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Infants with Intrauterine Growth Restriction Have Impaired Formation of Docosahexaenoic Acid in Early Neonatal Life: A Stable Isotope Study

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ABSTRACT

This study evaluated the arachidonic acid (AA) and docosahexaenoic acid (DHA) formation from d5-labeled linoleic acid (d5-LA) and α-linolenic acid (d5-LNA) precursors in infants with intrauterine growth restriction (IUGR) compared with control groups matched by gestational age (GA) or birth weight. We compared DHA and AA formation from deuterated precursors d5-LA and d5-LNA in 11 infants with IUGR with 13 and 25 control subjects who were appropriate for GA and matched by GA and by birth weight, respectively. After an enteral administration of d5-LA and d5-LNA, we determined unlabeled and d5-labeled fatty acids at 24, 48, and 96 h in plasma. Absolute concentrations and area under the curve (AUC) over the 96-h study were used for analysis. Absolute concentration of d5-DHA and the product/precursor ratio of the d5-labeled AUCs indicated a less active DHA formation from LNA in infants with IUGR compared with their GA-matched (2-fold) and birth weight–matched (3-fold) control subjects. The ratios of eicosapentaenoic and n-3 docosapentaenoic acid to DHA were also affected. Similar evaluation for the n-6 series was not significant. DHA metabolism is affected in infants with IUGR; the restricted DPA to DHA conversion step seems to be principally responsible for this finding. **(*Pediatr Res* 58: 735–740, 2005)**

Abbreviations

AA, arachidonic acid
AGA, appropriate for gestational age
AUC, area under the curve
DHA, docosahexaenoic acid
d5-LA, deuterated linoleic acid
d5-LNA, deuterated α-linolenic acid
DPA, docosapentaenoic acid
EFA, essential fatty acids
EPA, eicosapentaenoic acid
GA, gestational age
IUGR, intrauterine growth restriction
LA, linoleic acid
LCP, long-chain polyunsaturated fatty acids
LNA, α-linolenic acid
PGC-1, peroxisome proliferator-activated receptor-γ coactivator-1
PPAR, peroxisome proliferator-activated receptor
SGA, small for gestational age

Intrauterine growth restriction (IUGR) is the result of the failure of the placenta to provide the necessary nutrients required by the fetus to maintain adequate growth (1). During the last trimester of gestation, there is a significant accumulation of long-chain polyunsaturated fatty acids (LCPs) in the fetus and an increase in the relative content of both n-6 and n-3 LCPs in the human brain and retina. Fetal tissue content of LCPs is dependent on maternal intake and on an adequate placental transfer. Thus, a greater risk for restricted *in utero* LCPs supply may occur in pregnancies that are complicated with abnormal placental function affecting nutrient transfer. Data derived from IUGR animal models indicate that placental insufficiency is associated with abnormalities in fetal lipid metabolism of skeletal muscle and liver (2,3). Cetin *et al.* (4) reported that fetuses with IUGR have a lower proportion of long-chain n-6 and n-3 fatty acids, arachidonic acid (AA), and docosahexae-

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noic acid (DHA) relative to the precursors linoleic acid (LA) and α-linolenic acid (LNA) in fetal blood in comparison with maternal blood than is found in control infants who are appropriate for gestational age (AGA). Studies that have evaluated essential fatty acids (EFA) status in infants with IUGR, most of them premature, compared with control subjects using umbilical cord blood, the umbilical artery-vein wall, or the placenta reported lower EFA status in infants with IUGR (5–7). Studies in term infants that included infants who were small for gestational age (SGA) failed to corroborate the findings described in preterm infants with IUGR, reporting an even higher biochemical EFA status in infants who were classified as SGA compared with their large for GA counterpart (8). This served to highlight the vulnerability of the maternal/placental supply of EFA to the fetus not only as a result of poor maternal intake and/or placental transfer as a result of placental insufficiency but also of the increased demands as a result of higher EFA accretion by the heavier fetus.

Several studies in human neonates have used stable isotopes, $^{2}H_{5}$- or ^{13}C-labeled LA or LNA, to assess *in vivo* metabolic conversion reflecting EFA elongation and desaturation. These studies have provided clear evidence that term and preterm infants are able to synthesize AA and DHA from their respective dietary EFAs, LA and LNA, respectively, albeit in small amounts (9,10). Formation of LCPs in infants with IUGR has not been characterized. The objective of this study was to evaluate the AA and DHA formation from d5-labeled LA and LNA precursors in infants with IUGR compared with control groups that were matched by GA or birth weight.

METHODS

Between March 1994 and October 2001, preterm and full-term infants who were admitted to the neonatal unit at Hospital Sótero del Río in Santiago were invited to participate in the study to evaluate the effect of birth weight and GA on LCP formation in neonates, excluding those with major malformations or those who were unable to receive the enteral dose of tracer fatty acids for any reason (metabolic disorders or maternal diabetes). GA was assessed by last menstrual period or using age of conception on the basis of early ultrasound and confirmed by the modified Ballard evaluation conducted soon after birth (11). They were appropriate for GA (AGA) according to the Lubchenco standard or considered to have IUGR on the basis of fetal ultrasound with postnatal confirmation of SGA defined as weight below the 10th percentile for GA and clinical evidence (12,13). Most preterm infants studied presented with mild to moderate forms of respiratory distress; term infants often had mild forms of hypoxic insult or transient respiratory problems. Infants who were selected for these analyses had the isotope administered in the first few days of life and not beyond the first 2 wk of life. Feeding was generally started within the first 2 d after birth, and the type of feeding varied depending on the mothers' willingness to provide breast milk. When breast milk was not available, term infants received Nan (Nestle), a formula that is devoid of LCP and contains LA 11.7% and LNA 1.1% of total lipids. Preterm infants received either PreNan (Nestle) or Similac Special Care (Ross), both of which were devoid of LCP and had EFA content of LA 12.8% and LNA 1.1%, and LA 12.6% and LNA 1.2% of total lipids, respectively (14). DHA/AA content in breast milk from Chilean mothers after 15 d of lactation range from 0.4 to 0.7% DHA, whereas AA is 0.5–0.8% of total fatty acid weight; the ratio of AA to DHA is close to 1. Patients who could not be fed enterally received parenteral glucose starting soon after birth; crystalline amino acids were added by day 3 of life. None of the patients included in this study received i.v. lipids, before or during the study period. Written informed consent was obtained by a trained research nurse supervised by a neonatologist shortly after birth or soon after feedings were commenced. The study was approved by INTA's ethics committee and by the research committee at Hospital Sótero del Río that is responsible for supervising research involving human subjects. The protocol was also approved by the National Institutes of Health/National Institute on Alcohol Abuse and Alcoholism Institutional Review Board and received a Single Project Assurance from the Office for the Protection from Research Risks, Department of Health and Human Services, for international collaboration.

Isotope administration. Patients received 50 mg/kg each of deuterated linoleic acid (d5-LA) and deuterated α-linolenic acid (d5-LNA) as ethyl esters provided by Cambridge Isotope Labs (Andover, MA) by the nasogastric route ranging from the first to the 15th day of life. Blood was drawn (0.5 mL) from a peripheral vein or umbilical catheter into a tube that contained EDTA as anticoagulant at baseline and at 24, 48, and 96 h after dosing (baseline sample was discontinued after the first five patients presented nondetectable concentrations of interfering substances for the d5-labeled fatty acids measured). Plasma was separated by centrifugation shortly after sampling, aliquotted, and kept frozen until lipids were analyzed.

Fatty acid and deuterated fatty acid metabolite assay. The method for fatty acid analysis has been published but is described briefly (15); lipids were extracted from 100 μL of plasma using the method of Folch *et al.* (16) adding 50 μg of BHT per sample to prevent oxidation. Fatty acids were methylated using boron trifluoride–methanol according to Morrison and Smith (17) as modified by Salem *et al.* (18). Quantification was achieved by capillary gas chromatography (model 5890; Hewlett Packard, Wilmington, DE) using flame ionization detection. Samples were air-shipped on dry ice to the National Institute on Alcohol Abuse and Alcoholism Laboratory of Membrane Biochemistry and Biophysics for measurements of the concentrations of d5-fatty acids as previously described (15). A model 5973 Mass Spectrometer with Model 6890 Gas Chromatograph (Agilent, Wilmington, DE) using a FFAP bonded-phase capillary column (60 m × 0.25 mm I.D., film thickness 0.25 mm; J & W Scientific, Folsom, CA) served to separate the d5-labeled fatty acids from the unlabeled products of LA and LNA elongation/desaturation. Results are expressed in terms of d5-labeled fatty acids concentrations in μg/mL of plasma at 24, 48, and 96 h. All data were coded and managed using computerized spreadsheets and analyzed in a blinded manner.

The change, appearance or disappearance, over time for the d5-labeled products of interest was measured at given times and also integrated over the 96 h of the study. The area under the curve (AUC) was determined for each labeled fatty acid by application of a time-integrated algorithm (19) to the plasma concentration–time course curve. For each time point, trapezoidal areas are calculated by a formula in which the area equals one half the sum of the concentrations multiplied by the time interval in hours. The sum of the trapezoids from 0 to 96 h is the AUC).

Group selection. A total of 80 patients were studied; two previous publications have reported data from 51 patients who were evaluated from 1994 until April 1998 (10,15). The present analysis was conducted in 30 patients from this group (nine in the IUGR and 21 in the AGA matched control groups) and in an additional 14 patients from 29 studied up to August 2001. Thus, the group being reported now corresponds to the 44 patients who received the isotope before 15 d of life from the total of 80 patients studied.

The present analysis differs from the previously published work in that it limited inclusion to patients who were studied before 15 d to minimize the confounding effect of feeding and established two control groups for the IUGR group; one matched by GA and a second group matched by birth weight. Matching criteria were defined as ±100 g for the birth weight group (n = 25) and equal GA expressed in weeks for the GA control group (n = 13). The present analysis of the data were based on actual concentration of deuterated precursors and products rather than isotope enrichment to avoid the effect of changes in the unlabeled fatty acids in defining the responses. Finally, we assessed formation of DHA and AA on the basis of product:precursor ratios of AUC over the complete study period rather than as ratios from single time points.

Statistical analysis. Statistical analysis of data included measures of central tendency and distribution. Means and SDs for the relevant variables for the three groups were computed. Specific data points that were identified as outliers from the group data were examined for potential errors in data management or chemical analysis. Comparison among the three groups was performed using a nonparametric test given that the distribution of all evaluated parameters of fatty acid metabolism was nonnormally distributed.

RESULTS

The birth characteristics and anthropometrics for IUGR and control groups are shown in Table 1. As expected, the IUGR group differs in birth weight by nearly 1000 g with the GA-matched control and is 4 wk older in gestation relative to the birth weight–matched control group (Table 1). The lack of differences in length, head circumference, and ponderal index

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Table 1. *Biodemographic and anthropometric indices of infants included in the three groups*

		Control Groups	
	IUGR (n = 11)	GA (n = 13)	BW (n = 25)
Gestational Age (wks)	34.0 ± 3.4*	34.4 ± 3.5	30.6 ± 1.7§
Birth Weight (g)	1650 ± 398	2526 ± 839§	1540 ± 327
Length (cm)	41.6 ± 3.6	46 ± 3.6§	40.7 ± 2.7
Head circumference (cm)	29.8 ± 2.1	32.8 ± 2.6	29.5 ± 2.4
Ponderal Index (kg/m3)	22.6 ± 1.8	25.2 ± 3.5§	22.5 ± 1.8
Gender (M/F)	5/6	6/7	16/9

* Mean ± SD. Comparison between IUGR and the GA and BW matched control groups was conducted using One way anova followed by post-hoc test with Bonferroni correction for gestational age, birth weight, length, head circumference and ponderal index. Difference in gender was assessed using pearson Chi-2.

§ $p < 0.01$.

between the IUGR study group and the birth weight–matched control group despite the 4-wk difference in gestation indicates that these are infants with symmetric growth restriction as opposed to typical asymmetry observed in term infants with IUGR. This pattern suggests that growth restriction had been sustained over a long period.

The age at study entrance for the GA-matched control group was relatively similar to the IUGR group, whereas the infants in the birth weight–matched control group were older than those in the IUGR group [median (range): IUGR 1 d (0–15 d); GA-matched control group 0 d (0–10 d); birth weight–matched control group 10 d (0–15 d)]. Most of the infants included in this analysis were premature (nine of 11 in the SGA group and 33 of 38 in the AGA group). Regarding the feeding received by the patients at the time of evaluation, the majority were not receiving enteral feeds: 10 in the IUGR group, eight in the GA control group, and 10 in the birth weight control group. The type of feeding received by the GA and birth weight control groups was predominantly a mix of breast milk and LCP-free milk formula, with a few of them receiving exclusively formula or breast milk.

Comparison of concentrations of unlabeled (d0) and d5-labeled (d5) fatty acids for the n-3 and n-6 series measured at 24, 48, and 96 h after tracer administration is presented in Table 2. Comparison of infants with IUGR in relation to the two control groups was done using nonparametric tests. The concentration of unlabeled LNA measured at 24 h is significantly lower in the infants with IUGR when compared with the birth weight control infants, suggesting poor transplacental transfer or lower dietary intake. Similar effects are found in LA concentrations at 24 h, but differences are not significant. This difference disappears by 96 h, indicating possible mobilization from tissue pools or increasing dietary intake. The trend in the infants with IUGR indicates a time-related increase in concentration of the unlabeled n-3 fatty acids, whereas the concentration of LNA metabolite EPA in the two control groups remains stable or slightly decreases. The concentrations of d5-labeled fatty acids of infants with IUGR were not significantly different from those of control groups at 24, 48, and 96 h for the precursors d5-LNA and d5-LA. The d5-labeled concentration for the main product of interest, DHA in the IUGR group, presents lower values than for their matched control infants. These differences are significant at 24 h compared with the GA control infants and at 24 and 48 h for the birth weight counterpart. There was also a significantly greater concentration of plasma d5-AA at 24 h in the birth weight–matched group compared with the IUGR group.

Table 3 provides the results of d5-fatty acid integrated over time for all of the fatty acids analyzed. Integration of the plasma concentration–time course curve provides the AUC. The values for the AUC for the d5-labeled n-3 and n-6 precursors and intermediary metabolite between the IUGR and

Table 2. *Unlabeled (d0) and labeled (d5) fatty acid concentration (μg/ml plasma) at 24 h, 48 h and 96 h after isotope fr do*

		IUGR/SGA (n = 11)		GA Controls (n = 13)		BW Controls (n = 25)	
	Hrs	d0	d5	d0	d5	d0	d5
18:3 n-3	24	0.52§ (0.28–3.01)	0.32 (0.10–0.79)	1.04 (0.7–4.95)	0.52 (0.26–0.75)	4.11* (0.8–10.29)	0.304 (0.14–0.46)
	48	2.01 (1.46–5.3)	0.13 (0.08–0.42)	2.1 (1.42–4.13)	0.16 (0.05–0.31)	3.94 (1.35–8.86)	0.07 (0.04–0.16)
	96	4.5 (2.9–10.93)	0.09 (0.02–0.16)	3.32 (1.92–8.63)	0.05 (0.02–0.09)	4.7 (2.11–8.86)	0.02* (0.01–0.05)
20:5 n-3	24	2.4 (1.47–9.94)	0.09 (0.01–0.14)	3.5 (2.38–5.7)	0.11 (0.06–0.32)	4.84 (3.5–9.82)	0.06 (0.03–0.21)
	48	3.47 (2.45–7.29)	0.1 (0.04–0.1798)	3.17 (2.83–7.04)	0.12 (0.04–0.18)	5.03 (3.25–8.95)	0.04 (0.02–0.14)
	96	4.42 (3.72–8.16)	0.10 (0.02–0.13)	3.57 (2.50–7.61)	0.05 (0.01–0.12)	5.03 (3.1–8.8)	0.02 (0.01–0.06)
22:6 n-3	24	23.09 (19.24–41.8)	0.009 (0.0–0.02)	29.2 (20.05–34.31)	0.02* (0.01–0.07)	30.83 (20.47–38.6)	0.06* (0.02–0.27)
	48	28.93 (22–37.56)	0.02 (0.01–0.0319)	28 (16.13–40.33)	0.03 (0.02–0.07)	28.14 (15.61–39.87)	0.07* (0.02–0.22)
	96	27.1 (22.68–35.09)	0.05 (0.01–0.07)	25.20 (17.65–29.47)	0.03 (0.02–0.12)	27.2 (17.87–35.5)	0.08 (0.02–0.12)
18:2 n-6	24	78.91 (38.7–180.7)	0.513 (0.19–1.11)	80.19 (65.78–258.81)	0.793 (0.46–0.99)	258 (59–437)	0.61 (0.11–0.93)
	48	117.9 (80.19–183.97)	0.46 (0.18–0.9995)	144.77 (73.4–224.13)	0.52 (0.3–1.51)	254.24 (72.03–367.52)	0.32 (0.05–0.54)
	96	194.38 (128.9–337.58)	0.25 (0.08–0.61)	224.95 (124–361.32)	0.167 (0.07–0.58)	308 (118.1–401.29)	0.14 (0.05–0.25)
20:3 n-6	24	20.05 (12.12–35.3)	0.01 (0.002–0.01)	23.79 (19.05–28.4)	0.017 (0.01–0.04)	25.23 (19.83–36.42)	0.015 (0.01–0.0317)
	48	24.48 (15.49–30.23)	0.02 (0.01–0.0311)	25.58 (16.26–28.67)	0.03 (0.01–0.05)	22.89 (18.78–34.57)	0.02 (0.01–0.03)
	96	22.6 (17.59–30.08)	0.03 (0.01–0.04)	20.91 (16.74–31.36)	0.03 (0.01–0.05)	24.9 (19.24–34.5)	0.012 (0.01–0.05)
20:4 n-6	24	81.46 (36.76–112.7)	0.00031 (0–0.0013)	83.1 (66.4–91.06)	0.003 (0–0.01)	107.98 (77.54–119.02)	0.003* (0.0004–0.01)
	48	77.28 (65.86–98.02)	0.01 (0.01–0.0078)	92.96 (63.02–113.31)	0.01 (0.01–0.01)	96.86 (61.2–138.06)	0.01 (0.01–0.02)
	96	79.815 (68.37–87.8)	0.012 (0.003–0.03)	84.50 (64.23–109.42)	0.02 (0.004–0.04)	90.4 (63.33–119.54)	0.01 (0.004–0.04)

§ Values presented as median (p25-p75).

Differences between the unlabeled and d5-labeled fatty acids concentrations at 24 and 96 hours in the Gestational Age matched controls and Birth Weight matched controls compared to the IUGR group are significantly different using a non-parametric test (Kruskal-Wallis).

* $p \leq 0.05$.

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Table 3. *Area under the plasma concentration-time course curves for IUGR/SGA and reference groups*

	IUGR	GA Controls	GA *vs* IUGR (*p* values)*	BW Controls	BW *vs* IUGR (*p* values)
n-3					
d5-18:3	18.12§ (5.21–37.91)	17.98 (8.96–39.89)	0.41	12.19 (6.40–17.98)	0.31
d5-20:5	7.69 (2.80–14.04)	9.48 (3.85–17.28)	0.25	2.90 (2.05–12.90)	0.71
d5-22:5	1.88 (0.65–2.65)	1.60 (1.04–2.58)	0.77	1.70 (0.93–2.60)	0.64
d5-22:6	2.12 (0.61–3.45)	2.94 (1.47–7.17)	0.37	6.30* (2.65–17.55)	0.01
n-6					
d5-18:2	36.01 (10.2–65.14)	35.52 (23.94–95.82)	0.79	31.59 (10.92–49.90)	0.5
d5-20:2	0.62 (0.42–1.04)	0.58 (0.40–1.17)	0.43	0.86 (0.45–1.52)	0.97
d5-20:3	1.62 (0.66–2.55)	2.39 (1.26–3.83)	0.97	1.36 (0.46–3.82)	0.23
d5-20:4	0.41 (0.15–0.95)	0.80 (0.40–1.38)	0.11	0.46 (0.27–1.57)	0.31

§ Data presented as median (p25-p75) of the integrated area under the plasma concentration-time course curve (AUC) over the 96 hours of the study (arbitrary units).

* *p* values obtained using non parametric test (Kruskal-Wallis).

the two control groups did not reach statistical significance. However, for DHA, the value is significantly lower in the IUGR group; it is approximately one third that observed in the birth weight–matched control infants.

The ratios of products to precursors may be used to compare differences in metabolism across groups. The AUC values presented in Table 3 were used to compute these ratios. This parameter provides some insight into the relative formation of AA and DHA in the IUGR and the two reference groups. The result of product:precursor ratios using the AUC values for the d5-labeled n-6 fatty acids does not reveal differences between IUGR and either reference group (data not shown). The AUC values for the various isotopic fatty acids of the n-6 series were similar across all groups.

The results of product:precursor ratios for the n-3 pathway that form DHA are shown in Fig. 1. There are significantly lower values in the IUGR group relative to the control groups for DHA formed from all three main precursors (Fig. 1*A*). For DHA formation from LNA, the difference is significant for the birth weight–matched control group but not for the GA control group ($p = 0.07$). The ratio of DHA to LNA is 2-fold greater in the GA control infants and 3-fold higher in the birth weight control infants relative to the infants with IUGR. We performed analysis of covariance to assess the effect of differences in gender between the IUGR and birth weight–matched control groups in the reported finding. The *p* value for the observed effect of IUGR in the evaluated isotopic DHA:LNA, DHA:EPA, and DHA:DPA ratios remain significant after adding gender to each DHA/precursor model.

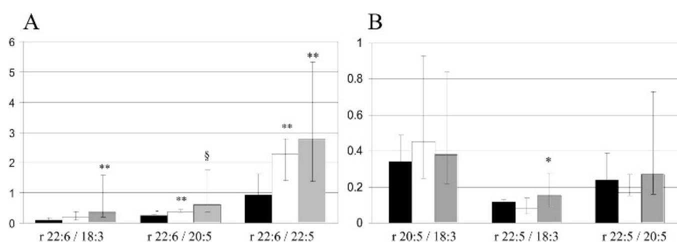

Figure 1. Product:precursor ratio after integration of the d5-labeled n-3 fatty acid plasma concentration–time course curves. This figure depicts the product-:precursor ratios using the AUCs of the respective plasma concentration–time course curves. (*A*) From left to right, the ratios of the product of interest d5-DHA (d5-22:6 n-3) to the precursor d5-LNA (d5 18:3 n-3) and the intermediate metabolites d5-EPA (d5-20:5 n-3) and d5-DPA (d5-22:5 n-3). (*B*) From left to right, the ratios along the n-3 series metabolic pathway of d5-EPA (d5-20:5 n-3) and d5-DPA (d5-22:5 n-3) from the parental EFA d5-LNA (d5-18:3 n-3) and the d5-EPA (d5-20:5 n-3) to d5-DPA (d5-22:5 n-3) ratio. Bars represent the median and interquartile (p25–p75) range. Symbols over the bars indicate a significant difference when comparing IUGR(■, n =11) with GA-(□, n = 13) and birth weight(▩, n = 25)-matched control groups using Kruskal-Wallis. *$p < 0.05$; **$p < 0.01$; §$p < 0.001$.

Figure 1*B* examines the portion of the metabolic pathway that leads from LNA to EPA and DPA. There were no differences in the ratios of EPA:LNA or of DPA:LNA for the IUGR *versus* the GA matched control infants. However, there was an increased ratio for the DPA:LNA for the birth weight group relative to the IUGR group. The elongation step forming DPA from EPA seems to be relatively insensitive to birth weight or GA. The large differences in the DHA:DPA ratio indicate that this may be the metabolic step that is most restricted in infants with IUGR.

DISCUSSION

The present work addresses specifically LCP metabolism in infants with IUGR compared with neonates of equivalent birth weight but shorter gestation or similar gestation but who are significantly smaller at birth. Our current results suggest lower formation of DHA in infants with IUGR and complement our previous observations (10,15) and those of others (9,20) that demonstrate higher LCP formation in neonates of lower birth weight and AGA. From these studies, we have been able to show that term and preterm infants synthesize DHA and AA from LNA and LA and that preterm infants seem to have an overall metabolic conversion greater than that found in term infants. The decrease in DHA and not in AA formation in infants with IUGR suggests that the metabolic abnormality may be related to the final metabolic steps that include the insertion of the sixth double bond and a partial β-oxidation to form DHA, because AA formation does not require these steps in its metabolism. The relative conversions observed in the n-3 pathway as shown in Fig. 1*A* support this concept, because it seems that the most restricted step is conversion of DPA to DHA. This conversion includes an elongation, a $\Delta 6$ desaturation, and a partial β-oxidation, which is considered to occur

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mainly in the peroxisome (21). On the basis of this finding, we speculate that infants with IUGR may have an impaired peroxisomal β-oxidation that explains the lower DPA to DHA conversion observed in this group of infants compared with the AGA control groups.

There is evidence that neonates with IUGR have altered maturation or inactive enzymes related to lipid metabolism (2,3,22). The work of Lane *et al.* (22) using rat models with uteroplacental insufficiency documents that the expression of the peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1 (PGC-1) mRNA varies according to muscle groups, suggesting differences in oxidative capacity of muscle fibers. The PGC-1 expression is gender specific. Higher expression of carnitine palmitoyl transferase I, an enzyme that regulates fatty acid β-oxidation as well as its transcriptional co-activator PGC-1, has also been documented in the skeletal muscle of rats with IUGR (22). These results highlight evidence of impaired expression of key enzymes in lipid metabolism that are mediated by the family of the nuclear receptor PPARs (PPAR-γ, PPAR-α, and PPAR-β). This group of transcriptional factors is known by its important regulatory effect on adiposity differentiation and lipid metabolism (23,24) and offers a possible explanation to the limited peroxisomal β-oxidation suggested by our results.

Considering the important role that DHA plays in neural and retinal development, the finding of a lower formation of DHA in infants with IUGR may have important implications as it can be a contributing factor to the abnormalities in neurodevelopment described in infants with IUGR (25,26). Also, DHA supplementation may improve DHA status in infants with a demonstrated DHA deficiency, such as premature infants or those with Zellweger syndrome. Taken together, these findings suggest a potential benefit for early nutritional interventions with DHA supplementation to improve neurologic outcome for infants with IUGR. Several clinical studies have shown the benefits of DHA supplementation in visual function of premature infants, improvement that correlates with better DHA status in red blood cells and plasma (27,28) More recently, Sarkadi-Nagy *et al.* (29) were able to document a positive correlation between DHA supplementation and DHA content in the brain, retina, and liver in 4-wk-old (adjusted age) baboons. The work of Martinez *et al.* (30) on 13 patients who had Zellweger and whose vision, muscle tone, social contact, and liver function and brain myelination improved after being supplemented with DHA ethyl ester (100–500 mg/d), offers further evidence in favor of the effect of DHA supplementation in patients with a DHA deficit.

There is an important difference in the feeding status of the infants with IUGR and the two control groups at the time of study. The effect of this difference in the reported results is difficult to assess because of limitations imposed by the small sample size. Nevertheless, that none of the patients in the IUGR group had received DHA-containing milk formula makes it unlikely that differences in the type of feeding explain the finding of lower DHA formation in infants with IUGR compared with the two AGA control groups. The possible effect of the age difference at study entrance between the infants with IUGR and the birth weight–matched control group on the results works in the opposite direction of the observed effects, because it would favor a higher DHA formation in the infants with IUGR compared with the 1 wk postnatally older birth weight–matched infants (10,20).

Finally, we consider selected methodologic aspects to be taken into account in the analysis of results. The simple model of AUC integration assumes that we are able to model concentrations over time using a linear interpolation between data points forming a trapezoid. Thus, we will underestimate the area when data points form a convex curve and conversely will overestimate the area when the curve is concave. The limited number of samples obtained in this study during the initial 24 h will predictably underestimate the precursor concentrations over time. There was also an underestimation of product formation because the study was restricted to 96 h. In our more recent studies, we have verified that, in some cases, DHA appearance in the plasma peaks at ~96 h but may continue for up to 168 h. This could account in part for the apparent lower rate of DHA formation observed in infants with IUGR; it is clear that there is a slower decline in LNA and a slower rate of rise in DHA on the basis of the observed isotopic fatty acid concentrations at 24, 48, and 96 h. Thus, perhaps the process may be slower than normal for IUGR but cumulative DHA formation over time may be unchanged. The limited blood sampling possible in this study does not allow a more complex kinetic analysis of fatty acids considering the fractional rate of synthesis and catabolism. The *in vivo* physiology and biochemistry of LCP metabolism are sufficiently complex such that even with presently available stable isotope methods, care must be exercised in the interpretations of the parameters available. The combined use of radioactive isotopes ([1-^{11}C]AA and [1-^{11}C]DHA) and *in vivo* detection of labeled metabolites using positron emission topography may permit in the future a more dynamic evaluation of DHA metabolism coupled to specific anatomic/functional loci within the CNS and specific cognitive functions (31). That stable isotope studies are based on the plasma pool has been considered an important limitation of this type of studies on the basis of the idea that plasma fatty acid status may not necessarily reflect the LCP accretion of critical organs such as the CNS and the retina (29,32,33).

CONCLUSION

In conclusion, this study extends our previous report of enhanced DHA and AA formation in neonates with lower GA and birth weight indicating that less mature infants with adequate fetal growth have greater LCP formation from EFA precursors. The finding of significantly lower DHA formation in the infants with IUGR relative to neonates who were 1 kg greater in birth weight is in contrast to the prediction based on the observed enhanced metabolic activity in preterm infants of lower birth weight. That this restriction occurs only in the formation of DHA and not of AA suggests that the effect may be related to metabolic steps that are specific to DHA. Our results lead us to suggest that infants with IUGR have a specific impairment in the final biosynthetic pathway that leads to the synthesis of DHA. We speculate that this metabolic defect

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could contribute to the abnormalities in neurodevelopment observed in neonates with IUGR.

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