Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning1-4

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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.

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 of 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...
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 individual sleep measures 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 in 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 PUFA, 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.

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

<table>
<thead>
<tr>
<th>Race</th>
<th>Parity</th>
<th>Infant birth length</th>
<th>Maternal education (y)</th>
<th>Length of gestation (wk)</th>
<th>Infant head circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>0</td>
<td>29.20 ± 5.24</td>
<td>13.90 ± 2.33</td>
<td>40.40 ± 0.96</td>
<td>33.88 ± 1.34</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
<td>24.8 ± 5.12</td>
<td>11.57 ± 5.41</td>
<td>39.00 ± 1.86</td>
<td>33.98 ± 1.34</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>High-DHA group(^{(n = 10)})</th>
<th>Low-DHA group(^{(n = 7)})</th>
<th>% by wt of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>20:3n-6</td>
<td>3.26 ± 0.56</td>
<td>2.74 ± 0.41(^{4})</td>
<td></td>
</tr>
<tr>
<td>20:4n-6</td>
<td>10.64 ± 1.50</td>
<td>10.01 ± 1.04(^{4})</td>
<td></td>
</tr>
<tr>
<td>22:4n-6</td>
<td>0.55 ± 0.13</td>
<td>0.50 ± 0.17(^{4})</td>
<td></td>
</tr>
<tr>
<td>22:5n-6</td>
<td>1.69 ± 0.51</td>
<td>1.25 ± 0.19(^{4})</td>
<td></td>
</tr>
<tr>
<td>Σ n-6 PUFAs(^{4})</td>
<td>34.15 ± 5.16</td>
<td>35.65 ± 2.31(^{4})</td>
<td></td>
</tr>
<tr>
<td>Σ n-6 LCPUFAs(^{4})</td>
<td>16.33 ± 1.73</td>
<td>14.64 ± 1.59(^{4})</td>
<td></td>
</tr>
<tr>
<td>n-3</td>
<td>20:5n-3</td>
<td>0.29 ± 0.07(^{4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22:5n-3</td>
<td>0.57 ± 0.12(^{4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22:6n-3</td>
<td>3.98 ± 0.66(^{4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ n-3 PUFAs(^{7})</td>
<td>5.01 ± 0.56(^{4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ n-3 LCPUFAs(^{7})</td>
<td>4.72 ± 0.54(^{4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ n-6 LCPUFAs(^{7})</td>
<td>3.47 ± 0.29(^{4})</td>
<td></td>
</tr>
<tr>
<td>n-9</td>
<td>20:3n-9</td>
<td>0.19 ± 0.05(^{4})</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

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.

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).

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).^

<table>
<thead>
<tr>
<th>Sleep measures</th>
<th>P1</th>
<th>P2</th>
<th>Group effect</th>
<th>Days effect</th>
<th>Group-by-day interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiet sleep (%)</td>
<td>Low-DHAJ</td>
<td>12.48 ± 5.07</td>
<td>12.49 ± 3.62</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Active sleep (%)</td>
<td>High-DHAJ</td>
<td>4.82 ± 1.68</td>
<td>3.62 ± 1.48</td>
<td>0.12</td>
<td>0.71</td>
</tr>
<tr>
<td>Sleep-wake transition (%)</td>
<td>Low-DHAJ</td>
<td>4.84 ± 2.21</td>
<td>5.93 ± 2.15</td>
<td>0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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.

Sleep measures are expressed as the percentage of time spent in the crib in that sleep state.

Maternal plasma phospholipid DHA >3.0% by wt of total fatty acids.

Maternal plasma phospholipid DHA ≤3.0% by wt of total fatty acids.

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 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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REFERENCES