Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1073–1077, February 1993
Applied Biological Sciences

Arachidonic acid status correlates with first year growth in preterm infants

SUSAN E. CARLSON*†‡§, SUSAN H. WERKMAN*, JEANETTE M. PEEPLES*, RICHARD J. COOKE*†, AND ELIZABETH A. TOLLEY¶

Departments of *Pediatrics, †Obstetrics and Gynecology, ‡Biochemistry, and ¶Biostatistics and Epidemiology, The University of Tennessee, Memphis, TN 38163

Communicated by Ralph T. Holman, October 5, 1992 (received for review December 18, 1991)

ABSTRACT **Diets deficient in the ω-6 fatty acid linoleic acid reduce arachidonic acid (Ach) concentrations and retard growth of developing animals and humans. Nevertheless, plasma phosphatidylcholine Ach concentrations declined from 84 ± 23 mg/liter at birth to a nadir of 38 ± 11 mg/liter at 4 mo of age in preterm infants fed commercial formulas with linoleic acid, and weight normalized to that of term infants fell progressively beginning at 2 mo of age. The nadir of plasma phosphatidylcholine Ach (31 ± 7 mg/liter) and growth were further reduced by formula containing marine oil compared with the commercial formulas. Ach status (defined as the mean plasma phosphatidylcholine Ach concentration at 2, 4, and 6.5 mo) correlated with one or more measures of normalized growth through 12 mo. Ach status and maternal height accounted for as much as 59% of the weight variance and 68% of the length variance in infants fed standard formulas. Better Ach status was not from higher energy intakes. A conditional Ach deficiency in preterm infants may contribute to growth over the first year of life. On the strength of the relationship between Ach status and growth, we hypothesize that dietary Ach could improve first year growth of preterm infants.**

Despite recent advances in neonatal intensive care, preterm infants do not achieve first year growth equivalent to that of infants born at term (1–5). Moreover, preterm infants remain smaller than term infants after the first year of life (6, 7), and poorer individual growth is associated with poorer mental and motor performance (8–11). That infants with chronic lung disease have poorer growth after discharge is well known (12–14); however, preterm infants free of chronic illness and fed standard formulas had progressive declines in normalized weight, weight-to-length ratio, and head circumference beginning 2 mo after expected delivery (15), as has been reported (1, 3).

Erythrocyte phospholipid arachidonic acid (Ach) in these infants also fell progressively, reaching a nadir 4 mo after expected term delivery (16) below that seen in formula-fed term infants of equivalent age (17). The nadir in plasma phospholipid Ach concentration coincided with the onset of the decline in normalized weight. The mean concentration of Ach in plasma phosphatidylcholine (PtdCho) of preterm infants at birth is 84 ± 23 mg/liter (mean ± SD, range 43–152, $n = 86$, unpublished data). When these infants were 3 weeks old, this value was 67 mg/liter (range 41–136 mg/liter, $n = 59$), and by 4 mo after term this value was 38 ± 11 mg/liter ($n = 29$). Direct evidence that normalized growth might relate to Ach status came from the observation that marine oil-supplemented formula further decreased the concentrations of plasma PtdCho Ach (31 ± 7 mg/liter) (16) and normalized weights (15) compared with standard formula. Plasma PtdCho linoleic acid (Lin) concentration remained high and was unaffected by marine oil supplementation.

Diets deficient in the essential ω6 fatty acid, Lin, cause declines in phospholipid Ach (18) and impair growth in developing animals (19, 20) and human infants (21). Ach restored growth in ω6-deficient rats (22) better than Lin, and diets with Ach compared with those without Ach increased tissue Ach and growth in young alligators (23). Holman and coworkers (24–27) have shown that plasma phospholipid Ach declines in a number of diseases despite apparently good Lin intakes (24–27). In this study, the growth of premature infants in the first year of life was compared to plasma PtdCho Ach concentration, which declined with both time and low intakes of marine oil ω3 fatty acids (0.25% of energy) in infants consuming up to 16% of energy as Lin (16).

SUBJECTS AND METHODS

Selection of Patients. Preterm infants (>725 g and <1400 g) were eligible for this study if they were admitted to the Newborn Center at The University of Tennessee, Memphis. Although infants in this birth-weight range are at high risk for medical complications, those selected for this study had fewer and milder complications than the population as a whole. Infants were not eligible if they had (*i*) a weight for gestational age less than the fifth percentile, (*ii*) intraventricular hemorrhage >grade 2 (28), (*iii*) retinopathy of prematurity >stage 2 (29), (*iv*) a need for mechanical ventilation after achieving enteral intakes >454 kJ (>110 kcal/kg per day), (*v*) surgical intervention for necrotizing enterocolitis, or (*vi*) a maternal history of cocaine or alcohol abuse. Infants were enrolled after obtaining parental consent, according to an Institutional Review Board-approved protocol and randomly assigned to receive either commercially available formulas [Similac Special Care (PT-A) followed by Similac With Iron (T-A); Ross Laboratories, Columbus, OH] or experimental formulas identical except containing marine oil ω3 fatty acids as 0.5% of total fatty acids (Table 1) (16). Infants were enrolled and followed until 12 mo (note that this and all ages described were calculated from the age of expected delivery).

Data from a total of 59 infants were available for analyzing the relationship between growth and essential fatty acid status. Twenty additional infants were enrolled, but data were not available because they failed to complete follow-up through 6.5 mo ($n = 13$), did not receive their assigned study formula consistently to 9 mo ($n = 5$), or were outliers (age at follow-up or biochemistry) ($n = 2$). The neonatal/perinatal characteristics of the 59 infants are shown in Table 2 by formula assignment.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ach, arachidonic acid; Lin, linoleic acid; PtdCho, phosphatidylcholine; ω3, fatty acids of the ω-3 family derived from and including linolenic acid; ω6, fatty acids of the ω-6 family derived from and including Lin.

§To whom reprint requests should be addressed at: Newborn Center, Room 201, 853 Jefferson Avenue, Memphis, TN 38163.

Table 1. Fatty acid composition of infant formulas

	Formula,* g/100 g of total fatty acid			
	Preterm		Term	
	PT-A	PT-B	T-A	T-B
6:0–24:0	68.0	67.1	44.4	44.0
18:1	9.9	9.8	17.1	17.0
18:2ω6	19.1	18.7	33.2	32.6
18:3ω3	3.0	3.1	4.8	4.9
20:5ω3		0.3		0.3
22:5ω3				
22:6ω3		0.2		0.2

Fatty acids are designated as number of carbons:number of double bonds, ω6 or ω3 family of fatty acids.
*Formulas contained ≈48% of energy from fat. PT-A was control formula (Similac Special Care); PT-B was experimental formula. Term (T) formula (Similac With Iron) had the same ω3 fatty acid supplementation as corresponding preterm (PT) formula.

Experimental Design. Infants were enrolled shortly after reaching intakes >454 kJ (110 kcal)/kg per day of a preterm formula. At enrollment, they were assigned a gestational age based on dates and ultrasound before assignment to formula as described earlier. If any discrepancy of >2 weeks occurred between these assessments and physical and neurological assessment of maturity at birth (30), the assessments were averaged. Infants were fed control (PT-A) or experimental (PT-B) formula until ≈1.8 kg. Thereafter, a term formula with the same ω3 fatty acid supplementation as their assigned preterm formula (T-A or T-B, Table 1) was fed ad libitum until 9 mo of age. At term, 2, 4, 6.5, 9, and 12 mo of age, dietary intake, length, weight, and head circumference were recorded, and blood was obtained by venipuncture.

Analytical Methods. Plasma was fractionated, erythrocytes were washed and stored, lipids were extracted and purified by TLC, and phospholipid fatty acids were methylated and quantitated by capillary gas/liquid chromatography as described (16, 31). Infants were weighed undressed on a calibrated infant balance. Length was measured in duplicate by use of a measuring board with infants supine (Jim's Instrument Manufacturing, Coralville, IA). Head circumference was measured with paper measuring tape.

Statistical Methods. The National Center for Health Statistics' normal values for growth by age and gender of term infants (32) were used to generate percentiles, and growth of these premature infants was normalized against these percentiles (33). The growth index generated is equivalent to a *Z*-score with weight, length, and head circumference expressed as ± SD from the 50th percentile. Because *Z*-scores can be manipulated arithmetically, values for an individual infant can be averaged across time or values within a dietary group can be averaged at a given age.

Table 2. Characteristics of study infants

	Control (n = 29)	Marine ω-3 supplemented (n = 30)
Birth weight, g	1054 ± 193	1139 ± 164
Gestational age, wk	28.9 ± 1.7	29.7 ± 1.8
Enrollment weight, g	1268 ± 152	1337 ± 133
Enrollment age, day	25 ± 10	24 ± 10
i.v. support, day	17 ± 8	18 ± 10
Mechanical ventilation, hr*	14.8 ± 6.6	6.6 ± 7.3
Total oxygen, hr*	63.7 ± 3.8	52.0 ± 4.6
Gravida, no.	2.4 ± 1.3	2.4 ± 1.4
Maternal height, cm	163.1 ± 7.7	164.8 ± 7.9
Maternal age, yr	23 ± 6	23 ± 6
Race, Black/Caucasian	26/3	25/5

*Mean ± SD of individual logarithm of hr converted to hr (mean ± SD).

Infants were ranked into quartiles on the basis of their plasma PtdCho Ach concentration at 2 mo, and the mean plasma PtdCho Ach concentrations of each quartile were compared (mg/liter) by repeated-measures ANOVA for time (2, 4, and 6.5 mo), quartile ranking, and interaction of time and rank effects. For each quartile, mean plasma PtdCho Ach concentrations did not differ significantly over time—i.e., Ach concentrations tracked between 2 and 6.5 mo. Accordingly, the mean concentration of plasma PtdCho Ach at 2, 4, and 6.5 mo was defined as Ach status and correlated with normalized growth from 2 to 6.5 mo (Fig. 1) and at each age from 2 through 12 mo (see Table 4).

A stepwise multiple regression procedure was used to determine the independent factors that affected normalized growth at each age. Birth weight was included as a potentially influential variable in all analyses. Other potential factors were maternal height, birth order, Ach status, and marine oil supplementation as a dummy variable with zero for control and one for supplemented infants. Independent variables that met the $P < 0.05$ significance level are reported (Tables 5 and 6).

Groups of infants, ranked by quartile on the basis of Ach status during the period from 2 to 6.5 mo, were compared for birth weight and energy intake from formula at 2, 4, and 6.5 mo (Table 3). Growth (weight, length, weight-to-length ratio, and head circumference) at 2–6.5 and 9–12 mo was plotted for each quartile (Fig. 2).

RESULTS

Diet Histories/Energy Intake. Most infants received energy exclusively from formula through 4 mo. By 6.5 mo, almost all were consuming a mixed diet but with ≥0.72 liter of formula

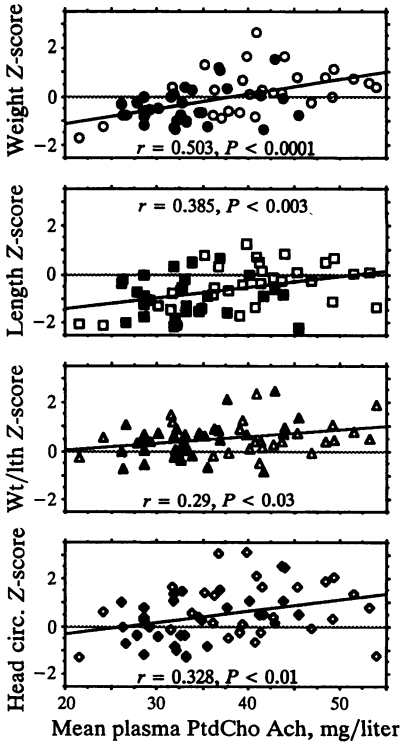

FIG. 1. Correlation of average (2, 4, and 6.5 mo) weight, length, weight-to-length (wt/lth) ratio, and head circumference (circ.) *Z*-scores with Ach status (average concentration of plasma PtdCho Ach at 2, 4, and 6.5 mo). Open symbols represent infants fed standard formula; filled symbols represent those supplemented with marine oil.

Table 3. Ach status by quartile: Plasma PtdCho Ach, birth weight, and reported energy intakes from formula at 2, 4, and 6.5 mo

Quartile rank (*n*)	Plasma PtdCho Ach, mean mg/liter (2–6.5 mo)	Birth weight, g	Reported energy intake from formula, kcal/kcal per kg		
			2 mo	4 mo	6.5 mo
Highest (15)	48.6 ± 3.9	1094 ± 158	655/136	708/118	675/92
Second (15)	39.1 ± 1.9	1052 ± 219	663/142	708/109	698/90
Third (14)	33.6 ± 1.4	1150 ± 154	572/126	663/110	691/100
Lowest (15)	28.1 ± 2.8	1098 ± 196	565/130	667/120	760/111
ANOVA by Ach quartile	$P < 0.0001$	NS	NS/NS	NS/NS	NS/NS

NS, not significant.

per day. After 9 mo, most received a mixed diet and cow's whole milk instead of infant formula. All quartiles for Ach status had the same birth weight and energy intake from formula (kcal or kcal/kg) at 2, 4, or 6.5 mo of age (Table 3).

Ach Status and Growth Achievement. Individual weight and length *Z*-scores correlated significantly ($r = 0.27$–0.53) with Ach status at 2, 4, 6.5, 9, and 12 mo (Table 4). Plasma PtdCho Ach accounted for 7–28% of the variance (r^2) in weight and length at these ages. The weight-to-length ratio at 2, 4, and 6.5 mo also correlated ($r = 0.25$ to 0.28) with Ach status. Head circumference was related to Ach status at 2 and 4 mo ($r = 0.34$–0.37) but did not relate thereafter.

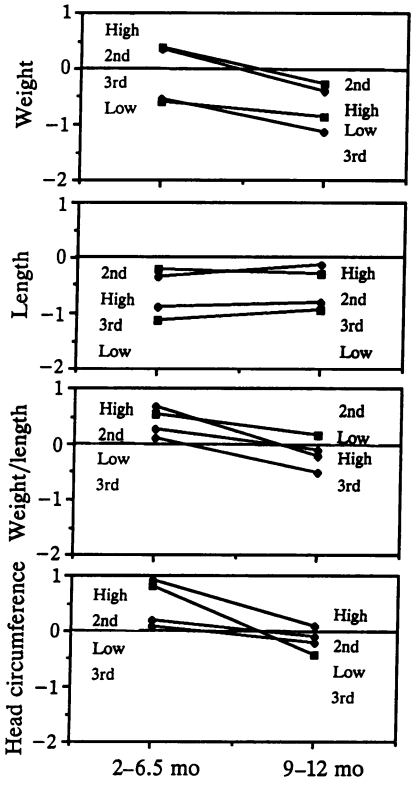

FIG. 2. Average-growth *Z*-scores (2–6.5 mo and 9–12 mo) of infants by quartile of Ach status (average plasma PtdCho Ach concentration from 2 to 6.5 mo). Between-groups ANOVA: (*i*) 2–6.5 mo—weight, $P < 0.0005$; length, $P < 0.01$; weight/length, not significant; head circumference, $P < 0.01$; (*ii*) 9–12 mo—weight, $P < 0.04$; length, not significant; weight/length, $P < 0.05$; head circumference, not significant. Between-quartile differences by Fisher probable least-significant difference: (*i*) weight *Z*-score: high and second vs. third and low at 2–6.5 mo and high and second vs. third at 9–12 mo, $P < 0.05$; (*ii*) length: high and second vs. low at 2–6.5 mo and 9–12 mo, $P < 0.05$; (*iii*) weight-to-length: high and second vs. third at 2–6.5 mo and second vs. third at 9–12 mo, $P < 0.05$; (*iv*) head circumference: high and second vs. low at 2–6.5 mo, $P < 0.05$.

Fig. 1 illustrates the relationship between Ach status and growth during the period from 2 to 6.5 mo. Each point in this figure represents the average of three measures of plasma PtdCho Ach concentration related to the average of three consecutive measures for normalized growth (*Z*-score). Plasma PtdCho Ach concentration accounted for 8–25% of the variance in growth during this period.

Because we knew that ω3 supplementation had decreased plasma Ach, we looked also at the relationship between Ach status and growth in infants fed only standard formulas ($n = 29$). These infants are designated by open symbols in Fig. 1. Their Ach status correlated significantly with their average normalized weight ($r = 0.43$, $P < 0.02$) and length ($r = 0.44$, $P < 0.02$) between 2 and 6.5 mo but did not correlate with their weight-to-length ratio or head circumference.

First year growth of term infants has been related to maternal height (34) and inversely related to birth order (34). These two variables, plus marine oil supplementation and Ach status, were included as independent variables in regression analyses of normalized weight (Table 5) and length (Table 6) achievement during infancy. Any potential effects of differences in birth weight were corrected by including birth weight in all models of multiple regression analyses. Some combination of Ach status, maternal height, and marine oil supplementation accounted for 21–44% of the variance in weight of the 59 study infants. Some combination of Ach status and maternal height accounted for 40–59% of the variance in weight of the 29 infants fed only commercial (control) formulas (Table 5) through 6.5 mo of age. In the

Table 4. Correlation of Ach status with normalized growth (*Z*-score) in the first year of life

Growth	*n*	Age, mo	Correlation, *r*	*P*
Weight	59	2	0.48	<0.001
	59	4	0.53	<0.001
	59	6.5	0.41	<0.001
	58	9	0.31	<0.02
	54	12	0.27	<0.05
Length	59	2	0.35	<0.01
	58	4	0.419	<0.001
	59	6.5	0.31	<0.02
	58	9	0.351	<0.01
	54	12	0.32	<0.02
Wt/length	59	2	0.28	<0.03
	58	4	0.26	<0.05
	58	6.5	0.25	<0.06
	58	9	0.33	NS
	54	12	0.13	NS
Head circ.	59	2	0.37	<0.01
	58	4	0.34	<0.01
	59	6.5	0.24	<0.07
	56	9	0.19	NS
	54	12	0.08	NS

Ages are from expected term delivery. Wt, weight; NS, not significant; circ., circumference.

Table 5. Normalized weight achievement in the first year of life

Age	Formula	Independent variable	*F*	*P*	Model r^2
Term	Both	Ach status (+)	10.66	0.002	0.21
	Control	Ach status (+)	7.78	0.01	
		Maternal height (+)	9.46	0.006	0.56
	Marine	Birth order (−)	7.34	0.02	0.23
2 mo	Both	Ach status (+)	22.41	0.0001	
		Maternal height (+)	6.52	0.01	0.35
	Control	Ach status (+)	16.73	0.0005	
		Maternal height (+)	9.44	0.006	0.59
	Marine	Birth order (−)	6.39	0.02	0.20
4 mo	Both	Ach status (+)	12.94	0.0007	
		Marine oil ω3 (−)	5.74	0.02	
		Maternal height (+)	5.90	0.02	0.44
	Control	Ach status (+)	13.19	0.002	
		Maternal height (+)	5.76	0.03	0.50
	Marine	Birth order (−)	10.69	0.007	0.26
6.5 mo	Both	Marine oil ω3 (−)	13.92	0.0005	
		Birth order (−)	4.92	0.03	0.30
	Control	Ach status (+)	5.21	0.03	
		Maternal height (+)	5.85	0.02	0.40
9 mo	Both	Marine oil ω3 (−)	12.64	0.0008	0.20
12 mo	Both	Marine oil ω3 (−)	11.28	0.002	0.19

The stepwise regression model included birth weight (forced), maternal height, birth order, marine oil ω3 supplementation, and Ach status. Only significant variables are noted with sign (+ or −) to indicate the relationship to growth.

marine oil-supplemented infants, higher birth order was negatively related to weight in early infancy. At 9 and 12 mo, ω3 supplementation had a negative effect on weight that accounted for 20% of the variance in weight seen in the combined group of infants (Table 5).

Ach status and maternal height also accounted for 25–39% of the length variance in the 59 study infants and 58–68% of the variance in infants fed only commercial formulas (Table 6). At 2 and 6.5 mo of age, the negative influence of ω3 supplementation replaced the positive influence of Ach status on length in the entire group. Only maternal height was positively related to length in ω3-supplemented infants, but that relationship was not consistent. Because ω3 supplementation significantly reduced the concentration of plasma PtdCho Ach (16), its entrance into these models as a negative influence on weight and length is consistent with a positive influence of Ach status on growth.

Table 6. Normalized length achievement in the first year of life

Age	Formula	Independent variable	*F*	*P*	Model r^2
Term	Both	Ach status (+)	7.39	0.009	
		Maternal height (+)	4.59	0.04	0.25
	Control	Maternal height (+)	14.01	0.001	
		Ach status (+)	6.23	0.02	0.58
2 mo	Both	Maternal height (+)	9.26	0.004	
		Marine oil ω3 (−)	11.87	0.001	0.29
	Control	Maternal height (+)	19.24	0.0002	
		Ach status (+)	10.63	0.004	0.61
4 mo	Both	Maternal height (+)	16.18	0.0002	
		Ach status (+)	18.13	0.0001	0.39
	Control	Ach status (+)	19.53	0.0002	
		Maternal height (+)	25.99	0.0001	0.68
6.5 mo	Both	Maternal height (+)	9.88	0.003	
		Marine oil ω3 (−)	14.22	0.0004	
		Birth order (−)	4.74	0.03	0.37
	Control	Maternal height (+)	4.92	0.04	0.19
	Marine	Maternal height (+)	4.89	0.04	0.20
9 mo	Both	Ach status (+)	11.14	0.002	
		Maternal height (+)	12.07	0.001	0.29
	Control	Ach status (+)	13.92	0.001	
		Maternal height (+)	22.38	0.0001	0.61
12 mo	Both	Maternal height (+)	17.62	0.0001	
		Ach status (+)	9.73	0.0003	0.33
	Control	Maternal height (+)	11.05	0.003	0.34

The stepwise regression included birth weight (forced), maternal height, marine oil ω3 supplementation, and Ach status. Only significant variables are noted with sign (+ or −) to indicate the relationship to growth.

Infants in the two higher quartiles for Ach status achieved better normalized weight, length, and head circumference compared with the two lower quartiles (Fig. 2). The two higher quartiles had weights above the 50th percentile from 2 to 6.5 mo. Although growth in all categories, except length, declined for all quartiles of Ach status between early and late infancy, the higher quartiles compared with lower Ach quartiles continued to have higher weights and lengths at 9–12 mo (Fig. 2).

DISCUSSION

Although these preterm infants received Lin, Ach in plasma and erythrocyte phospholipids declined for months after birth (16) and remained low for ≈5 mo. This 5-mo period was chosen as the period of poorest individual Ach status. Large interindividual differences in both Ach status and normalized growth were seen during this 5-mo period, and Ach status and growth correlated significantly. Moreover, Ach status was one of two independent variables (the other was maternal height) that consistently accounted for a large proportion of the variance in the first-year growth achievement.

Human infants can accumulate Ach from maternal/placental transfer, consumption of human milk, and synthesis from Lin. These infants were born early in the last trimester and fed formulas without Ach; thus normal intrauterine and extrauterine accumulation of preformed Ach could not occur. Infants in this study did receive Lin well in excess of the known requirement for growth and development. This study does not address the ability of preterm infants to synthesize Ach; however, declines in Ach status after birth lead us to speculate that the rate of biosynthesis does not keep pace with the need for Ach in formula-fed, prematurely born infants. This result does not necessarily mean that these infants have poorer biosynthesis compared with term infants, older children, or adults because either an increased need for Ach or inadequate intrauterine accumulation of Ach could cause the same decline. These data do suggest that a conditional deficiency of Ach occurs despite good intakes of Lin.

After these analyses were complete, we looked at the relationship between growth and the concentrations of Lin, eicosapentaenoic acid, and docosahexaenoic acid in plasma PtdCho. Like Ach, plasma PtdCho Lin concentration (2–6.5 mo) correlated with weight and length; however, in contrast to plasma PtdCho Ach, plasma PtdCho Lin concentration was consistently high (>175 mg/liter) and did not decline with time or marine oil feeding. When Lin concentration was included with Ach status as a potential independent variable for normalized weight and length (Tables 5 and 6), Ach, but not Lin, entered the models.

Any dietary fatty acids would be expected to positively relate to growth because greater intakes of the fatty acid occur with greater energy intakes. This fact is likely the basis for the positive relationship between growth and Lin concentration, as well as our failure to find a negative relationship between growth and the concentrations of eicosapentaenoic acid and docosahexaenoic acid, even though Ach status was decreased by docosahexaenoic acid and eicosapentaenoic acid intake (16). The relationship of Ach status to growth was not confounded by intake of Ach or related to

differences in energy intake (Table 3). Only the relationship between Ach status and growth is reported here.

Differences in nutritional status may account for some variability in Ach status found at each age within infants fed the same formula. For example, the common practice of discharging preterm infants at <2 kg on formulas designed to meet the nutritional needs of much larger term infants slows nutritional recovery (35), which may reduce the ability of preterm infants to elongate/desaturate Lin to Ach (36–38). Because infants vary a great deal in plasma PtdCho Ach concentration at birth, intrauterine influences could play a role in individual Ach status. Koletzko and Braun (39) recently reported a positive relationship between plasma triglyceride Ach and body weight of premature infants at birth.

Dietary ω3 fatty acids are known to reduce phospholipid Ach (40, 41), probably by a combination of reduced Ach synthesis and simple physical replacement of phospholipid Ach by eicosapentaenoic acid and docosahexaenoic acid. Marine oil significantly decreased plasma PtdCho Ach in the half of these study infants randomized to receive supplemented formula (16). Marine oil supplementation also reduced growth (15), direct evidence that poorer Ach status was growth limiting in these infants. It is important to emphasize, however, that marine oil supplementation alone did not account for the relationship between growth and Ach status seen in these very low-birth-weight infants: Among infants fed only the commercial (control) formulas, Ach status and maternal height accounted for a large part of the variance (40–68%) in weight and length (Tables 5 and 6). Because control infants were managed according to standard practices of nutritional care, poor Ach status may relate to the routine observation that preterm infants fail to achieve normal growth in infancy. Diets containing Ach should improve Ach status (17, 42).

In conclusion, growth (15) and very long-chain ω3 and ω6 status (16) were studied in preterm infants at low risk for growth failure due to medical problems. The concentrations of plasma PtdCho Ach throughout infancy were well below those seen at birth, and erythrocyte phospholipid Ach (16) was lower than that of term infants (17). Growth equivalent to term infants was not achieved in the first year of life (15), and Ach status predicted a significant part of the variance in first year growth. Despite good intakes of the essential ω6 fatty acid, Lin, preterm infants may need dietary Ach for optimal growth.

This work was supported by the National Institutes of Health (R01-EY08770) and Ross Laboratories.

1. Karniski, W., Blair, C. & Vitucci, J. S. (1987) *Am. J. Dis. Child.* **141**, 520–526.
2. Gayle, H. D., Dibley, M. J., Marks, J. S. & Trowbridge, F. L. (1987) *Am. J. Dis. Child.* **141**, 531–534.
3. Georgieff, M. K., Mills, M. M., Zempel, C. E. & Chang, P.-N. (1989) *J. Pediatr.* **114**, 288–292.
4. Casey, P. H., Kraemer, H. C., Bernbaum, J., Tyson, J. E., Sells, J. C., Yogman, M. W. & Bauer, C. R. (1990) *J. Pediatr.* **117**, 298–307.
5. Ernst, J. A., Bull, M. J., Rickard, K. A., Brady, M. S. & Lemons, J. A. (1990) *J. Pediatr.* **117**, S156–S166.
6. Kitchen, W. H., Ford, G. W. & Doyle, L. W. (1989) *Arch. Dis. Child.* **64**, 379–382.
7. Robertson, C. M. T., Etches, P. C. & Kyle, J. M. (1990) *J. Pediatr.* **117**, 19–26.
8. Hack, M., Merkatz, I. R., Gordon, D., Jones, P. K. & Fanaroff, A. A. (1984) *Am. J. Dis. Child.* **138**, 370–375.
9. Ross, G., Lipper, E. G. & Auld, P. A. M. (1985) *J. Pediatr.* **107**, 284–286.
10. Lipper, E. G., Lee, K. S., Gartner, L. M. & Grellong, B. (1981) *Pediatrics* **67**, 502–505.
11. Ross, G., Lipper, E. G. & Auld, P. A. M. (1990) *J. Pediatr.* **117**, 307–309.
12. Markestad, T. & Fitzhardinge, P. M. (1981) *J. Pediatr.* **98**, 597–602.
13. Sauve, R. S. & Singhal, N. (1985) *Pediatrics* **76**, 725–733.
14. Groothuis, J. R. & Rosenberg, A. A. (1987) *Am. J. Dis. Child.* **141**, 992–995.
15. Carlson, S. E., Cooke, R. J., Werkman, S. H. & Peeples, J. M. (1992) *Lipids* **27**, 901–907.
16. Carlson, S. E., Cooke, R. J., Rhodes, P. G., Peeples, J. M., Werkman, S. H. & Tolley, E. A. (1991) *Pediatr. Res.* **30**, 404–412.
17. Putnam, J. C., Carlson, S. E., DeVoe, P. W. & Barness, L. A. (1982) *Am. J. Clin. Nutr.* **36**, 106–114.
18. Holman, R. T. (1978) in *CRC Handbook Series in Nutrition and Food, Section E: Nutritional Disorders*, ed. Rechcigl, M., Jr. (CRC, Boca Raton, FL), Vol. 2, pp. 491–515.
19. Burr, G. O. & Burr, M. M. (1929) *J. Biol. Chem.* **82**, 345–367.
20. McAmis, A. J., Anderson, W. E. & Mendel, L. B. (1929) *J. Biol. Chem.* **82**, 247–262.
21. Hansen, A. E., Haggard, M. E., Boelsche, A. N., Adam, D. J. D. & Wiese, H. F. (1958) *J. Nutr.* **66**, 565–576.
22. Mohrhauer, H. & Holman, R. T. (1963) *J. Lipid Res.* **4**, 151–159.
23. Staton, M. A., Edwards, H. M., Briskin, I. L., Joanen, T. & McNease, L. (1990) *J. Nutr.* **120**, 674–685.
24. Holman, R. T. & Johnson, S. B. (1981) *Prog. Lipid Res.* **20**, 67–73.
25. Holman, R. T. (1986) *J. Am. Coll. Nutr.* **5**, 236–265.
26. Cerra, F. B., Alden, P. B., Negro, F., Billiar, T., Svingen, B. A., Licari, J., Johnson, S. B. & Holman, R. T. (1988) *J. Parenter. Enteral Nutr.* **12**, 63S–68S.
27. Holman, R. T., Johnson, S. B. & Hatch, T. F. (1982) *Am. J. Clin. Nutr.* **35**, 617–623.
28. Papile, L. A., Burstein, J., Burstein, R. & Koffler, H. (1978) *J. Pediatr.* **92**, 529–534.
29. Committee for the Classification of Retinopathy of Prematurity (1984) *Pediatrics* **74**, 127–133.
30. Dubowitz, L., Dubowitz, V. & Goldberg, C. (1970) *J. Pediatr.* **77**, 1–10.
31. Carlson, S. E., Rhodes, P. G., Rao, V. S. & Goldgar, D. E. (1987) *Pediatr. Res.* **21**, 507–510.
32. Hamill, P. V., Drizd, T. A., Johnson, C. L., Reed, R. B., Roche, A. F. & Moore, W. M. (1979) *Am. J. Clin. Nutr.* **32**, 607–629.
33. Dibley, M. J., Goldsby, J. B., Staehling, N. W. & Trowbridge, F. L. (1987) *Am. J. Clin. Nutr.* **46**, 5736–5748.
34. Wingerd, J. (1972) *Hum. Biol.* **42**, 105–131.
35. Carlson, S. E., Peeples, J. M., Cooke, R. J. & Werkman, S. H. (1991) *Pediatr. Res.* **29**, 292A (abstr.).
36. Cunnane, S. C. (1988) *Br. J. Nutr.* **59**, 273–278.
37. de Tomas, M. E., Mercuri, O. & Rodrigo, A. (1980) *J. Nutr.* **110**, 595–599.
38. Huang, Y. S., Cunnane, S. C. & Horrobin, D. F. (1986) *Proc. Soc. Exp. Biol. Med.* **181**, 399–403.
39. Koletzko, B. & Braun, M. (1991) *Ann. Nutr. Metab.* **35**, 128–131.
40. Holman, R. T. (1964) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **23**, 1062–1067.
41. Morita, I., Saito, Y., Chang, W. C. & Murota, S. (1983) *Lipids* **18**, 42–49.
42. Koletzko, B., Schmidt, E., Bremer, H. J., Haug, M. & Harzer, G. (1989) *Eur. J. Pediatr.* **148**, 669–675.