



# Synthesis of long-chain polyunsaturated fatty acids in preterm newborns fed formula with long-chain polyunsaturated fatty acids<sup>1-3</sup>

Virgilio P Carnielli, Manuela Simonato, Giovanna Verlato, Ingrid Luijendijk, Mario De Curtis, Pieter JJ Sauer, and Paola E Cogo

## ABSTRACT

**Background:** Docosahexaenoic acid (DHA) and arachidonic acid (AA) are long-chain polyunsaturated fatty acids (LCPs) that play pivotal roles in growth and neurodevelopment.

**Objective:** We aimed to quantify the synthesis of LCPs in preterm infants fed infant formula containing LCPs.

**Design:** Twenty-two preterm infants were randomly assigned to either the no-LCP group (fed formula without LCPs;  $n = 11$ ) or the LCP group (fed formula with LCPs;  $n = 11$ ). Dietary LCPs had higher  $^{13}\text{C}$  content than did the endogenously synthesized LCPs, which were derived from linoleic and  $\alpha$ -linolenic acids. The  $^{13}\text{C}$  content of major selected plasma fatty acids was measured by using gas chromatography–isotope ratio mass spectrometry at birth and at age 1, 3, and 7 mo. Absolute LCP synthesis and the percentage of LCP synthesis relative to dietary intake were calculated.

**Results:** Percentage AA synthesis was  $67.2 \pm 7.8\%$ ,  $35.9 \pm 9.8\%$ , and  $29.0 \pm 10.3\%$ , and that of DHA was  $41.7 \pm 14.9\%$ ,  $10.5 \pm 8.1\%$ , and  $7.4 \pm 6.2\%$  at 1, 3, and 7 mo old, respectively. Absolute AA synthesis was  $26.7 \pm 4.2$ ,  $14.4 \pm 3.9$ , and  $11.6 \pm 4.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  and that of DHA was  $12.6 \pm 4.5$ ,  $3.2 \pm 2.5$ , and  $2.3 \pm 1.9 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at 1, 3, and 7 mo old, respectively. AA and DHA synthesis decreased significantly ( $P < 0.01$ ) with time, and AA synthesis was significantly ( $P < 0.01$ ) greater than DHA synthesis.

**Conclusions:** By this novel approach, we measured endogenous LCP synthesis in infants receiving dietary LCPs over long periods. By age 7 mo, LCP synthesis was dramatically lower in preterm infants fed LCPs. *Am J Clin Nutr* 2007;86:1323–30.

**KEY WORDS** Preterm infant, long-chain polyunsaturated fatty acids, LCPs, natural abundance, endogenous synthesis

## INTRODUCTION

Docosahexaenoic (DHA; 22:6–3) and arachidonic (AA; 20:4–6) acids are the predominant long-chain polyunsaturated fatty acids (LCPs) in the structural phospholipids of the human brain and retina (1–3), and both occur in human milk. Animal and human studies have indicated that DHA is important to the development of the central nervous system (4, 5). The LCP content in human milk is somewhat variable, depending mostly on the maternal diet and on the stage of lactation (6). Information on the effect of the variable LCP composition of human milk fat on infant neurodevelopment is limited, but infant formulas marketed in Europe and, more recently, in North America contain LCPs in amounts similar to the mean values in human milk in Western countries (7).

Information on the endogenous synthesis of LCPs in the newborn infant and on the contribution of such synthesis to the overall availability of LCPs for tissue growth and development is limited. Studies in preterm infants and term neonates, conducted by using the LCP precursors linoleic acid (LA) and  $\alpha$ -linolenic acid (ALA) labeled with stable isotopes (deuterium or  $^{13}\text{C}$ ), indicate that 1) LCP turnover is very slow; 2) body stores at birth are not negligible; 3) LCP synthesis occurs even in small preterm infants; 4) synthesis appears to be higher in preterm than in term infants; and 5) infants who are small-for-gestational-age may have less LCP synthesis than those who are normal-size-for-gestational-age (8–12). Despite these studies, reliable quantitative data on endogenous synthesis are still lacking.

We report here a novel approach to measuring endogenous AA and DHA synthesis over a very long periods by using the “stable isotope natural abundance” approach (13, 14). The method is based on the measurement of the natural variation in the  $^{13}\text{C}$  content of selected nutrients. Plants with the C3 or C4 photosynthetic pathway show different discrimination against  $^{13}\text{C}$  during photosynthesis. C3 plants, such as wheat and sugar beets, exhibit a lower ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  ( $^{13}\text{C}:^{12}\text{C}$ ) in their organic compound than do the C4 plants, such as corn and sugar cane (15). A careful selection of the nutrient source thus provides unique opportunities for performing metabolic studies. In regions such as Europe, most foods consumed are based on C3 plants, a basis that lead to  $^{13}\text{C}$  enrichment values in humans similar to the background diet. In this context, when nutrients from C4 plants are used, they behave as natural tracers, and changes in isotopic enrichment can be measured in plasma and breath by using high-precision isotope ratio–mass spectrometry. With the use of this approach, it

<sup>1</sup> From the Division of Neonatology, Institute of Maternal-Infantile Sciences, Polytechnic University of Marche, Ancona, Italy (VPC); the Department of Pediatrics, University of Padua, Padua, Italy (MS, GV, and PEC); the Laboratory of Biochemistry, Erasmus University, Rotterdam, Netherlands (IL); the Department of Gynecology, Perinatology and Child Health, La Sapienza University, Rome, Italy (MDC); and the Department of Pediatrics, Beatrix Children’s Hospital, University Medical Center Groningen, Groningen, Netherlands (PS).

<sup>2</sup> Nutricia Research donated the study formulas.

<sup>3</sup> Reprints not available. Address correspondence to VP Carnielli, Istituto di Scienze Materno Infantili, Università Politecnica della Marche, Azienda Ospedaliera Universitaria Ospedali Riuniti Ancona, SOD Neonatologia, Via Corridoni 11 62123 Ancona, Italy. E-mail: lab2@pediatria.unipd.it.

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**TABLE 1**Composition of the Study Feedings and  $\delta^{13}\text{C}$  content of selected methyl ester fatty acids from no-LCP and LCP formulas<sup>1</sup>

	No-LCP group (n = 11)	LCP group (n = 11)	$\delta^{13}\text{C}$ no-LCP formula	$\delta^{13}\text{C}$ LCP formula
			% PDB	% PDB
Energy (kcal/L)	800	800		
Protein (g/L)	22	22		
Carbohydrates (g/L)	80	80		
Lactose (g/L)	40	40		
Dextrine maltose (g/L)	40	40		
Fat (g/L)	44	44		
Fatty acid (% by wt)				
8:0	2.29	2.20		
10:0	1.92	1.88		
12:0	14.08	14.34		
14:0	6.72	6.47		
16:0	9.33	8.65	-27.6 ± 0.1 <sup>2</sup>	-27.5 ± 0.2
18:0	4.35	4.48	-29.9 ± 0.04	-29.9 ± 0.04
20:0	0.27	0.29		
16:1n-7	0.21	0.17		
18:1n-9	43.15	42.49	-30.1 ± 0.1	-29.6 ± 0.2
18:1n-7	0.63	0.63		
18:2n-6 (LA)	14.14	14.05	-29.9 ± 0.4	-29.6 ± 0.1
20:4n-6 (AA)	0	0.84	ND	-16.8 ± 0.01
18:3n-3 (ALA)	1.23	1.21	-30.3 ± 0.3	-29.8 ± 0.1
20:5n-3	0	0		
22:6n-3 (DHA)	0	0.64	ND	-17.3 ± 0.3
Total n-6 LCPs (% by wt)	0	0.97		
Total n-3 LCPs (% by wt)	0	0.64		

<sup>1</sup> LCP, long-chain polyunsaturated fatty acids; no-LCP group, the infants fed the formula without LCPs; LCP group, the infants fed the formula with LCPs; PDB, Pee Dee Belemnite; LA, linoleic acid; AA, arachidonic acid; ND, not detectable; ALA,  $\alpha$ -linolenic acid; DHA, docosahexaenoic acid.

<sup>2</sup>  $\bar{x} \pm \text{SD}$  (all such values).

was possible to evaluate the contribution of diet to plasma glucose and plasma lipids (13, 14). In the present study, we proposed an extension of this approach that enabled us to assess the contribution of both dietary and endogenously synthesized LCPs to plasma fatty acids. We obtained novel information on absolute endogenous synthesis of LCPs in preterm infants fed formula that included LCPs from age 1 to 7 mo.

## SUBJECTS AND METHODS

### Subjects

This study was part of a randomized clinical trial conducted at the Sophia Children's Hospital (Rotterdam, Netherlands) from 1992 to 1995. Preterm infants whose mothers decided not to or were unable to provide their own breast milk were randomly assigned to 2 study formulas with identical fatty acid composition and differing only in LCP supplementation (16). The composition of the study formulas is shown in **Table 1**. Infants enrolled in the study were healthy, normally growing, and exclusively fed the study formulas until age 7 mo, when they were weaned according to local practices and with local foods. Clinical characteristics are shown in **Table 2**.

Participation in the study was voluntary, and written informed consent was obtained from each infant's parents. The project was approved by the Ethics Committee of Sophia Children's Hospital, Erasmus University Rotterdam (Rotterdam, Netherlands), in accordance with the principles of the Declaration of Helsinki.

### Clinical design

Subjects were randomly assigned to the study formula after the maternal decision not to breastfeed. Researchers who conducted the clinical and laboratory evaluation and analysis were blinded to dietary assignment. Study formulas were produced exclusively by Nutricia Research (Zoetermeer, Netherlands) and were identical with the exception of the addition of the LCPs in the supplemented formula (LCP formula). The fat blend of the LCP formula contained DHA and AA derived exclusively from the single-cells oils (DHASCO and ARASCO; Martek Biosciences Corporation, Columbia, MD).

Data on infant formula composition and on the intestinal fat and fatty acid absorption were reported elsewhere (16). EDTA-containing tubes (Vacutainer; Becton Dickinson, Rutherford,

**TABLE 2**Clinical characteristics of the study infants<sup>1</sup>

Characteristic	No-LCP group (n = 11)	LCP group (n = 11)	P
Sex (M/F)	4/7	6/5	
Birth weight (kg)	1.16 ± 0.27 <sup>2</sup>	1.15 ± 0.36	0.48
Gestational age (wk)	31 ± 2	31 ± 2	0.92
Weight at 7 mo (kg)	6.7 ± 0.3	6.7 ± 0.5	0.75

<sup>1</sup> LCPs, long-chain polyunsaturated fatty acids; no-LCP, formula with no added LCPs; LCP, formula with LCPs. Data were compared by using a *t* test.

<sup>2</sup>  $\bar{x} \pm \text{SD}$  (all such values).

NJ) were used to collect blood (0.5 mL) from the mother, from the umbilical cord at the time of the delivery (or from the infant within 30 min after birth), and from the infants at 1, 3, and 7 mo of age. Plasma lipid classes and fatty acids were measured as reported previously (17).

### <sup>13</sup>C content in the formula and plasma fatty acids

The <sup>13</sup>C enrichment of the fatty acid methyl esters derived either from plasma lipid classes or from the formula fat blend were analyzed by using gas chromatography–combustion inter-phase isotope ratio–mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany). Separation of the methyl esters was achieved on a DB225 column (length: 30 m; internal diameter: 0.25 mm; film thickness: 0.25 μm; J & W Scientific, Folsom, CA). Injections were performed in splitless mode at an injector temperature of 250 °C.

The <sup>13</sup>C contents of individual fatty acids were expressed as δ<sup>13</sup>C values relative to Pee Dee Belemnite (PDB) carbonate (18). The following formula was used to calculate δ<sup>13</sup>C:

$$\delta^{13} = [(R_x - R_s) - 1] \times 1000 \quad (1)$$

where  $R_x$  is the isotopic ratio of the sample, and  $R_s$  is the isotopic ratio of the reference standard. The defined reference standard for <sup>13</sup>C has been PDB with <sup>13</sup>C:<sup>12</sup>C of 0.0112372.

To determine <sup>13</sup>C values relative to the PDB reference, CO<sub>2</sub> samples with a known δ<sup>13</sup>C value (−61.5079) were introduced at appropriate time points during chromatography. All samples were analyzed in duplicate or triplicate. A baseline-to-baseline peak separation was obtained for all major fatty acids, including AA and DHA, that had an SD of ≤0.3%.

The percentage of endogenous synthesis was calculated by using the following equation:

$$\text{Percentage endogenous synthesis} = 100 - [(A - C/A - B) \times 100] \quad (2)$$

where  $A$  is the δ<sup>13</sup>C value of the parent dietary fatty acid (LA or ALA),  $B$  is the δ<sup>13</sup>C value of the dietary LCP (AA or DHA), and  $C$  is the δ<sup>13</sup>C value of the plasma phospholipid LCP (AA or DHA).

Absolute AA or DHA synthesis (in mg · kg<sup>−1</sup> · d<sup>−1</sup>) was then calculated by using the following equation:

$$\text{Synthesis (mg)} = (D \times E \times F) / 10\,000 \quad (3)$$

where  $D$  is the daily intake of AA or DHA (in mg · kg<sup>−1</sup> · d<sup>−1</sup>),  $E$  is the intestinal absorption (in %), and  $F$  is the endogenous synthesis (in %). Estimation of endogenous synthesis was also based on the following 2 assumptions: first, there was no metabolic discrimination between dietary and endogenously synthesized LCPs, and, second, LCP oxidation was negligible.

### Statistical analysis

The  $t$  test was used to compare the clinical characteristics of study groups and the composition of the study formulas. Plasma fatty acids, isotopic enrichment, percentages, and absolute AA and DHA synthesis values were compared by using 1- or 2-factor repeated-measures analysis of variance. The Bonferroni test was used for post hoc analysis. All statistical analyses were performed with SPSS (version 13.0; SPSS Inc, Chicago, IL) and

MICROSOFT EXCEL (version 2000; Microsoft Corp, Redmond, WA) software. Significance was set at 0.05. Data were expressed as means ± SDs.

## RESULTS

### Characteristics of infant formulas

The fatty acid composition of the study formulas is reported in Table 1. In the no-LCP formula, AA and DHA were undetectable, whereas they were 0.84% by wt and 0.64% by wt of the fat blend of the LCP formula. With the exception of AA and DHA, no significant differences in concentration or in the <sup>13</sup>C enrichment of major selected fatty acids were found between the 2 formulas. This information confirms that the 2 formulas had the same fat blend and differed only as a result of the addition of the ARASCO and DHASCO oils. Identical <sup>13</sup>C enrichment of the major formula fatty acids, including LA and ALA, rules out minor “isotopic contamination” by small amounts of non-LCPs from the ARASCO and DHASCO oils. The <sup>13</sup>C enrichment of dietary LA and ALA did not differ significantly, but their enrichment, as expected by study design, was significantly different from that of AA and DHA ( $P < 0.001$  for both). The <sup>13</sup>C of formula AA and DHA did not differ significantly ( $P = 0.12$ ).

### Characteristics of the study infants

Clinical characteristics of the study infants are reported in Table 2. There were no differences between groups at birth and during the study. All infants were exclusively fed the study formulas, and no other nutrients were allowed during the study period. This feeding pattern was verified at each follow-up visit. Formula volume and energy intake did not differ significantly between groups. All infants grew normally during the first 7 mo of life, and no significant difference between groups was found in weight gain at any of the study time points.

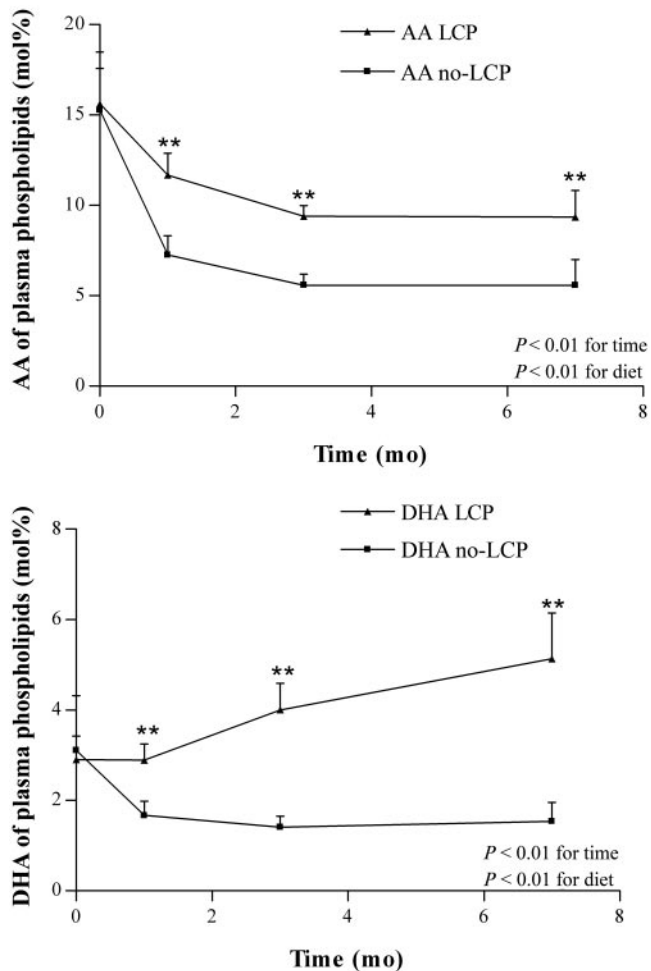
### Effect of diet on plasma phospholipid fatty acids

AA and DHA of plasma phospholipids (mol%) in infants at 1, 3 and 7 mo old are reported in Figure 1. AA declined significantly from birth to age 7 mo in both groups: from 15.3 ± 3.2 mol% to 5.6 ± 1.4 mol% in the no-LCP group and from 15.6 ± 1.9 mol% to 9.4 ± 1.5 mol% in the LCP group ( $P < 0.01$  for both). DHA also changed significantly from birth to age 7 mo in both groups: it declined from 3.1 ± 1.2 mol% to 1.5 ± 0.4 mol% in the no-LCP group, and it rose from 2.9 ± 0.5 mol% to 5.1 ± 1.0 mol% in the LCP group ( $P < 0.01$  for both). The time effect, diet effect, and interaction were significant ( $P < 0.01$  for all).

The plasma phospholipids AA and DHA in infants fed the no-LCP formula were significantly ( $P < 0.01$ ) lower than those in infants fed the LCP formula. No major differences were found for saturated and monounsaturated fatty acids (data not shown).

### Maternal compared with cord blood fatty acid isotopic enrichments

The <sup>13</sup>C enrichment values of LA, ALA, AA, and DHA from maternal blood and from cord blood did not differ significantly. Nor were significant differences found among the other major fatty acids (data not shown).



**FIGURE 1.** Arachidonic acid (AA) and docosahexaenoic acid (DHA) (in mol%) of plasma phospholipids in preterm infants fed formula with or without long-chain polyunsaturated fatty acids (LCP or no-LCP formula). AA and DHA decreased significantly during the study period ( $P < 0.01$ , 1- and 2-factor ANOVA) in both groups. AA and DHA were significantly ( $P < 0.01$ ) lower in the no-LCP group. The time effect, diet effect, and interaction were significant ( $P < 0.01$ ). \*\*Significantly different from the no-LCP group,  $P < 0.01$ .

### Changes in isotopic enrichment during the study

The  $^{13}\text{C}$  enrichment of plasma phospholipids LA, ALA, AA, and DHA in the individual study patients is depicted in **Figure 2** and **Figure 3**.

#### Linoleic acid

The  $^{13}\text{C}$  enrichment of LA did not differ significantly during the study or between the study groups (**Figure 2**). None of these enrichment values differed significantly from the  $^{13}\text{C}$  enrichment of dietary LA (ie,  $-29.7 \pm 0.3$ ).

#### Linolenic acid

The determination of the  $^{13}\text{C}$  enrichment of ALA in plasma phospholipids was not feasible in several samples because of the low ALA content of plasma phospholipids. ALA  $^{13}\text{C}$  enrichment was measured more consistently in plasma triacylglycerols. Paired measurements of phospholipids and triacylglycerol ALA were feasible in 24 plasma samples and yielded identical results

(data not shown). Enrichment of ALA in the LCP and no-LCP infants was fairly constant throughout the study (**Figure 3**).

#### Arachidonic acid

The AA  $^{13}\text{C}$  enrichment in cord blood phospholipids did not differ significantly between the study groups. In the no-LCP group, AA enrichment values did not differ significantly during the 7-mo study ( $P = 0.168$ ) (**Figure 2**). In the LCP group, the  $^{13}\text{C}$  enrichment became less negative, closer to the dietary value ( $P < 0.01$ ). The difference in the LCP group was already significant at 1 mo ( $-26.2 \pm 1.9$ ;  $P < 0.01$ ) and between 1 and 3 mo ( $P < 0.01$ ) but not between 3 and 7 mo ( $P = 1.000$ ) (**Figure 2**).

#### Docosahexaenoic acid

Cord blood phospholipid DHA enrichment did not differ significantly between the study groups ( $-29.6 \pm 1.7$  and  $-29.8 \pm 1.8$  in the no-LCP and LCP groups, respectively). In the no-LCP group, the enrichment did not change significantly during the study ( $P = 0.215$ ) (**Figure 3**). In the LCP group, we observed a significant ( $P < 0.01$ ) change toward less negative values (closer to the enrichment of the dietary DHA). The difference was already significant at 1 mo ( $-23.6 \pm 2.5$ ;  $P < 0.01$ ) and between 1 and 3 mo ( $P < 0.01$ ), but no significant difference was seen between 3 and 7 mo ( $P = 1.00$ ) (**Figure 3**).

### Estimation of endogenous synthesis of long-chain polyunsaturated fatty acids

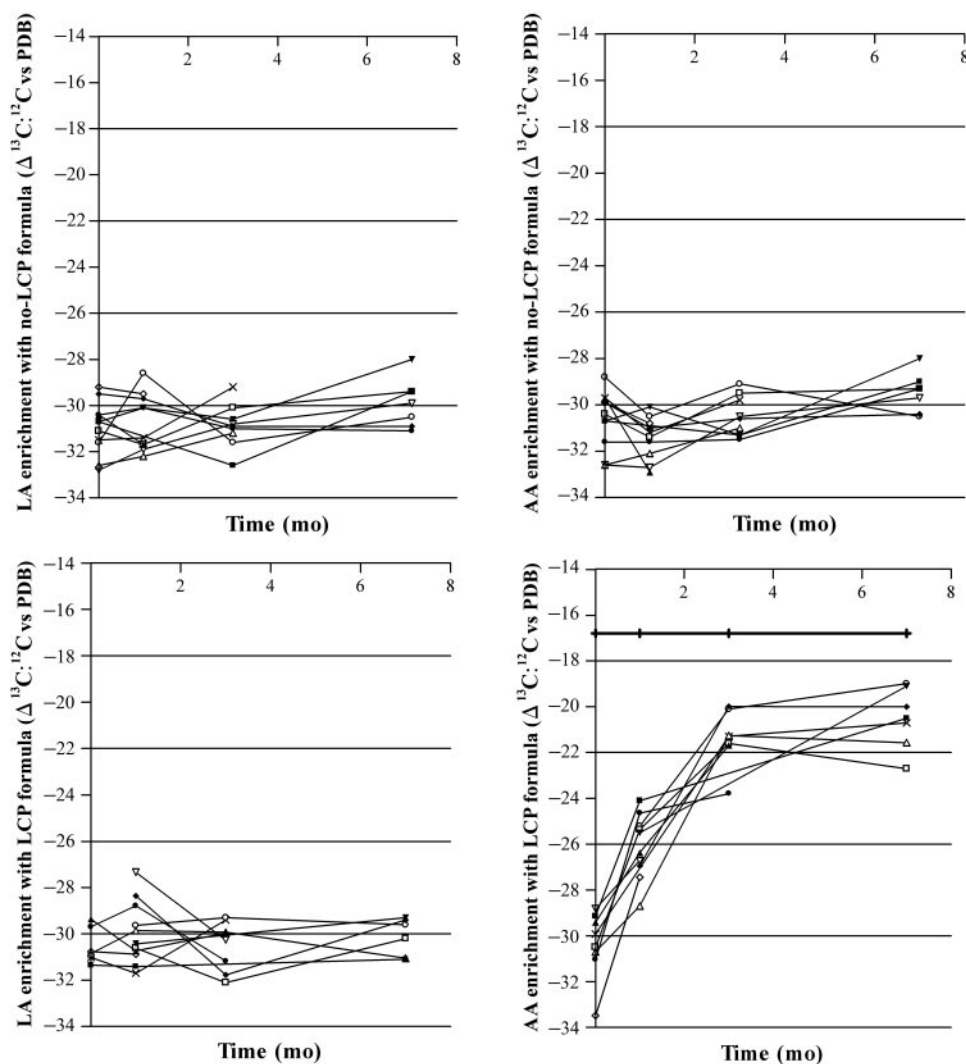
Percentages of AA and DHA endogenous synthesis relative to the metabolizable dietary intakes are depicted in **Figure 4**. There was a significant time effect and a significant difference between AA and DHA ( $P < 0.01$  for both) but no significant interaction ( $P = 0.852$ ).

Absolute amounts of endogenous LCP synthesis were calculated from the net amount of intestinal absorption, which was measured at 1 mo by the classical fat balance studies (16) (**Figure 4**). We used the individual data measured at 1 mo for the 3 and 7 mo calculations. Absolute AA endogenous synthesis was estimated to be  $27 \pm 4$  (range: 20–32),  $14 \pm 4$  (10–22), and  $12 \pm 4$  (7–18)  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (**Figure 4**) at age 1, 3, and 7 mo, respectively. DHA synthesis was  $13 \pm 4$  (8–21),  $3 \pm 2$  (0.8–7), and  $2 \pm 2$  (0.2–5)  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at the 3 time points (**Figure 4**). There was a significant time effect and difference between AA and DHA ( $P < 0.01$  for both) but no significant interaction ( $P = 0.256$ ).

## DISCUSSION

### General considerations

We report here on the feasibility of measuring endogenous LCP synthesis over long periods in preterm infants fed formula containing LCP. This approach, using the isotopic natural abundance technique, has been used in the past only in “acute” studies with the aim of measuring the metabolic fate of nutrients (13, 14). To the best of our knowledge, the natural abundance technique has never been used to assess the endogenous synthesis of nutrients. When body lipids are investigated, especially in short studies, only qualitative information on metabolic processes can be obtained, mainly because of the long time needed to reach isotopic steady state. Lipids have a slow turnover and also long



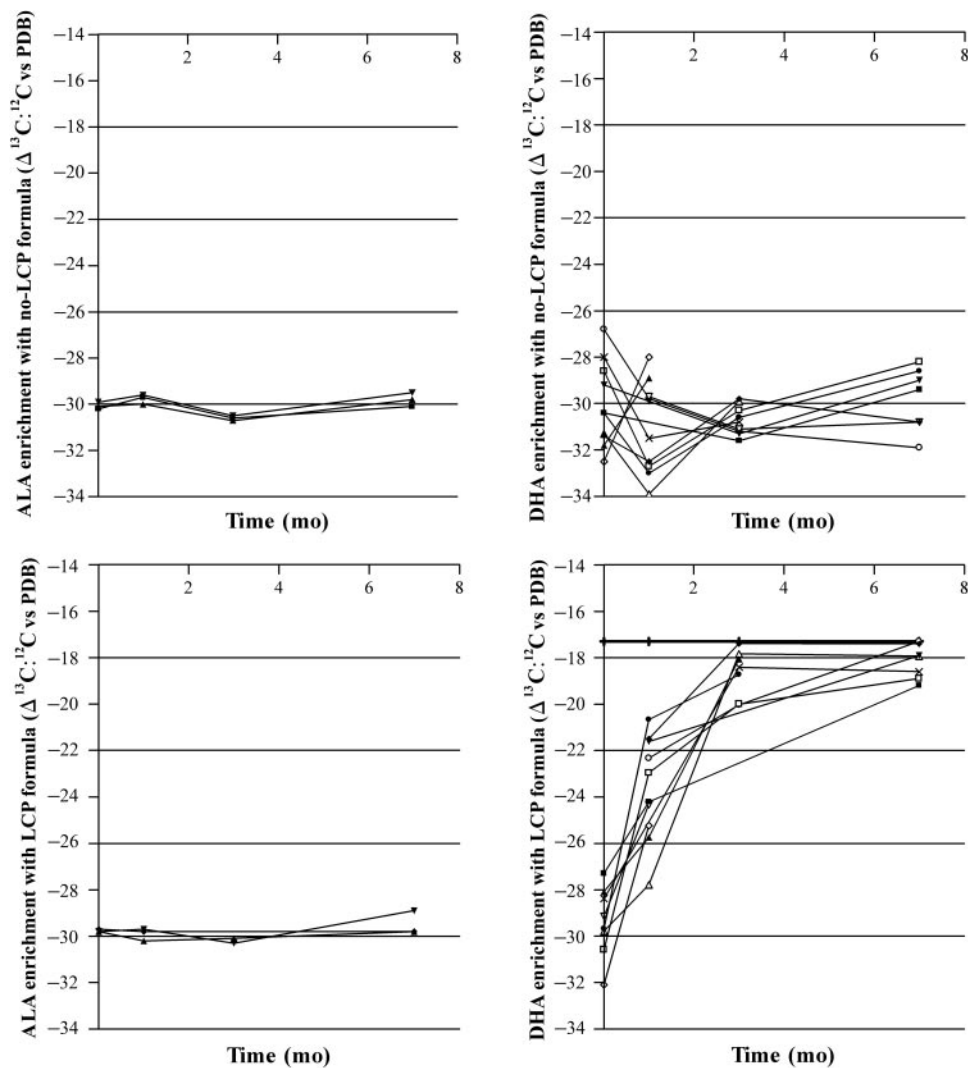
**FIGURE 2.** The  $^{13}\text{C}$  enrichment of plasma phospholipid linoleic acid (LA) and arachidonic acid (AA) in preterm infants fed formula with or without long-chain polyunsaturated fatty acids (LCP or no-LCP formula). Each symbol represents a different subject.  $\Delta$ , change; PDB, Pee Dee Belemnite (reference). The LA  $^{13}\text{C}$  enrichment values did not change during the study in either the no-LCP or the LCP group. The bold line in the lower righthand panel represents AA  $^{13}\text{C}$  enrichment in the diet. AA  $^{13}\text{C}$  enrichment did not change significantly in the no-LCP group ( $P = 0.168$ ), but it became less negative (ie, closer to the enrichment of dietary AA) in the LCP group ( $P < 0.01$ ).

equilibration times with tissue lipids. To circumvent this problem, we applied the natural abundance approach to a unique population—newborn infants. We took advantage of the fact that newborns are normally fed a constant diet over very long periods. We studied LCP biosynthesis because of the major importance of these nutrients for growth and neurodevelopment in early life.

We did not calculate endogenous synthesis on the basis of absolute plasma concentrations of labeled or unlabeled molecules, as was done in other studies (8, 19). We used isotopic enrichment, which is conceptually similar to the tracer-to-tracee ratio. In our model, we assume the system to be in steady state and the isotopic enrichment to better reflect the proportion of dietary LCPs to endogenous synthesis. Absolute plasma concentrations of the tracer and the tracee, as used in other, more acute studies, are more prone to calculation errors when steady state is not fully achieved. For instance, in case of less clearance of plasma lipids, higher plasma concentrations of the newly synthesized, labeled LCPs would result in an overestimation of the synthesis. Our method offers several major advantages: there is no need for

highly enriched and expensive tracers or for alteration of the diet; blood sampling is limited in comparison with that required in classic kinetic studies (10, 12, 19); and sufficient time is allowed for equilibration between the plasma pool and the tissues. Limitations mainly have to do with the availability of the analytic instrumentation and of suitable nutrients, which behave as tracers.

If the first 3 advantages are self-explanatory, the long equilibration time needed with tissues rich in fat appears to be a more complex issue. Uncertainties exist about the interpretation of the classical lipid kinetic because of the slow equilibration between the small plasma pool and the huge, very-slowly-turning-over body fat pools (mainly brain and adipose tissue). The approach described in this report represents an advantage over the classic tracer studies in that it allows much more time for equilibration. Sufficient time is allowed for the contribution of LCP molecules that are released via lipolysis after storage. It is difficult to correct for this issue, especially during the first few days of life. If significant amounts of depot LCPs are present at birth, as has



**FIGURE 3.** The  $^{13}\text{C}$  enrichment of plasma phospholipid linolenic acid (ALA) and of docosahexaenoic acid (DHA) in preterm infants fed formula with or without long-chain polyunsaturated fatty acids (LCP or no-LCP formula). Each symbol represents a different subject.  $\Delta$ , change; PDB, Pee Dee Belemnite (reference). ALA  $^{13}\text{C}$  enrichment values did not change significantly in either the no-LCP or LCP group. The bold line in the lower righthand panel represents DHA  $^{13}\text{C}$  enrichment in the diet. DHA  $^{13}\text{C}$  enrichment did not change significantly in the no-LCP group ( $P = 0.215$ ), but it became less negative (ie, closer to the enrichment of dietary DHA) in the LCP group ( $P < 0.01$ ).

been reported or hypothesized (20), their contribution to plasma fatty acids from lipolysis could be significant. Were this to be the case, it would result in an overestimation of endogenous LCP synthesis. This is why we choose to draw the first blood sample at when the infant is 1 mo of age. However, unlike in stress conditions (21), lipolysis, should be very low in the growing, fully fed infant, who is actively depositing fat.

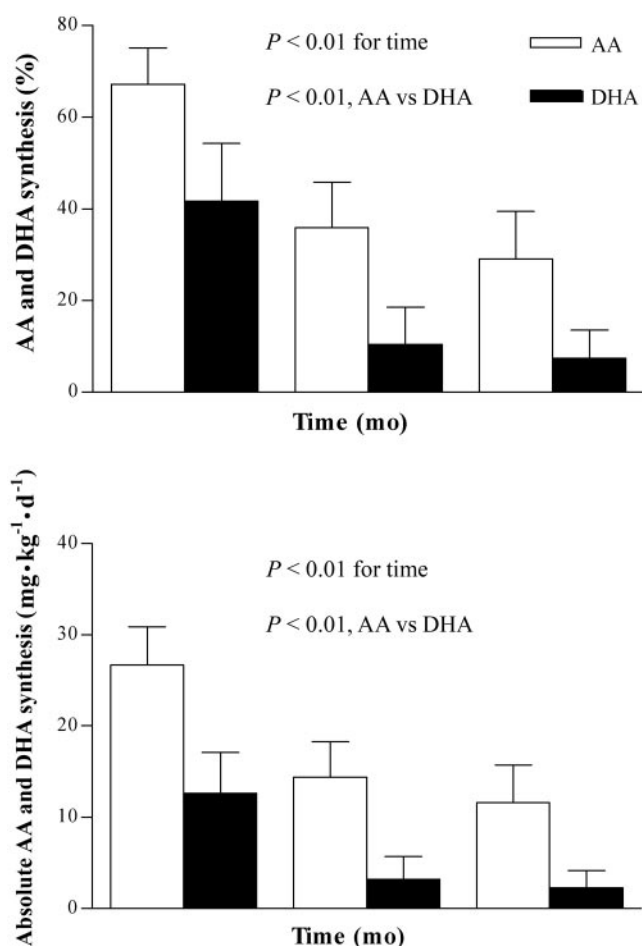
Endogenous synthesis could be overestimated in case of an uncontrolled dietary intake of LCP from sources other than the study infant formula. We carefully controlled this aspect of the protocol, and no infant received feeds other than the study formula. Variation of  $^{13}\text{C}$  due to time of sampling and feeding also was unlikely, because all infants were fed 5–6 times/d. In addition, infants have slow gastric emptying, and lipid absorption peaks slowly—a few hours after feeding. Isotopic measurements were performed on plasma phospholipids, which have a lower turnover than do plasma triacylglycerols (22), and sampling was conducted just before feeding. This protocol makes the study

design very close to a steady state condition. Moreover, we had the opportunity to measure a few additional samples that were collected at various times from the feedings, and the isotopic enrichments were mostly constant (data not shown).

Overestimation of synthesis could have occurred if dietary lipids were to be oxidized at a different rate than endogenously synthesized lipids. No data exist to support this notion; in addition, the oxidation of LCPs was found to be somewhat low in growing preterm infants (10).

We wish to comment briefly on the precision of our estimates. We achieved analytic precisions of 0.3‰, which translates into an error of 2.3% and 2.4% in the endogenous synthesis of AA and DHA, respectively. Biological error due to sampling time should account for  $\leq 1.5\%$  of errors, and errors in estimating LCP intakes from infant formula volume should also account for  $\leq 3\%$  of errors. The coefficient of intestinal fat absorption carries, however, a much larger variability because of the well-recognized imprecision of the metabolic balance studies, and that





**FIGURE 4.** Percentage and absolute synthesis of arachidonic acid (AA) and docosahexaenoic acid (DHA) in the group fed formula with long-chain polyunsaturated fatty acids (LCP formula). The percentage synthesis was significantly reduced with time ( $P < 0.01$ ); there was a significant ( $P < 0.01$ ) difference between AA and DHA but no significant interaction ( $P = 0.852$ ). Absolute endogenous synthesis also was significantly ( $P < 0.01$ ) reduced with time; there was a significant ( $P < 0.01$ ) difference between AA and DHA but no significant interaction ( $P = 0.256$ ). Data are expressed as means  $\pm$  SDs.

could have contributed to the interindividual variability in LCP synthesis. Because AA and DHA are obtained from LA and ALA by chain elongation reactions (1 acetyl-CoA molecule for AA and 2 acetyl-CoA molecules for DHA), it is theoretically possible that changes in isotopic enrichment could occur by the addition of carbon atoms. This process however, has a negligible effect on our calculation. Even when we assumed that all acetyl-CoA molecules used for LCP synthesis were from C3 plant products (which is extremely unlikely), only a change of 0.2‰ to 0.6‰ would be produced. More important, we did not observe any change in isotopic enrichment in the no-LCP group during the 7-mo study period. It should be noted that all potential sources of errors taken together could have caused a maximum error of 3–4  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for AA and of 1–2  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for DHA. These figures are much smaller than the biological differences found in the present study.

#### Clinical considerations

The major findings of this study are that LCP synthesis is far from trivial in early life and that it decreases with age and becomes somewhat low at 7 mo. Mean endogenous synthesis of AA

decreased from  $\approx 27 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at 1 mo to 12  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at 7 mo, and that of DHA increased from  $\approx 13 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at 1 mo to 2  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at 7 mo. In the present study, the use of the LCP formula resulted in metabolizable dietary intakes of  $\approx 40 \text{ mg} \text{ AA} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  and of 30  $\text{mg} \text{ DHA} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  up to the age of 7 mo. The information that endogenous synthesis decreases with age is new, and repeated measurements of the endogenous LCP synthesis in the same subjects have not previously been performed. Previous studies showed that, shortly after birth, synthesis was higher in preterm infants than in term infants (23). Information on the somewhat low synthesis at 7 mo seems of particular interest, and more studies are in progress. These studies should answer the question of whether this is a developmentally regulated phenomenon or whether the dietary LCP supplementation reduces the endogenous LCP synthesis.

In summary, we report here a novel approach in assessing the synthesis of LCPs over long periods. This approach enabled us to measure in vivo the synthesis of LCPs in formula-fed preterm infants up to the age of 7 mo. The synthesis of AA was significantly higher than that of DHA, and both decreased with age.

The authors' responsibilities were as follows: VPC: designed the experiment and conducted the study; MS, GV, and IL: contributed to the collection and analysis of data; MDC and PS: provided significant advice on the study design and contributed to subject recruitment; PEC: contributed significantly to the analytic methods and to the data analysis; and all authors: contributed to the writing of the manuscript. None of the authors had a personal or financial conflict of interest.

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