0031-3998/02/5103-0263
PEDIATRIC RESEARCH
Vol. 51, No. 3, 2002
Printed in U.S.A.

COMMENTARY

Arachidonate Metabolism in Neonates

Commentary on the article by Wijendran *et al.* on page 265

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Neonates have a higher requirement for polyunsaturated fatty acids (PUFA) than adult animals. Over the past 30 years, there has been intensive research into establishing the extent to which the infant is dependent on an exogenous supply of docosahexanoate (DHA, 22:6ω3) and arachidonate (AA, 20:4ω6). Two large, recent studies indicate that preterm but not term infants benefit neurodevelopmentally by consuming formulas supplemented with AA and DHA (1, 2). These trials reaffirmed the safety of appropriate amounts of AA and DHA (0.4–0.5 and 0.1–0.3% of total formula fatty acids, respectively) given together, whether from fish/fungal or egg sources. They also supported earlier evidence that has not found a beneficial effect of supplementation with AA and DHA in term infants compared with those consuming breast milk containing similar amounts of AA and DHA. Whereas both term and preterm infants have some capacity to synthesize AA and DHA, term infants are born with about 1 g each of AA and DHA, so it is not entirely surprising that they respond less to AA and DHA supplementation than preterm infants with 10% to 20% of the fat stores of term infants.

Much of the interest in determining why PUFA are required for normal development has centered on the ω3 PUFA while much less attention has been paid to the ω6 PUFA. This interest in the ω3 PUFA has arisen partly because of the relatively large amounts of DHA, in gray matter of the brain and in the photoreceptors of the eye, and partly because behavioral deficits in various animal models are inducible by dietary ω3 PUFA deficiency and can be corrected or prevented by supplemental ω3 PUFA. However, dietary ω-3 PUFA deficit often has to be prolonged, severe, and even multigenerational to achieve the developmental and behavioral abnormalities that researchers use to assess the role of ω3 PUFA in the nervous and sensory systems. Notwithstanding these concerted efforts, it is often difficult for one lab to reproduce effects reported by another lab. Hence, throughout mammalian evolution, it appears that mechanisms have developed to tenaciously retain what little tissue ω3 PUFA may be available, thereby frustrating efforts to understand their function.

Despite the fact that we have known about the essential role of AA since the 1930s, it seems we have learned even less about how the ω6 PUFA contribute to normal development than we have about the role of ω3 PUFA. Wijendran *et al.* (3) in this issue describe an elegant carbon-13 (^{13}C) tracer approach to studying AA metabolism in neonatal baboons. Radiolabeled AA has been available for many years but radiotracers are less and less popular for whole animal studies owing to the logistical requirements for containment and disposal. Unlike several other fatty acids including DHA, ^{13}C-AA cannot easily be produced by ^{13}C labeling of algal biomass, which adds a further and usually prohibitive financial challenge to obtaining this tracer. Wijendran *et al.* (3) overcame this challenge with corporate support and chemists capable of inserting four methylene-interrupted double bonds into their synthetic AA, all in the *cis* configuration. Using sensitive and precise isotope ratio mass spectrometry, they were able to use small amounts of ^{13}C-AA to address a key question of metabolic and human nutritional importance – dietary PUFA are almost exclusively provided as esters, in either phospholipids or triglycerides, but does it matter whether the carrier lipid for AA is triglyceride or phospholipid? Over 90% of AA (and DHA) in human milk is in the triglyceride form but AA carried by phospholipid is a potentially attractive source of AA for formulating human milk substitutes.

Wijendran's study (3) showed that, *as a % of dose*, about twice as much ^{13}C-AA (4.5% compared with 2.1%) reached the brain if the AA starts out as a phospholipid. Since the milk formula used to feed their baboons contained 92% of the AA in the triglyceride form, in *absolute terms*, about 5 times more preformed (dietary) AA reaching the brain was in the triglyceride compared with phospholipid form. They did not report the effect of a different ratio of dietary AA as triglyceride compared with phospholipid but if the % dose data can be extrapolated to a formula containing, say 50% of AA as triglyceride and 50% as phospholipid, it would appear that only half as much AA would have to be given in the phospholipid form to achieve the same brain ^{13}C-AA uptake. Such an experiment is important because it is essential to establish that the tracer behaves like the dietary fatty acid in question; if the phospholipid form of AA is twice as accessible to the brain, would it actually raise brain AA to an adverse extent or could half as much AA carried by phospholipid be provided to achieve the same brain AA as with triglyceride? These types of studies are important to establish the safety and palatability of new formulations of human milk substitutes. They will also

help us understand how these important fatty acids are normally used.

Wijendran's study (3) also paves the way to explore why there is apparently greater retention of AA in the brain during dietary ω-6 PUFA deficiency than DHA retention during ω-3 PUFA deficiency. One potential reason that bears further investigation and for which ^{13}C is especially well suited is the oxidation and 'carbon recycling' of these fatty acids. The same lab has recently demonstrated in a similar primate model that as much 1/3 of dietary DHA is oxidized and the carbon skeleton recycled into newly synthesized saturated and monounsaturated fatty acids appearing in brain, liver and elsewhere (4). This recovery of recycled DHA does not include the proportion that would inevitably be lost to complete oxidation during the recycling process. Hence, at least 50% of dietary DHA appears to be lost during normal development. Carbon recycling is more extensive for 18 carbon PUFA than for DHA (4, 5) but, so far, has not been reported for AA. In our unpublished and preliminary work, no ^{13}C from an oral dose of ^{13}C-AA was recovered in saturated or monounsaturated fatty acids in piglet brain, suggesting that oxidation and carbon recycling of AA is less extensive than for DHA. Less oxidation and recycling of AA than DHA may not fully account for the apparently more tenacious retention of AA than DHA in brain but it would contribute to this effect.

Using their ^{13}C data, Wijendran *et al.* (3) also calculated that preformed AA in the diet would contribute fewer than 50% of the AA appearing in the baboon brain during their 10-day study. This clearly suggests that both preformed and newly synthesized AA both contribute to brain AA. However, what happens if there is no preformed dietary AA, *i.e.* in infants on milk substitutes containing no AA? Is brain AA lower in this case or is it more efficiently obtained from body stores or by synthesis from linoleate? Autopsy data suggest brain AA in infants is resistant to depletion (6), suggesting the capacity to synthesize AA can make up the difference or that recycling of carbon from AA is lower than for DHA. Gas chromatography-isotope ratio mass spectrometry methods now allow this to be tested experimentally.

In any event, Wijendran's paper (3) is an innovative application of tracer technology toward addressing potentially important aspects of the metabolism and utilization of key PUFA needed for normal infant development.

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