converstion to arachidonic acid in the pregnant and fetal baboon.** ***J. Lipid Res.*** 40: **1304–1311.**

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Linoleic acid (18:2n–6) is an essential fatty acid (EFA) (1) in part because of its role as the precursor of n–6 long chain polyunsaturated fatty acids (LCP), particularly arachidonic acid (20:4n–6) and di-homo-γ-linoleic acid (20:3n–6), and possibly docosapentaenoic acid (22:5n–6). These fatty acids are components of membrane structural lipids, and the C20 fatty acids are immediate precursors of eicosanoids. In addition, 20:4 is the most prominent polyunsaturated fatty acid (PUFA) in neural tissue and in the brain, comprising about 11% of total fatty acids, by weight (2–4). Arachidonate rapidly accumulates in the brain during development (5, 6), which in humans takes place from the beginning of the third trimester of gestation up to about 2 years of age (7, 8). Deficiency of unsaturated fatty acids results in a well-known suite of overt symptoms including growth retardation, skin lesions, and reproductive failure (9), some of which are directly attributable to deficiency of 18:2 (10) and some related to eicosanoid function.

Arachidonate is indispensable for growth in developing animals (11), where it plays important roles in cell division (12–14) and signaling (12, 15). Four studies in humans have now been reported that implicate 20:4 as a critical factor for growth in premature infants (16–19) and, by extension, in fetal life. The role of 20:4 as an eicosanoid precursor is central to proper signal transduction in a variety of processes including immune and inflammatory responses (12, 15, 20).

Because mammals cannot synthesize n–6 fatty acids de novo, either 18:2 or 20:4 must cross the placenta to supply fetal demand. Arachidonate is synthesized in adult mammals by sequential Δ6-desaturation, elongation, and Δ5-desaturation, resulting in the addition of 2 carbons and the insertion of 2 double bonds. This metabolic pathway is most active in the liver but is known to operate in other tissues, including the brain (21, 22). However, it is not yet established whether the primate fetus has the ability to synthesize 20:4 from 18:2 in vivo, and thus it is not known whether the conversion is predominately done by the mother prior to transport across the placenta or by the fetus

Abbreviations: AFE, atom fraction excess; BHT, butylated hydroxytoluene; CS, cesarean section; EFA, essential fatty acids; FA, fatty acids; FAME, fatty acid methyl esters; GC–FID, gas chromatography–flame ionization detection; GCC–IRMS, gas chromatography–combustion isotope ratio mass spectrometry; LCP, long chain polyunsaturated fatty acids; NEFA, nonessential fatty acids; PUFA, polyunsaturated fatty acids; RPE, retinal pigment epithelium.

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directly. Pregnant women tend to have lower plasma PUFA concentrations than in the non-pregnant, non-lactating state, suggesting a higher demand during pregnancy (23, 24) which could be related to fetal demands or those of the maternal conceptus.

There is some evidence that excess dietary 18:2 levels may not promote optimal health during pregnancy. High fat intakes are related to increased cancer risk (25), which may be related to increased circulating estrogen levels (26, 27) induced by 18:2n−6. Recent data suggest that high 18:2n−6 intake in pregnant rats doubles the risk for 7,12-dimethylbenz(a)anthracene-induced breast cancer in female progeny compared with low fat fed dams (26), in a manner similar to that of estrogen-injected control pups. In humans, high maternal 18:2 consumption is negatively associated with head circumference at birth (28).

These considerations suggest there is a range of 18:2 intake during pregnancy that optimizes the fetal and neonatal health. The goals of this study were to *1*) define the maternal plasma kinetics of an intravenous dose of [U-^{13}C]-18:2 (18:2*) and its functionally important n−6 fatty acid metabolites, *2*) determine whether 18:2* is a major n−6 fatty acid transported across the primate placenta in vivo, and *3*) define fetal kinetics of a single 18:2* dose administered to the mother, including long chain n−6 fatty acid metabolites, in visceral organs (liver, kidney, heart, lung) and neural organs (brain, retina, retinal pigment epithelium (RPE)). From the plateau brain 20:4* concentration we estimate the proportion of maternal dietary 18:2 used to support fetal brain development under conditions where the diet contains no LCP during late gestation, the time of accelerated brain growth.

MATERIALS AND METHODS

Animals

Pregnant baboons (*Papio cynocephalus*) were bred at the Southwest Foundation for Biomedical Research (San Antonio, TX; 6 animals) or were from the University of Illinois at Chicago (Chicago, IL; 4 animals), and transported to the Laboratory for Pregnant and Newborn Research at Cornell University (Ithaca, NY). The care of animals was approved by the Cornell Institutional Animal Care and Use Committee and the facility was approved by the American Association for Laboratory Animal Care. A complete veterinary examination was performed on all pregnant baboons upon arrival. They were housed individually in cages in sight of at least one other baboon and a video screen showing other baboons. The room temperature and humidity were maintained at 24°C and 70%, respectively, with a 14-h light and 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 were instrumented with maternal femoral artery and vein catheters to permit ready access to the bloodstream via a tether described in detail elsewhere (29–31).

Diets and dose

The influence of uncontrolled dietary fatty acid intake on endogenous fatty acid concentration can be a serious confounding factor in tracer experiments. Substrate and product feedback are well known in a wide variety of processes in physiology and biochemistry. In addition, uncontrolled tracer dilution by dietary tracee (n−6 fatty acids) or other fatty acids would lead to unreliable kinetics as well as uncharacterized effects on conversion due to competition for desaturation and elongation enzymes with n−3 fatty acids (32–34). To minimize possible effects of LCP on 18:2 to 20:4 conversion, mother baboons were fed for the last 8 weeks of pregnancy an LCP-free diet containing controlled levels of 18:2n−6 and 18:3n−3. The diet had 2% of energy as 18:2n−6, and 0.2% of energy as 18:3n−3 (18:2/18:3 = 10) (Harlan Teklad, Madison, WI). The diet fatty acid composition has been reported previously (35). Animals consumed this diet for 8 ± 1 weeks (mean ± SD) before the administration of labeled dose and continued until cesarean section (CS).

A tracer dose of 19.9 ± 2.8 mg [U-^{13}C]-18:2 (>98% chemical purity, >95% isotopic purity) was administered to nine pregnant baboons in their third trimester (term = 182 days) by chronic indwelling catheter. The tenth baboon was used for baseline samples and received no dose. One baboon only was used for baseline assessment because, in the absence of artificially enriched isotopes, baseline ^{13}C/^{12}C are constant within about 10 ppm for subjects on a controlled diet (36).

Animal details are presented in **Table 1**. The 18:2* was purified from a [U-^{13}C]-algal oil (Martek Biosciences, Columbia, MD) as described previously (37). The 18:2* dose was sonicated into 2.5 ml of 20% Intralipid (KabiVitrum, Franklin, OH), an intravenous emulsion consisting primarily of LCP-free soybean oil with trace LCP (<1% of total fatty acids) added incidentally as a component of lecithin emulsifier. It was diluted with 7.5 ml of sterile saline (Abbott Laboratories, North Chicago, IL) prior to use.

Sampling

Maternal plasma kinetic curves were derived from five animals. Baseline samples prior to dosing were drawn from the femoral artery catheter, then after dosing once per hour for 8 h and once per day until CS or 20 days post-dose. Pregnancies were allowed to continue until myometrial activity indicated that labor was imminent. CS was then performed under halothane general anesthesia; the times between 18:2* dose administration and CS are shown in Table 1. The fetus was killed by exsanguination under halothane general anesthesia and fetal tissues were collected immediately. The brain (occipital lobes), liver, heart, kidney, and lung were removed quickly, weighed, wrapped in aluminum foil, and frozen in liquid N_2. Retina and retina pigment epithelium (RPE) were immediately dissected from the eyes, separated, and stored in saline. All samples were kept at −80°C until analysis.

Lipid extraction, fatty acid, and tracer analysis

Total lipids were extracted from tissue homogenate by the method of Bligh and Dyer (38). Fatty acid methyl esters (FAME) were 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 chromatography 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 (39). Tracer analysis was performed using a high precision gas chromatography–combustion isotope ratio mass spectrometer (GCC-IRMS), described in detail previously (40, 41).

Calculations

The concentration of tracer in tissues was calculated from the quantity of fatty acids determined by GC-FID and from the

TABLE 1. Characteristics of animals used in this study

Baboon	Dose[a]	Age at Dosing[b]	Age at CS[c]	Dosing Period	Maternal Weight[d]	Fetal Weight	Dosing[e]	Fetal Gender	Samples Collected[f]
	mg			*d*	*kg*	*g*			
1	24.15	175	176	1	17	n.r.[g]	IV	F	f
2	19.50	150	152	2	15	560	Catheter	M	f
3	17.80	116	121	5	19	392	IV	F	f
4	15.01	130	139	9	14	400	Catheter	F	f,m
5	22.30	143	155	12	19	710	Catheter	F	f,m
6	22.47	142	156	14	16	650	Catheter	F	f,m
7	19.50	141	162	21	17	875	IV	M	f
8	17.83	130	159	29	18	625	Catheter	F	f,m
9	20.14	126				600	Catheter	M	m
10[h]	No Dose		158			n.r.			f

[a] [U-^{13}C]-18:2n−6 was in a free fatty acid form, blended with Intralipid.
[b] Age of fetus (days of gestation) when dose is administered.
[c] Age of fetus (days of gestation) at cesarean section.
[d] Material weight was recorded at the maternal catheterization or at cesarean section.
[e] Dose was either injected by syringe into the femoral vein or via catheter to femoral vein. Maternal plasma was collected by catheter from the femoral artery.
[f] Fetal tissues (f) collected were brain, retina, retina pigment epithelium (RPE), liver, heart, kidney, and lung. Maternal plasma (m) was taken by catheter prior to dosing, then once per hour for 8 h and daily thereafter as available.
[g] Not recorded.
[h] Samples used to establish baseline isotope values for fetal tissue.

isotopic enrichment of fatty acids measured by GCC-IRMS (42). The molar amount of 18:2* present in any fatty acid pool is calculated as the product of the total fatty acid per unit tissue and the atom fraction excess (AFE), corrected for the ratio of C in analyte fatty acid to that in 18:2* (42, 43). For 18:2 this factor is 18/18 = 1; for C20 and C22 fatty acids it is 20/18 and 22/18, respectively. The final reported tracer concentration therefore refers to the moles of nascent (dose) 18:2* to have entered a particular pool.

Percent of dose (%Dose) was calculated for each labeled n–6 fatty acid (FA*) in each pool, normalized to pool units (per liter plasma or per whole organ(s)). %Dose adjusts for differing dose sizes among animals, and insures that differences are related to function rather than experimental protocol. At each time point, total %Dose was calculated for each organ in units of whole fetal brain, liver, heart, per both kidneys, per both lungs, per single retina, and per single RPE. Percent of total FA* (%Total) was then calculated for each labeled fatty acid at each time point. %Total reflects the distribution of the 18:2* dose that appears in each n–6 fatty acid, and is most useful for assessing shifts in labeled metabolites over time within a pool. Least-squares fits through the data are presented to aid the eye only, using Excel 7.0 for Windows95 (Microsoft, Seattle, WA).

RESULTS

Maternal plasma kinetics

Maternal plasma kinetic curves for n–6 labeled fatty acids are shown in **Figs. 1A** and **1B**, in units of %Total. Fig. 1A shows that 18:2* peaks on day 1 and drops to baseline at about 14 days. Intermediary 18 and 20 carbon fatty acids rise starting on day 1, with the most prominent intermediate, 20:3* peaking to above 20% of total FA* on day 6 post-dose. Labeled 20:4 rises to a plateau measured at about 70% of total FA* at 10 days. Figure 1B displays kinetics of n–6 intermediates over the first 8 h post-dose. At 1 h the immediate product of δ6-desaturation, 18:3*, is about 2.5% of total FA*, and its elongation product 20:3* is already above baseline. Labeled 20:4 (20:4*) begins to rise at 3 h but remains below its immediate precursor 20:3*. The C22 FA* both remain very close to baseline levels throughout the first 8 h.

Fetal organ FA* kinetics

Figure 2 presents the sum of labeled fatty acids found in fetal brain and the visceral organs over 29 days, expressed as %Dose. For all fetal tissues, each point is derived from samples of a single animal, and the time point is plotted as the interval between dosing and CS. At 1–2 days post-dose FA* in liver are a factor of 3 greater than in the brain, followed by the other organs, consistent with the liver's role as the primary site of dietary 18:2 metabolism. After day 2, the FA* concentration falls in all organs except the brain, which rises and shows the highest n–6 FA* concentration. These data suggest a unique conservation of n–6 fatty acids in brain compared with other organs. As shown below, the increase in brain FA* after about 5 days post-dose corresponds to the increasing predominance of 20:4*, suggesting selective conservation of 20:4* compared with 18:2*.

Fetal liver, kidney, heart, and lung

The time course of incorporation of 18:2* and 20:4* in the fetal liver is shown in **Fig. 3** which shows that 20:4* rose as a %Total FA* up to day 15 post-dose. Intermediary FA* were omitted for clarity. From day 15 until day 29, 20:4* plateaued at about 60% of liver labeled fatty acids, while 18:2* dropped to 20%. As 20:4* as a %Dose is constant at 0.0062 ± 0.0013 throughout this period, the increase can be attributed to a drop in 18:2*. Labeled

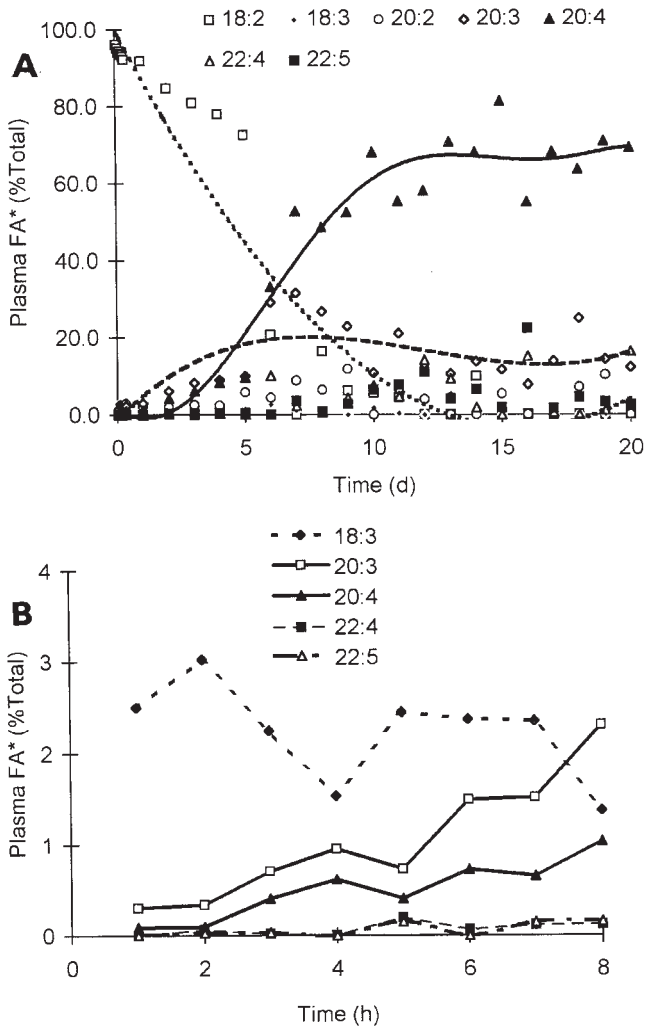

Fig. 1. A) Maternal plasma kinetics of labeled n−6 FA over 20 d after an intravenous dose of [U-^{13}C]-18:2 (18:2*), expressed as % of total (%Total) labeled fatty acids at each time point, which n = 3, 4, or 5. In this and all subsequent figures, trendlines are plotted to aid interpretation. The tracer dose fatty acid, 18:2*, disappeared by about 14 days post-dose, while 20:4* plateaus by 10 days at 70%Total fatty acids. B) Expanded plot of maternal plasma kinetics up to 8 h post-dose for labeled n−6 metabolites. Labeled C18 and C20 FA* are observed within hours of the dose, while C22 FA* are not observed during the first 8 h.

22:4n−6 increased to 6% at 14 days while its desaturated analogue 22:5n−6* rose to 14% at 29 days.

Kinetics were similar for fetal kidney (**Fig. 4**), lung (**Fig. 5**), and heart (**Fig. 6**). In liver, kidney, and lung, the %Total least squares lines for 18:2* and 20:4* cross over around 10–12 days post-dose, while the heart lines crossed much later, around 29 days. In kidney, lung, and heart, 22:4* and 22:5* are below 10%Total through the whole dosing period. At 1–2 days, 18:2* was found in the fetal kidney, lung, and heart at 0.006, 0.007, and 0.012% dose, respectively. Liver, kidney, and heart 18:2* decreased by day 5 to 10–20% of their highest value, while lung 18:2* rose at day 2 and stabilized at about 0.002% dose at 9 days.

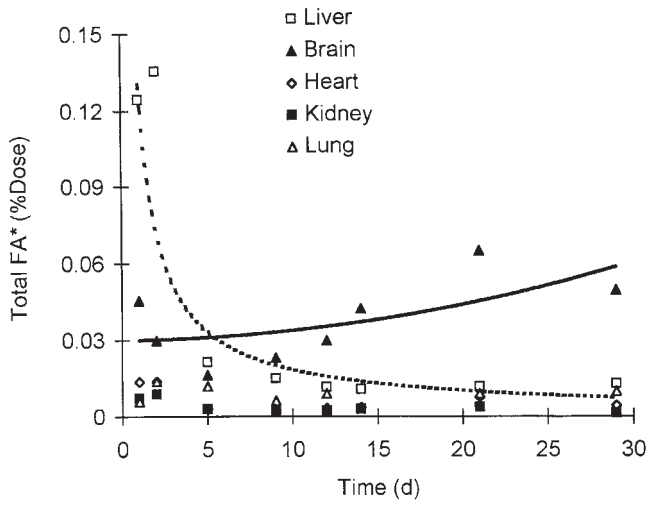

Fig. 2. Total label kinetics for 5 fetal organs, plotted as %Dose found in all FA*, over 29 d. FA* levels drop in all organs except brain which is stable or increasing at the end of the period.

Fetal brain, retina, RPE

The time course for appearance of FA* in fetal brain is presented in **Fig. 7A** and **7B**, in terms of %Dose and %Total, respectively. Labeled 18:2 is detected at about 0.028% of dose in brain 1 day post-dose. Intermediates 18:3* and 20:3*, not shown, were also detected at day 1, and no 20:4* or C22 FA* were detected. By day 2, 18:2* drops rapidly, while all labeled C20 and C22 fatty acids increase. As %Dose, 18:2* and 18:3* retain a ratio of about 9:1 over 29 days; as they are related as precursor/product via Δ6-desaturation, this observation suggests that the catalysis in this reaction is limited by substrate availability. The products 20:4*, 22:4*, and 22:5* all rise from 5 days

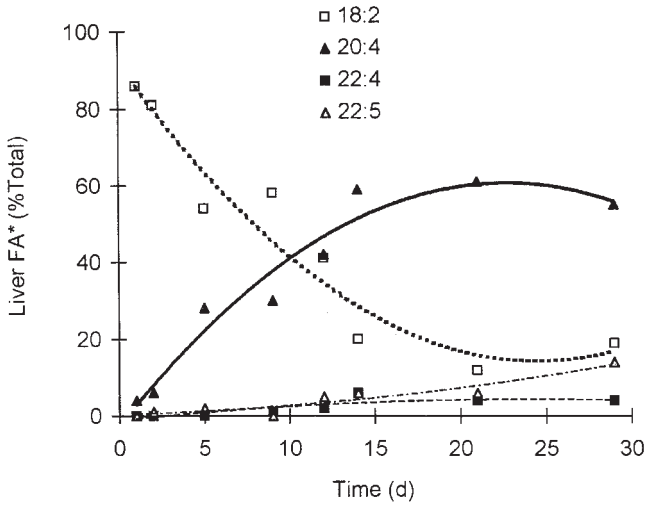

Fig. 3. Fetal liver FA* kinetics over 29 d after a dose of 18:2* presented as %Total. Labeled 18:2 is initially >85% of all FA* and decreases while 20:4* increases to be the predominant FA* after about 10 d. Labeled 22:4 rises to about 6%Total by 14 d while 22:5* is at 14%Total at 29 d.

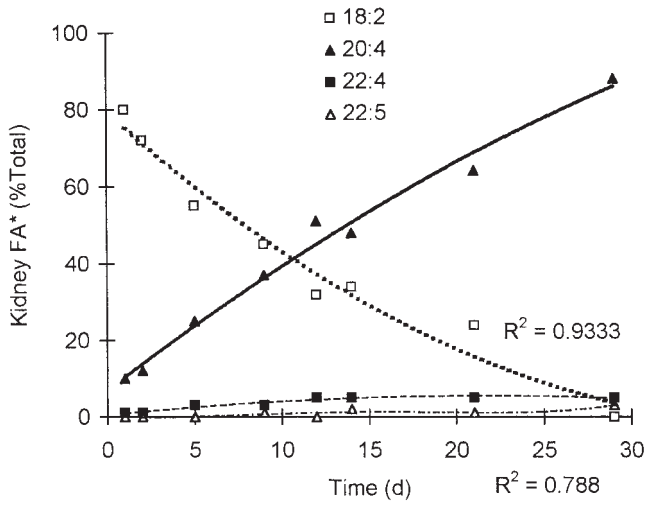

Fig. 4. Fetal kidney FA* kinetics as %Total. The crossover between 18:2* and 20:4* is similar to liver at about 10 d, but continues to rise at 29 days to >85%Total.

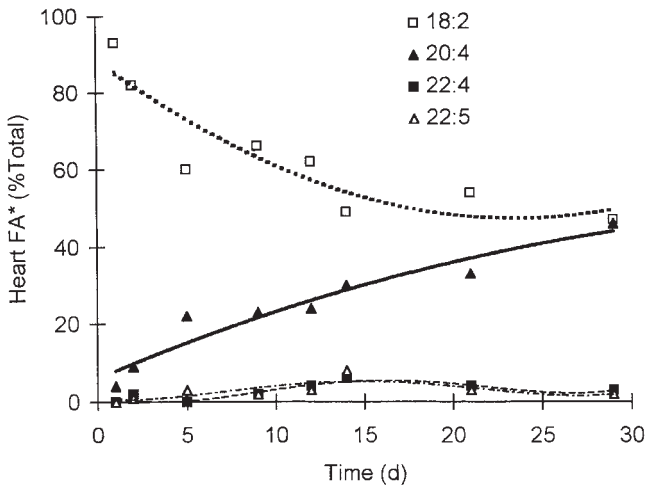

Fig. 6. Fetal heart FA* kinetics as %Total. The apparent conversion of 18:2* to 20:4* is slower than that in liver, kidney, or lung, as the crossover between the two is observed at about 29 d. Both 22:4* and 22:5* rise to about 6%Total by 14 d.

onward, reaching plateaus by 21 days. Expressed as %Dose, 20:4*, 22:4*, and 22:5* levels reach about 0.025%, 0.013%, and 0.008%, respectively, in whole brain; expressed as %Total, they are 45%, 22%, and 13%, respectively. The values for the 22 carbon FA* are more than double the %Total FA* found in visceral organs.

Retina and RPE FA* are similar, as shown in **Fig. 8** and **Fig. 9**. Least squares lines drawn through the kinetic points show that the crossover between the 18:2* and 20:4* occurs between 5 and 9 days. This is considerably earlier than observed for other tissues. Retina had the highest proportion of 20:4* observed at 1 day post-dose, about 30% Total. In contrast, RPE was more like other tissues as it rose slowly as a % Total to plateau at 60% at 12 days post-dose. Retinal 20:4* as a proportion of total FA* appears to increase at 29 days while 18:2* continues to fall.

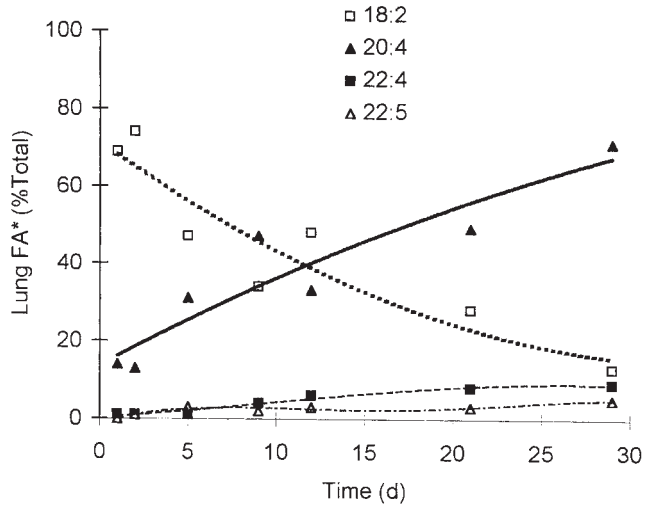

Fig. 5. Fetal lung FA* kinetics as %Total. The crossover between 18:2* and 20:4* occurs at about 12 d, and both 22:4* and 22:5* are below 10%Total.

DISCUSSION

The present study reports 18:2* kinetics and conversion to LCP in maternal plasma and in tissues of fetal baboons whose mothers were infused with 18:2* in late gestation. Until about 5 days post-dose the FA* at highest concentration in maternal plasma was 18:2*, thereafter 20:4* predominated. By day 1, fetal organs had accumulated their highest levels of 18:2*, thus indicating that 18:2 traverses the placenta and is readily incorporated into fetal tissue.

Visceral organs differed in their conversion kinetics between 18:2* and 20:4* primarily in the time required for 20:4* to become the predominant labeled fatty acid. The shift from 18:2* to 20:4* was slower in heart compared with liver, kidney, and lung. These results suggest that the oxidation of 18:2* is more important in late gestation in heart than its use as a substrate for 20:4* biosynthesis, implying enhanced oxidation or reduced conversion.

Brain kinetics differed markedly from visceral organs in several respects. In contrast to the decrease observed for other organs, the total FA* concentration, as %Dose, rose from day 5. C22 fatty acids were found in the brain at levels higher than that of visceral organs and appear to still rise in concentration at 29 days post-dose. The cross-over between brain 18:2* and 20:4* %Total label occurred at about 4 days, much earlier than in the visceral organs in which cross-over was observed at 10–12 days or later. In this respect the retina and RPE were very similar to the brain. It is known that 18:2 is rapidly transported across the blood–brain barrier (44) and that the brain has an active desaturation/elongation system. These data are consistent with rapid conversion in the brain, supported by previous measurements showing that Δ6-desaturase activity is higher in brain than in liver during prenatal and early post-natal development in the rat (45) and mouse (46).

We previously reported that the disappearance of a

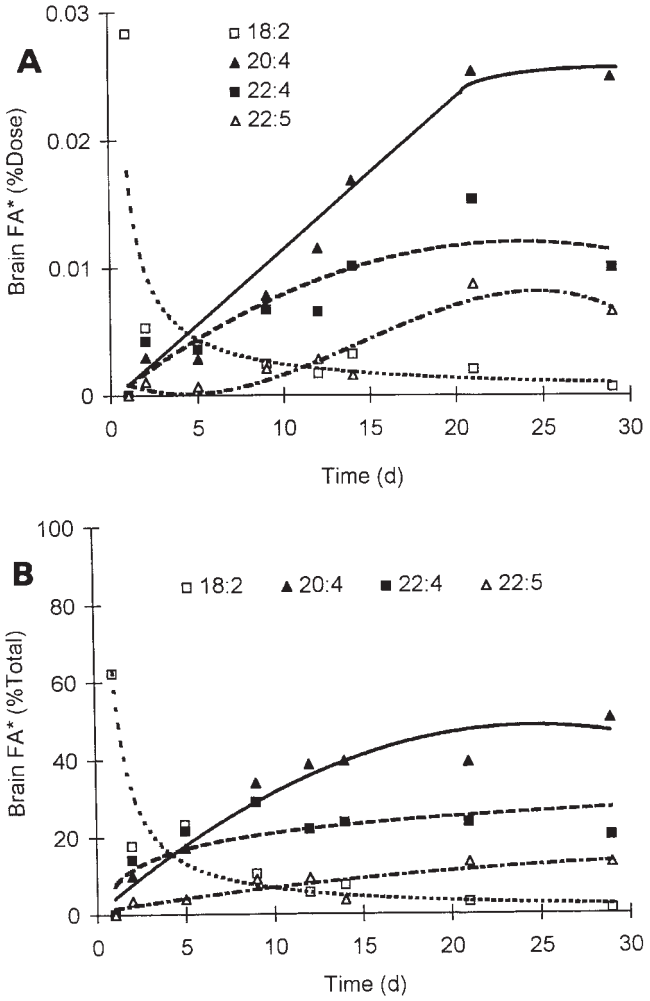

Fig. 7. A) Fetal brain FA* kinetics as %Dose. The brain %Dose of 20:4* rises to a plateau level of 0.025%Dose at about 21 days, while 18:2* is at 0.028%Dose at day 1 but drops to 0.005%Dose by day 2. Both C22 n−6 FA* increase through 21 d. B) Fetal brain FA* kinetics as %Total. At day 1 18:2* is >60%Total with no detectable label in major n−6 LCP; the balance of the label was found in intermediates (omitted for clarity). The crossover between 18:2* and 20:4* as dominant FA* occurs before 5 d. Labeled 22:4 is about 20%Total by 5 d, and 22:5* is >10%Total by 21 d.

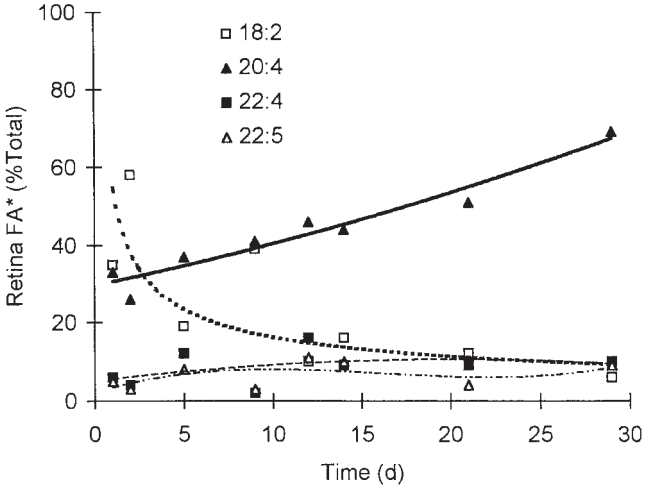

Fig. 8. Fetal retina FA* kinetics as %Total. Labeled 20:4* is about 30%Total at day 1 post-dose and rises to about 70%Total by 29 days. The crossover between 18:2* and 20:4* occurs by 3 d. C22 n−6 FA* are about 10%Total by 12 d.

dose of [U-^{13}C]-18:3n−3 from maternal plasma was nearly complete within 3 days post-dose, compared with the 14 days for 18:2* observed in this study (35). This may, in part, reflect slower metabolism and greater storage of 18:2 than 18:3n−3. In growing rats, minimally sufficient dietary levels of 18:2n−6 were stored at a rate of 24.5%, compared with 15.1% of 18:3n−3 (47). In primate studies, a dose of the LCP [U-^{13}C]-22:6n−3 was detectable in maternal plasma even at 30 days post-dose (35). Our previous study also showed that 0.075% of a 18:3n−3* dose was found in fetal brain as the active metabolite 22:6n−3* (35). This finding can be compared with the 0.025% of the 18:2* dose found as 20:4* in the present study. These factors can be directly compared to yield a factor of 3 (0.075% Dose vs. 0.025%Dose) advantage in production or retention of 22:6n−3 from C18 precursors than for 20:4n−6. It is possible that demand for metabolically active 20:4n−6-derived eicosanoids may drive a greater turnover of this FA than for metabolic products of 22:6.

Labeled 18:2 was detected in all fetal tissues at 1 day post-dose, before most of the conversion to 20:4*, and indicating that in vivo 18:2* is easily transported across the placenta. Though relative transport rates cannot be determined from our measurements, they do support recent findings for the perfused human placenta, showing that 18:2 is preferentially transported as compared with 20:4 (48). The 18:2* concentration dropped rapidly in all organs after 2 days except for brain 18:2*, which dropped after 1 day post-dose, while 20:4* slowly rose to become the predominant labeled fatty acid in most tissues by 15 days.

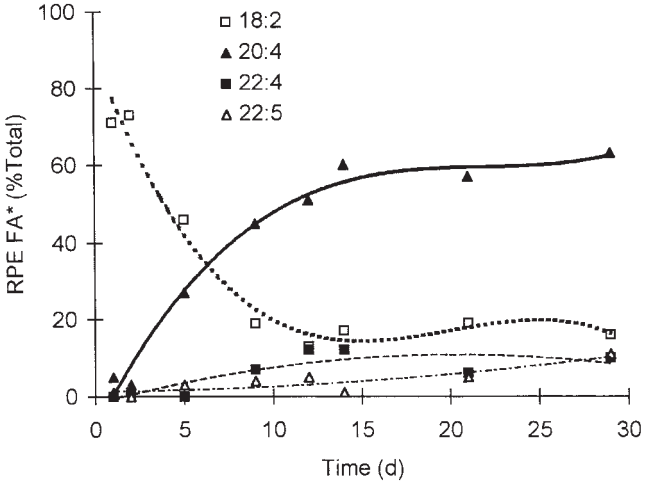

Fig. 9. Fetal RPE FA* kinetics as %Total. Labeled 20:4 is initially very low and rises to 60%Total by 14 d. The crossover between 18:2* and 20:4* occurs at about 6 d. C22 n−6 FA* are about 10%Total by 12 d.

Labeled 20:4 expressed as %Total was high at early times in retina, particularly compared with RPE. Retinal accumulation of total n–6 FA* was constant up to 29 days post-dose, while in RPE, the tracer gradually disappeared after dosing (data not shown). It has been proposed that RPE is a source of n–3 LCP for the retina (49–52), in part because of their intimate anatomical association. Whether this is tenable for n–6 LCP requires further study.

Estimate of dietary 18:2 requirements

As illustrated in Fig. 7A, an FA* plateau in the brain occurs at about 21 days post-dose. This result is similar to that observed for 18:3n–3–derived 22:6n–3* previously (35). Unlike 22:6n–3, 20:4n–6 is used as a substrate for essential metabolites such as eicosanoids that are subsequently excreted, and thus turnover of 20:4 in the brain is essential. However, our data indicate that this turnover is slow over 29 days. An estimate of the relative contributions of maternal dietary 18:2 and maternal 18:2 stores to fetal brain 20:4 accretion can be made assuming that the plateau level of 20:4* represents the overall accretion induced by the 18:2* dose, a manner analogous to that used to estimate 18:3n–3 requirements previously (35).

The baboon brain is about 80 g at term gestation of 182 days (53). Fetal baboon brain growth is negligible in the first half of gestation and approximately linear the second half of gestation (53). Fetal brain 20:4 was measured to be 4.5 μmol/g wet weight (1.37 mg/g) and was not correlated with gestation age. Combining these factors, we find that 3.96 μmol of 20:4 accumulated in brain per day in the last half of pregnancy.

Pregnant baboons consumed an LCP-free diet with 2% of energy as 18:2. Estimating total intake per day at about 1000 kcal, the dietary supply of 18:2 was 7.91 mmol (2.22 g) per day. At plateau, 0.025% of the 18:2 dose appeared as 20:4 in fetal brain. Thus, 1.98 μmol (50%) of fetal brain 20:4 was derived from diet, with the other 50% derived from maternal stores.

The functional relationship of dietary 18:2 to brain 20:4 accretion is not known for primates, though there are data relating dietary fatty acids to tissue fatty acids in rats, and prediction equations for adipose tissue fatty acid levels have been published for humans (54, 55). The functional relationship would permit estimation of the amount of dietary 18:2 sufficient to meet the requirements of the developing fetal brain. Prediction equations generally indicate that the relationship between plasma and adipose C18 unsaturated fatty acids is linear, while that for LCP is hyperbolic (55). Even if these relationships apply to brain accretion, the mathematical constants required to calculate absolute concentrations are not available. However, we observed that the major differences between linear and hyperbolic functions are at high intake levels, where the C18 fatty acids accumulate in proportion to dietary concentration, whereas the LCP concentrations plateau. At levels nearer to deficiency, hyperbolic functions show that tissue levels increase with dietary levels. Thus, assuming a linear relationship for LCP errs on the side of underestimation of tissue levels and hence dietary requirements. By this reasoning, a simple doubling of dietary 18:2 from 2% to 4% of calories is the minimum level required to support fetal brain 20:4 accretion requirements. For comparison, the average dietary 18:2 intake of adults in developed countries is 7% of energy (56).

As discussed previously (35), this calculation also assumes that dietary fatty acids are quantitatively absorbed, and that tracer fatty acids administered intravenously as non-esterified fatty acids (NEFA) reflect the metabolism of dietary fatty acids. Dietary 18:2 enters the circulation in chylomicrons, from which NEFA, including 18:2, are liberated by the action of lipoprotein lipase. Therefore, intravenous 18:2 as NEFA is a normal physiological form for dietary 18:2 transport, and the administration of a dose by this route circumvents experimental difficulties associated with quantitative oral administration to adult non-human primates.

Kinetics of any substrate within a pool can be considered as a function of total input and output. Our calculation also assumes that there is no turnover, or output, of 20:4 from the brain, the pool from which the estimate of accretion level is taken. For zero output, the plateau level corresponds to total input. This criteria is certainly violated for 20:4 as this FA is well known to be a substrate for several enzymes. Non-zero output implies that the plateau level corresponds to a lower bound for input. This is further reason to believe that 4% of energy as 18:2 represents a minimum to meet all fetal brain 20:4 in the absence of dietary 20:4.

The ratio of n–6 to n–3 fatty acids has long been known to affect metabolic conversion (33, 34). For this reason, it can be expected that a significant shift in dietary n–6/n–3 ratio from that used here, 10:1, will alter the requirements for 18:2. However, the ratio of 10:1 is within that used in infant formula and consumed by adults in the western world. Ratios less than 10:1 are likely to increase the requirement for 18:2, while ratios more than 10:1 are likely to decrease it. Effects of 18:2 levels on factors other than eicosanoids, such as modulation of plasma estrogen, cannot be estimated from existing data and require further study.

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