Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning[1–4]

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ABSTRACT

Background: The effect of docosahexaenoic acid (DHA) on the developing fetal central nervous system (CNS) and related functional outcomes in infancy remain unexplored. Sleep and wake states of newborns provide a tool for assessing the functional integrity of the CNS.

Objective: We investigated whether CNS integrity in newborns, measured with sleep recordings, was associated with maternal concentrations of long-chain polyunsaturated fatty acids, especially DHA.

Design: Plasma phospholipid fatty acid concentrations were measured in 17 women at parturition. On postpartum day 1 (P1) and day 2 (P2), a pressure-sensitive pad under the infants' bedding recorded body movements and respiratory patterns to measure sleep and wake states.

Results: Maternal plasma phospholipid DHA ranged from 1.91% to 4.5% by wt of total fatty acids. On the basis of previously published data and the median DHA concentration, the women were divided into 2 groups: high DHA (>3.0% by wt of total fatty acids) and low DHA (≤3.0% by wt of total fatty acids). Infants of high-DHA mothers had a significantly lower ratio of active sleep (AS) to quiet sleep (QS) and less AS than did infants of low-DHA mothers. Furthermore, the former infants had less sleep-wake transition and more wakefulness on P2. Correlations of maternal DHA status with infant sleep states were consistent with these data. Also, the ratio of maternal n−6 to n−3 fatty acids on P1 was inversely associated with QS and positively associated with arousals in QS. On P2, maternal n−6:n−3 was positively associated with AS, sleep-wake transition, and AS:QS.

Conclusion: The sleep patterns of infants born to mothers with higher plasma phospholipid DHA suggest greater CNS maturity. *Am J Clin Nutr* 2002;76:608–13.

KEY WORDS Long-chain polyunsaturated fatty acids, long-chain PUFAs, docosahexaenoic acid, central nervous system, infant sleep, sleep recordings, active sleep, quiet sleep, pregnancy, prenatal nutrition, maternal diet, phospholipids

INTRODUCTION

The phospholipids of the brain and retina are characterized by high contents of n−3 and n−6 long-chain polyunsaturated fatty acids (LCPUFAs), in particular docosahexaenoic acid (DHA; 22:6n−3). An adequate supply of LCPUFAs is necessary for the functional maturation of these tissues during embryonic and neonatal development. The fetus is capable of synthesizing LCPUFAs to some extent, but most of the LCPUFAs are obtained from the maternal circulation (1). During pregnancy, especially the third trimester, LCPUFAs are selectively transferred from the maternal blood to the fetal blood and subsequently to fetal tissues (2–4). After transfer across the placenta, the LCPUFAs are compartmentalized into lipid fractions that do not move back across the placental barrier and therefore are readily available to the developing fetal tissues (5). Thus, fetal accretion of the LCPUFAs is dependent upon maternal LCPUFA status.

The effects of LCPUFAs on visual development are well documented. Several studies showed that higher intakes of LCPUFAs, especially DHA, are associated with enhanced visual development (6, 7). Regarding brain development, suboptimal LCPUFA accretion into the developing central nervous system (CNS) is considered detrimental, because it might affect the child's emotional and intellectual development (8). However, the effect of fetal DHA status on the developing brain has not been investigated because of the lack of an appropriate assessment technique for use in the neonate. Because the developing nervous system is susceptible to potentially damaging factors of a biochemical or mechanical nature, any assessment technique must focus upon the functional integrity of the developing CNS (9). Measurements of the sleep and wake states of infants provide a tool for assessing the functional integrity of the CNS from the time of birth.

Infants' sleep patterns are an expression of central integrative control (10). Multiple mechanisms involving both neural and humoral processes in various regions of the brain interact to produce sleep and wakefulness (11). Studies of the relation between

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sleep-state organization and CNS function showed that changes in sleep architecture are associated with neurologic changes during development and that deviant sleep patterns are associated with neurologic deficits (12, 13).

In the present study, we tested the hypothesis that the integrity of the CNS in newborns, assessed with prolonged sleep recordings, would be associated with maternal LCPUFA status, especially DHA status.

SUBJECTS AND METHODS

Subjects

Healthy pregnant women (n = 17) were recruited from the Department of Obstetrics and Gynecology at a local hospital after they were admitted for delivery. We excluded women with a history of chronic hypertension, hyperlipidemia, renal or liver disease, heart disease, thyroid disorders, multiple gestations, or pregnancy-induced complications (eg, hypertension, preterm labor, or premature rupture of membranes). Women who were treated during labor with drugs that affect the respiration of newborns, such as magnesium sulfate and butorphanal, were also excluded from the study. Any infants with < 4 h of crib time in the first and second days postpartum were excluded from the study. Mothers were asked about past and current smoking and alcohol use, and this information was recorded. The University of Connecticut Human Subjects Review Committee and the Windham Hospital Institutional Review Board approved the protocol, and written, informed consent was obtained from each subject at the time of recruitment.

Blood sample collection

At delivery, maternal venous blood was collected into tubes containing EDTA. The plasma and erythrocytes were separated with centrifugation (700 × g at 4 °C for 10 min). The plasma was divided into aliquots and stored at −80 °C until analyzed for phospholipid fatty acids. Infant blood samples were not collected because previous studies in our laboratory showed positive associations between maternal PUFA and cord blood PUFA concentrations (14). Thus, maternal PUFA status reflects infant PUFA status. Data regarding mode of delivery, birth weight, head circumference, Apgar scores, and birth trauma were collected from the medical records.

Sleep recordings

The sleep recordings were obtained by using the Motility Monitoring System (MMS) throughout the infant's stay in the hospital. The basic principle underlying these sleep recordings is that each sleep or wake state involves a unique pattern of motility produced by respiration and body movements. This concept has been validated by comparing MMS data with direct observations and electroencephalogram recordings (15, 16).

The MMS consists of a capacitance-type sensor pad (12 inches × 24 inches × 1/8 inch thick) which is connected to an amplifier leading to a small 24-h data recorder. The sensor pad is placed under the sheet or mattress pad in the infant's crib. The amplifier and recorder are battery-driven and fit into a briefcase that is suspended on the crib. A single channel of analogue signals from the infant's respiration and body movements is transmitted from the sensor pad to the amplifier and then digitized (at 10 samples/s) and stored in the data logger. The data are transferred to a computer in the laboratory for processing and analysis. This procedure does not require the presence of an observer and permits continuous recording of data. The crib remains fully mobile during data collection. This method is nonintrusive because the instrumentation is not in direct contact with the infant's body.

Sample analysis: plasma phospholipid fatty acids

Plasma phospholipid fatty acid concentrations were analyzed with methods described previously (17). In brief, the total lipid was extracted from the maternal plasma by using a modified Folch procedure (18) with 2:1 (vol:vol) dichloromethane:methanol. Thin-layer chromatography was used to separate the phospholipids, which were methylated to form the fatty acid methyl esters. We used 17:0 as an internal standard to determine absolute concentrations in mg/L. Fatty acid methyl esters were separated via injection into a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with an Omegawax 250 capillary column (30 m × 250 mm × 25 μm; Supelco, Bellefonte, PA). Plasma phospholipid fatty acid methyl esters were identified by comparison with external standards and were expressed as both relative % by wt of total fatty acids and absolute concentrations (mg/L).

Analysis of sleep recordings

In the laboratory, the recorded data were entered into a computer and scored by using a pattern-recognition program. Each 30-s period of the recording was scored for the sleep states of quiet sleep (QS), active sleep (AS), sleep-wake transition, wakefulness, and time spent out of the crib.

The computer program that we used for scoring the sleep states from analogue signals was developed on the basis of a template-matching process that compares waveform signals directly. The data profile for each 30-s period in an infant's record is compared with data profiles from waveform regions that were selected as prototypical for each of the sleep states. The 19 standard templates were chosen from recordings of several infants from other studies, and > 1 template was chosen for each state because waveforms are not homogeneous for any of the states. The templates represent the range of motility patterns that occur in a behavioral state category. Computer assignment of a state to each successive 30-s period in the record is performed by comparing the profile for that period with the profile for each of the state templates in the file. The period is assigned to the state category of the template that it matches most closely. After an infant's record is scored in 30-s periods, it is smoothed by using the following rule: 6 successive periods of AS, QS, or wakefulness are required for a change of state; thus, when ≤ 5 periods of a different state occur, the ongoing state is not changed.

After the computer scoring of each record, the full 24 h of signals is printed out for visual editing. For each 24-h observation, the following measures are obtained: *1*) time spent in the crib, *2*) percentage of time spent in each of the sleep states (QS, AS, sleep-wake transition, and wakefulness), *3*) ratio of AS to QS, *4*) brief arousals in AS, and *5*) brief arousals in QS. Each of these measures was obtained separately for each infant on postpartum day 1 (P1) and day 2 (P2).

In previous studies, we repeatedly showed measurement reliability and validity for each of the sleep measures obtained from MMS recordings. Reliability of measurement was shown for preterm and full-term infants during the first 6 mo of life (10, 12, 19–22). Validity was shown by making comparisons across

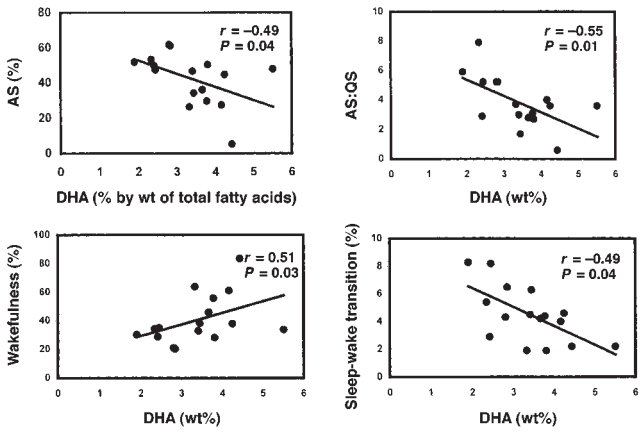

FIGURE 1. Associations of maternal plasma phospholipid docosahexaenoic acid (DHA) concentrations with infant sleep states on postpartum day 2; $n = 17$. Sleep states were measured as the percentage of time that the infant spent in that sleep state. AS, active sleep; QS, quiet sleep.

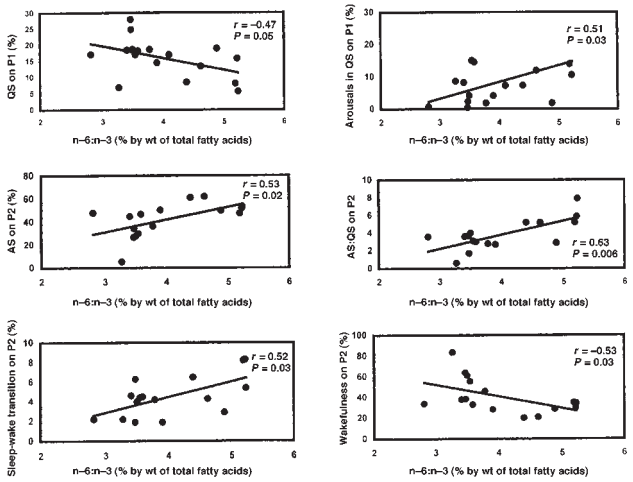

FIGURE 2. Associations of the maternal plasma ratio of n−6 to n−3 long-chain polyunsaturated fatty acids with infant sleep states on postpartum day 1 (P1) and day 2 (P2); $n = 17$. Sleep states were measured as the percentage of time that the infant spent in that sleep state. AS, active sleep; QS, quiet sleep.

species and procedures, including behavioral observations and electroencephalograms (16, 19, 23–25). Predictive validity of the sleep measures was found for newborns (26) and for infants during the early postnatal weeks (12, 23).

Statistical analyses

Simple linear regression analyses were used to describe the associations between maternal LCPUFA status and infant sleep measures, separately on P1 and P2. For maternal DHA concentrations, we divided the women into 2 categories: high DHA (> 3.0% by wt of total fatty acids; $n = 10$) and low DHA (≤ 3.0% by wt of total fatty acids; $n = 7$). The cutoff between the 2 categories was chosen on the basis of our previously published data on plasma phospholipid fatty acids of pregnant women in the third trimester (17) and the median DHA concentration (% by wt of total fatty acids) for this study population. Student's t tests were used to analyze for significant differences between the group means for the descriptive data (age, maternal education, length of gestation, infant birth weight, birth length, head circumference, and Apgar score). Analysis of variance (ANOVA) was used to compare the maternal fatty acid profiles of the 2 groups. Repeated-measures ANOVA was used to analyze for the main effects of group and day on the sleep measures, and group-by-day interactions. $P < 0.05$ was considered statistically significant for the main effects of group and day and $P < 0.10$ was considered significant for the interaction effect. Furthermore, ANOVA was used to compare individual sleep measures of the 2 groups separately on P1 and P2 for variables with significant group-by-day interactions in the repeated-measures ANOVA. We considered maternal age and maternal education to be possible confounding variables and therefore we used them as covariates in the ANOVAs for group differences in sleep measures. The statistical analyses were performed with SAS, version 6.09 (SAS Institute Inc, Cary, NC).

RESULTS

Regression analyses used to describe the associations of maternal plasma phospholipid fatty acid concentrations with infant sleep and wake states indicated that, among the n−6 and n−3 LCPUFAs, only the n−3 LCPUFAs, especially DHA, and the n−6:n−3 showed strong correlations ($P < 0.05$) on both days of measurement. The total n−6 and n−3 LCPUFA concentrations were not related to infant sleep measures.

The following correlations were the most significant among all the statistically significant correlations for this population. On P2, maternal DHA concentration was negatively associated with AS ($r = −0.49$, $P < 0.05$), AS:QS ($r = −0.55$, $P < 0.05$), and sleep-wake transition ($r = −0.49$, $P < 0.05$) and positively associated with wakefulness ($r = 0.51$, $P < 0.05$) (**Figure 1**). On P1, the ratio of n−6 to n−3 LCPUFAs in maternal plasma was negatively associated with QS ($r = −0.47$, $P = 0.05$) and positively associated with arousals in QS ($r = 0.51$, $P < 0.05$) (**Figure 2**). On P2, the ratio of n−6 to n−3 LCPUFAs in maternal plasma was positively associated with AS ($r = 0.53$, $P < 0.05$), AS:QS ($r = 0.63$, $P < 0.01$), and sleep-wake transition ($r = 0.52$, $P < 0.05$) and negatively associated with wakefulness ($r = −0.53$, $P < 0.05$) (Figure 2).

Maternal and infant characteristics of the high-DHA and low-DHA groups are shown in **Table 1**. There were no significant differences between the 2 groups.

Plasma phospholipid LCPUFA concentrations of the women in the high-DHA and low-DHA groups are shown in **Table 2**. Maternal plasma phospholipid DHA concentrations ranged from 1.91% to 4.5% by wt of total fatty acids. Women in the high-DHA group had significantly higher concentrations of dihomo-γ-linolenic acid (20:3n−6), osbond acid (22:5n−6), eicosapentaenoic acid (20:5n−3), docosapentaenoic acid (22:5n−3), DHA, Σ n−3 PUFAs, and Σ n−3 LCPUFAs. Also, women in the high-DHA group had a significantly lower ratio of total n−6 LCPUFAs to total n−3 LCPUFAs compared with the low-DHA group. Concentrations of mead acid (20:3n−9), the most unsaturated and elongated of the n−9 fatty acids, did not differ significantly between the high-DHA and low-DHA groups.

TABLE 1
Maternal and infant characteristics of the groups with high or low maternal docosahexaenoic acid (DHA) concentrations[1]

	High-DHA group[2] (n = 10)	Low-DHA group[3] (n = 7)
Maternal age (y)	29.20 ± 5.24[4]	24.28 ± 5.12
Race		
White	9	5
Hispanic	1	2
Parity		
0	6	5
1	3	2
2	1	0
Length of gestation (wk)	40.40 ± 0.96	39.00 ± 1.86
Maternal education (y)	13.90 ± 2.33	11.57 ± 5.41
Infant birth weight (kg)	3.66 ± 0.53	3.37 ± 0.63
Infant birth length (cm)	50.72 ± 2.46	49.32 ± 2.18
Infant head circumference (cm)	33.88 ± 1.34	33.98 ± 1.34
1-min Apgar score	7.00 ± 2.00	8.28 ± 0.95
5-min Apgar score	9.10 ± 0.56	9.14 ± 0.69

[1] There were no significant differences between the groups.
[2] Maternal plasma phospholipid DHA >3.0% by wt of total fatty acids.
[3] Maternal plasma phospholipid DHA ≤3.0% by wt of total fatty acids.
[4] $\bar{x}$ ± SD.

TABLE 2
Maternal plasma phospholipid long-chain polyunsaturated fatty acid (LCPUFA) concentrations in the groups with high or low maternal docosahexaenoic acid (DHA) concentrations[1]

Fatty acid	High-DHA group[2] (n = 10)	Low-DHA group[3] (n = 7)
	% by wt of total fatty acids	
n−6		
20:3n−6	3.26 ± 0.56	2.74 ± 0.41[4]
20:4n−6	10.64 ±1.50	10.01 ± 1.04
22:4n−6	0.55 ± 0.13	0.50 ± 0.17
22:5n−6	1.69 ± 0.51	1.25 ± 0.19[4]
Σ n−6 PUFAs[5]	34.15 ± 5.16	35.65 ± 2.31
Σ n−6 LCPUFAs[6]	16.33 ±1.73	14.64 ± 1.59
n−3		
20:5n−3	0.29 ± 0.07	0.19 ± 0.04[4]
22:5n−3	0.57 ± 0.12	0.42 ± 0.09[4]
22:6n−3	3.98 ± 0.66	2.53 ± 0.37[4]
Σ n−3 PUFAs[7]	5.01 ± 0.56	2.95 ± 0.43[4]
Σ n−3 LCPUFAs[8]	4.72 ± 0.54	3.07 ± 0.43[4]
Σ n−6 LCPUFAs:Σ n−3 LCPUFAs[9]	3.47 ± 0.29	4.80 ± 0.44[4]
n−9		
20:3n−9	0.19 ± 0.05	0.17 ± 0.03

[1] $\bar{x}$ ± SD.
[2] Maternal plasma phospholipid DHA >3.0% by wt of total fatty acids.
[3] Maternal plasma phospholipid DHA ≤3.0% by wt of total fatty acids.
[4] Significantly different from the high-DHA group, $P < 0.05$.
[5] Total n−6 PUFAs (18:2n−6, 18:3n−6, 20:2n−6, 20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6).
[6] Total n−6 LCPUFAs (20:2n−6, 20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6).
[7] Total n−3 PUFAs (18:3n−3, 20:5n−3, 22:5n−3, and 22:6n−3).
[8] Total n−3 LCPUFAs (20:5n−3, 22:5n−3, and 22:6n−3).
[9] Ratio of total n−6 LCPUFAs to total n−3 LCPUFAs.

The mean (± SD) values for the sleep measures in the high-DHA and low-DHA groups on days P1 and P2 are shown in **Table 3**. The values for the different sleep measures represent the percentage of time spent in the crib in that particular sleep state. These data were analyzed with ANOVA, with group at 2 levels (high- and low-DHA) and day as the repeated measure. The group effect was significant ($P < 0.05$) for AS and AS:QS. For QS, the group effect was nearly significant ($P = 0.06$). For the variables wakefulness and sleep-wake transition, the group-by-day interaction was significant ($P < 0.10$), so we performed simple effect testing to determine whether the high-DHA and low-DHA groups differed from each other within days (Table 3). Infants of mothers with high DHA concentrations had significantly less AS and had a lower AS:QS compared with infants of mothers with low DHA concentrations. Furthermore, infants in the high-DHA group had significantly less sleep-wake transition and more wakefulness than did infants in the low-DHA group on P2.

The high-DHA and low-DHA groups differed with regard to maternal age and education, and although these differences were not statistically significant in this sample, we considered the possibility that they might be operating as confounding variables. However, none of the results discussed above and shown in Table 3 changed when we included maternal age and maternal education as covariates in the ANOVAs for group differences in sleep measures.

DISCUSSION

An inadequate supply of LCPUFAs during development may be a causal factor in certain neurologic disorders (27, 28) and also in behavioral and other functional deficits (29–31). During the third trimester of pregnancy, n−6 and n−3 fatty acids accrue in the fetal tissues and rapid synthesis of brain tissue occurs. The rapid growth of brain tissue causes increases in cell number, cell size, and cell type. It is critical for the developing fetus to obtain the correct types and amounts of fatty acids to ensure complete and proper development of the brain (32).

Animal studies of n−3 deficiencies, especially DHA deficits, have provided the basis for studying the effects of different amounts of DHA on the functional status of human infants (33). Most of the human studies have focused on the effects of different amounts of DHA in the infant's diet (ie, breast milk containing LCPUFAs compared with formulas with and without LCPUFAs) on postnatal CNS development (34, 35). Lucas et al (35) showed that infant neurodevelopment is dependent on factors that are present in breast milk but not in formula. Although definitive evidence supporting the association is lacking, these factors include LCPUFAs, specifically DHA, which is recognized as necessary for maturation of the CNS (36). Because neuronal proliferation and synaptogenesis begin late in gestation and continue into postnatal life (37), it is crucial to study the factors that affect prenatal and postnatal brain development. The prenatal supply of DHA is one such factor and is mostly dependent on maternal DHA status (17).

Several studies suggest that intakes of essential fatty acids may modulate sleep (38–40). Malikova et al (38) reported that changes in the ratio of n−3 to n−6 fatty acids in the diet of guinea pigs affected the total sleeping time. Fagioli et al (39) characterized the effects of long-term deficiency of essential fatty acids on sleep organization by studying children who were maintained on fat-free total parenteral nutrition for 2–6 mo. These children had

TABLE 3
Sleep measures of the infants in the groups with high or low maternal docosahexaenoic acid (DHA) concentrations on postnatal day 1 (P1) and day 2 (P2)[1]

			P		
Sleep measures[2]	P1	P2	Group effect	Days effect	Group-by-day interaction effect
Quiet sleep (%)					
High-DHA[3]	18.20 ± 5.59[4]	12.49 ± 3.62	0.06	0.04	0.13
Low-DHA[5]	12.48 ± 5.07	10.85 ± 3.62			
Active sleep (%)					
High-DHA[3]	44.74 ± 7.31	34.86 ±13.16	0.004	0.52	0.21
Low-DHA[5]	52.35 ± 6.24	54.16 ± 6.03			
Active:quiet sleep					
High-DHA[3]	2.73 ± 1.10	2.88 ± 1.03	0.001	0.77	0.97
Low-DHA[5]	4.94 ± 2.42	5.38 ± 1.60			
Sleep-wake transition (%)					
High-DHA[3]	4.82 ± 1.68	3.62 ± 1.48	0.12	0.71	0.07
Low-DHA[5]	4.84 ± 2.21	5.93 ± 2.15[6]			
Wakefulness (%)					
High-DHA[3]	31.27 ± 8.39	48.20 ± 17.56	0.06	0.03	0.01
Low-DHA[5]	29.65 ± 6.79	28.33 ± 6.38[6]			

[1] $n = 10$ for the high-DHA group and 7 for the low-DHA group. The level of significance was set at $P < 0.05$ for the group and days effects and $P < 0.10$ for the interaction effect.
[2] Sleep measures are expressed as the percentage of time spent in the crib in that sleep state.
[3] Maternal plasma phospholipid DHA >3.0% by wt of total fatty acids.
[4] $\bar{x} \pm$ SD.
[5] Maternal plasma phospholipid DHA ≤3.0% by wt of total fatty acids.
[6] Significantly different from the high-DHA group, $P < 0.05$.

reduced amounts of slow-wave sleep compared with children who received total parenteral nutrition with essential fatty acids. These results suggest that intakes of essential fatty acids may modulate sleep (39). Furthermore, it was observed that breast-fed infants had a more mature pattern of CNS development than did formula-fed infants. The breast-fed infants had less AS and longer periods of QS (40). Our findings are consistent with these findings.

In a study of premature infants, Thoman and Whitney (12) found significantly different, distinctive sleep patterns in 4 groups of infants: those who showed normal development and those with a minimal mental deficiency, a neurologic disorder, or physical dysfunction. None of these infants had been diagnosed with an abnormality at the time of the early sleep recordings. In another study, Freudigman and Thoman (13) reported that a newborn's sleep characteristics were related to mental and motor development at 6 mo. Thus, sleep recordings in early infancy provide a uniquely sensitive indication of subtle prenatal compromise, which may not have any apparent effects until a later age.

The present study had limitations in that it was a correlational analysis with a descriptive, nonrandomized design. However, the results provide evidence that prenatal exposure to higher concentrations of DHA results in a more mature pattern of sleep and wake states. QS, as a percentage of total sleep, increases from the time of birth until it is predominant at 3 mo postterm and thereafter (41). It is well documented that the increase in QS with age is largely a function of maturation of the nervous system and is not environmental (42). Thus, in our study, the lower amounts of AS and the greater amounts of QS observed in the infants exposed prenatally to higher DHA concentrations suggest greater CNS maturity compared with the infants exposed to lower DHA concentrations. Furthermore, the lower AS:QS observed in the infants in the high-DHA group shows that their sleep organization soon after birth was approaching that of normal, older infants. Generally, consolidation of sleep increases with age, and therefore less sleep-wake transition and more wakefulness in the infants in the high-DHA group also reflects greater maturity.

In addition, the ratio of total n−6 to n−3 fatty acids significantly influenced the integrity of the CNS. Prenatal exposure to a higher ratio of n−6 LCPUFAs to n−3 LCPUFAs resulted in sleep patterns that were contrary to those seen with a lower ratio of n−6 LCPUFAs to n−3 LCPUFAs and higher DHA concentrations. The higher ratio of n−6 to n−3 fatty acids was associated with more AS, less QS, more sleep-wake transition, less wakefulness, and a higher AS:QS. Furthermore, there was a highly significant positive association between the ratio of n−6 to n−3 fatty acids and arousals in QS. Arousals in QS are defined as QS fragmentation and are associated with CNS vulnerability (13). Future studies should consider maternal nutrition, health, socioeconomic status, and other related factors as potential correlates of DHA status.

The marked differences between the sleep measures on P1 and P2 were expected on the basis of our previous studies (13, 26). Research has shown that the characteristic changes in sleep patterns over the first 2 d postpartum, as seen in this study, occur in several other species. These changes are not only found in human newborns but also in newborn rats and rabbits (24, 25).

In conclusion, our findings suggest that differences in the prenatal supply of LCPUFAs, especially DHA, may modify brain phospholipids and affect neural function. Furthermore, the MMS is a reliable instrument for studying the CNS maturity of an individual infant in relation to infant DHA status. To our knowledge, this is the first report documenting that maternal DHA status during pregnancy, which is significantly influenced by dietary DHA, is associated with CNS maturity of the infant at birth.

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Letters to the Editor

High serum ferritin is not identical to high iron stores

Dear Sir:

In an article in the December issue of the Journal, Fleming et al (1) report that some dietary factors are associated with an increased risk of development of high iron stores in the elderly. This finding contradicts data from our laboratory (2), which indicate that the absorption of dietary iron (heme and nonheme) cannot result in iron overload in otherwise healthy, iron-replete persons.

In the study by Fleming et al, high concentrations of serum ferritin (SF) in their sample of 614 subjects aged 68–93 y were equated with high iron stores. The normal ranges of serum ferritin were arbitrarily set to < 300 μg/L in men and to < 200 μg/L in women. About 13% of the sample had values that exceeded these concentrations, which indicated that the sample was iron overloaded.

Iron stores can only be directly quantitatively determined by measuring the amount of iron removed during repeated phlebotomies until anemia develops and then subtracting both the iron deficit induced and the amount of dietary iron absorbed during the phlebotomy period. In an analysis of the available data (3), an equation was derived to describe the relation between iron stores and SF.

Estimations of iron stores, however, can be made from direct chemical determinations of nonheme iron in liver samples. Total liver iron was determined in necropsy material from 136 healthy male subjects aged 10–95 y who died in accidents or committed suicide (4). Total iron was almost constant between 20 and 90 y. In the classic study of iron stores by Weinfeld (5), the amounts of stored iron were calculated from the concentration of nonheme iron in surgical liver specimens. Weinfeld concluded that iron stores in adult men remain within fairly constant limits with age. The same was found in an extensive study of necropsy materials from the livers of 3983 samples collected in 18 different countries. No effect of age on the concentration of storage iron in the liver was seen in men (6). In the United States, a study of 259 liver samples from persons who died acutely from disease or trauma was conducted. The concentration of nonheme iron in males did not increase with age (7). The conclusion to be drawn, on the basis of consistent evidence from these extensive studies, is that there is no increase with age in iron stores in men.

It was observed in the second and third National Health and Nutrition Examination Surveys that SF increases with age (8). The key question is whether this increase in SF with age is associated with higher iron stores. Several attempts have been made to find an explanation for the increase in SF observed with age. It is well known that the concentration of SF in plasma, which is a strong acute phase reactant, is influenced by various factors such as liver disease, infections, inflammatory conditions, renal failure, cardiovascular diseases, and high alcohol consumption. Thus, there are several reasons to be careful when translating SF concentrations into iron stores.

The findings by Fleming et al that certain dietary factors and intakes of dietary iron supplements are associated with higher SF concentrations seem to fit with our data and the data of others that iron balance is related to diet. Our objection is that there is no evidence of a relation between diet and iron overload and that the control systems for iron absorption from the diet, including from red meat, are overrun in otherwise healthy subjects.

It is reasonable that dietary factors influence serum ferritin concentrations and iron stores, as is outlined in our recent article (9). We object, however, to equating serum ferritin concentrations and iron stores. The question raised by Fleming et al is whether high iron stores in the elderly (ie, high SF concentrations) are associated with disease or whether the opposite—that certain diseases are associated with high SF—is true. We have found that even a very mild disease is associated with elevated concentrations of SF. In a study of 1670 teenagers aged 15–16 y, we found that 24.4% of those with a mild common cold and fever for ≥ 1 d during the preceding 3 wk had a significant shift in SF toward higher values (10).

The report that 13% of elderly Americans have an iron overload contrasts the finding of a low prevalence of iron deficiency (2.7%). This latter value, however, was derived by using multiple criteria that we found to markedly underestimate the true prevalence of iron deficiency (9). The only ways to clarify whether elevated serum ferritin concentrations in the elderly are really associated with increased iron stores are to perform phlebotomies for the measurement of iron stores and to obtain liver biopsy samples or to conduct postmortem analyses for the measurement of liver iron concentrations.

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Conflicting evidence of iron and zinc interactions in humans: does iron affect zinc absorption?

Dear Sir:

This letter is in reference to the article by Lind et al (1) on iron and zinc supplementation in infants that appeared in a recent issue of the Journal. I understand that the importance of the interactive effects of trace elements, particularly iron and zinc, on nutrient requirements is increasingly being recognized. The coexistence of multiple micronutrient deficiencies is a widespread public health problem in many regions of the world. Earlier human studies (2–7) provided evidence in favor of iron and zinc interactions at the site of absorption but gave conflicting results that were probably due to variations in protocol design or in the trace element status of subjects before the experiment.

The study by Lind et al was conducted in the same major population as that reported on earlier by Dijkhuizen et al (2)—ie, Indonesian infants $<$ 1 y old. It also used the same doses of iron (10 mg) and zinc (10 mg), and supplementation was provided over the same span of time. Rates of prevalence of anemia, iron deficiency, and reduced serum zinc concentrations were the same as those reported for this population by Dijkhuizen et al. Lind et al reported that there was a significant dose effect on serum zinc in the group supplemented with zinc alone, which indicated a negative effect of iron on zinc absorption. This finding does not agree with the observation of Dijkhuizen et al that iron supplementation does not depress serum zinc concentrations. What could be the reason for these conflicting results when the study by Lind et al seems to have no confounding variables compared with the study by Dijkhuizen et al? Both of these studies concluded that concurrent supplementation with iron and zinc is less efficacious in improving the iron and zinc status than is supplementation with either iron or zinc alone, but can single supplements promote catch-up growth in a stunted and wasted population, or must we look for any other limiting variables to explain these differences?

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Reply to B Sreedhar

Dear Sir:

Sreedhar addresses several interesting issues in his comparison between the study by Dijkhuizen et al (1) and our study (2). Both studies have a factorial design in which rural, marginally malnourished Indonesian infants were provided with iron, zinc, or a combination of iron and zinc or placebo. The 2 studies share the general conclusion that simultaneous supplementation with iron and zinc is less efficacious in improving iron and zinc status than is supplementation with either iron or zinc alone. However, Dijkhuizen et al concluded that supplementation with a combination of iron and zinc could be safe and effective, whereas we stated that such a supplement is not optimal.

There are some differences between the 2 studies that may contribute to the differences in results and interpretation. First, whereas supplementation in our study commenced when the infants were 6 mo old, supplementation in the study by Dijkhuizen et al started when the infants were 4 mo old. A recent study by Domellöf et al (3) of iron supplementation in infancy showed that the initiation of iron supplementation at age 4 mo instead of at age 6 mo had significantly different effects on the hemoglobin response. That study also showed that the initiation of supplementation at 4 mo increased hemoglobin irrespective of iron status, which indicated an immature response to iron supplementation at that age, whereas supplementation from age 6 mo did not have that effect. Effects on zinc status were not reported in that study. The different ages at the start of supplementation in the study by Dijkhuizen et al and in our study may thus make a comparison difficult.

Second, our infant population had generally lower serum zinc concentrations than did the population in the other study. The serum zinc concentrations that we found in the group receiving only iron were lower than those in the placebo group, but this difference was not significant. However, the prevalence of low serum zinc ($<$ 10.7 μmol/L) was significantly greater in the iron supplementation group than in the placebo group, which indicated a negative effect of iron supplementation on zinc status. It may be that this interaction is evident only in a more zinc-deficient population such as ours.

Third, our report included dose-effect analyses, which showed that an increased dose of combined supplement did not compensate for the lower efficacy of the combined supplement. This finding influenced our overall conclusion.

Sreedhar also inquired whether single supplements can promote catch-up growth in stunted and wasted populations or whether we have to look for other limiting factors. We are fully aware of the fact that these micronutrients are not the only limiting factors for growth: in our study, stunting increased despite the zinc supplementation. A meta-analysis on the subject showed that zinc supplementation improved growth in malnourished children (4). Findings of the growth effects of iron supplementation are also conflicting (5–8). The same is true for simultaneous iron and zinc supplementation (1, 9, 10); in one of those studies, Perrone et al (10) found an effect on growth if the iron and zinc supplements were consumed 12 h apart. Our data, not yet published, show positive effects of single zinc and iron supplementation on growth and psychomotor development but no such effect of combined supplementation. We therefore believe that, when a supplementation strategy is more feasible than is a food-based approach, innovative dose regimens for the provision of these micronutrients are needed.

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Erratum

Cheruku SR, Montgomery-Downs HE, Farkas SL, Thoman EB, Lammi-Keefe CJ. Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. Am J Clin Nutr 2002;76:608–13.

Page 610, Figure 1: The *n* value for postpartum day 2 should be 14. In addition, the *r* and *P* values reported in each of the 4 panels are incorrect; the correct values are as follows: active sleep ($r = -0.47$, $P = 0.09$), active sleep:quiet sleep ($r = 0.11$, NS), wakefulness ($r = 0.42$, NS), and sleep-wake transition ($r = -0.30$, NS).

Page 610, Figure 2: After a reanalysis of the data, the correct information no longer parallels the data shown in the panels of the original figure. The correct data are as follows. Postpartum day 1 ($n = 17$): quiet sleep ($r = -0.47$, $P = 0.056$), active sleep ($r = 0.49$, $P = 0.05$), and active sleep:quiet sleep ($r = -0.45$, $P = 0.08$). Postpartum day 2 ($n = 14$): active sleep ($r = 0.68$, $P = 0.01$), active sleep:quiet sleep ($r = -0.06$, NS), sleep-wake transition ($r = 0.51$, $P = 0.06$), and wakefulness ($r = -0.57$, $P = 0.03$).