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The Interaction between Diet and Neurobehavior in Very Low Birth Weight Infants

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Abstract

Background: Modulation of behavior and physiology by dietary perturbations early in life can provide clues to the pathogenesis of adult diseases. We tested the hypothesis that a period of early protein supplementation modulates sympathetic nervous system activity demonstrated indirectly by an increase in active sleep state distribution in very low birth weight (VLBW) infants.

Methods: VLBW infants (n=71) were randomized to a total parenteral nutritional regimen providing 18% of the energy intake as amino acids (AA) or a conventional regimen providing 12.5% to achieve targeted AA intakes of 4 grams/kilogram/day (0.004 kilocalories/kilogram/day) and 3 grams/kilogram/day (0.003 kilocalories/kilogram/day) respectively. Both groups were weaned to enteral feeding and advanced to provide similar AA intake of 4 grams/kilogram/day (0.004 kilocalories/kilogram/day). Six-hour daytime, behavioral sleep studies were performed when the infants reached full enteral intake (165 milliliters/kilogram/day).

Results: Infants in the high protein group spent more time in active sleep (77.2 ± 10.5% vs. 70.7 ± 11.8%), p<0.01 and less time in quiet sleep (12.9 ± 3.4% vs. 17.7 ± 7.0%, p<0.01) as compared to the conventional group. No group differences were observed for indeterminate sleep, awake or crying states.

Conclusions: These results suggest that dietary intake may indirectly influence sympathetic nervous system activity.

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Statement of Consent
Informed consent was obtained from the parent or guardian of each participant in the study.

Category of Study
This manuscript describes a clinical research study.

Introduction

Early diet can influence human development. It has been hypothesized that nutrition and growth during critical periods of development may program physiological control systems by yet unknown mechanisms resulting in environmentally-induced variations in health and behavior in later life (1, 2). Lucas and colleagues showed that infants fed human milk scored better on subsequent IQ tests than infants fed artificial formula (3). Other studies have demonstrated that deficiencies in both micronutrients and macronutrients can impact development including performance on educational testing and information processing tasks (4, 5. 6).

Additional work has looked specifically at the impact of nutrition on sympathetic nervous system activity. In experimental animals, brief prenatal or postnatal nutritional manipulations are known to induce both short-term and long-term changes in various physiological systems including the sympathetic nervous system (7, 8). In addition, experimentally-induced increases in protein synthesis are associated with increased active sleep duration and greater sympathetic nervous system activity (9). The potential association between nutrition and sleep state organization has been linked to the synthesis of sleep-related neurotransmitters that is dependent on the availability of specific amino acids (10). Careful assessment of modulation of behavior and physiology by dietary perturbations early in life can potentially provide clues to the pathogenesis of adult diseases.

Importantly, the organization and development of sleep wake states has been hypothesized to influence the neurodevelopment of premature infants (11, 12). The neonatal period is characterized by three prominent stages of sleep including active sleep, quiet sleep, and indeterminate sleep. The dominating active sleep in premature infants is distinguished by rapid eye movements, variability in respirations and heart rate, and increased physiological activity. In contrast, quiet sleep is characterized by the absence of rapid eye movements, regularity of respirations and heart rate, and energy maintenance. As premature infants approach term gestation, the percentage of time spent in quiet sleep increases (13, 14, 15). These differences in sleep states may be modulated by dietary intake. In an observational study, Butte and colleagues demonstrated that formula-fed infants spent a higher percentage of sleep time in active sleep compared with the breast-fed infants and conversely, breast-fed infants spent a higher percentage of sleep time in quiet sleep. Protein intake, which correlated positively with active sleep and negatively with quiet sleep in the study, was given as a possible explanation for behavioral entrainment (16). These data highlight the importance of evaluating the role of exogenous nutritional stimuli on modulating behavioral state organization in the early periods of development in order to further understand the pathogenesis of adult diseases.

In our previous work, low birth weight infants (BW 750–1600 grams) were randomized by birth weight stratification to varying quantities and qualities of enteral energy intake by varying carbohydrate intake (9.1–20.4 grams/kilogram/day (g/kg/d), 0.0091–0.02 kilocalories/kilogram/day (kcal/kg/d)) and fat intake (4.3–9.5 g/kg/d, 0.0043–0.0095 kcal/kg/d) but at the same protein intake (4 g/kg/d, 0.004 kcal/kg/d). A secondary analysis from this work showed no differences in the growth rates or macronutrient balances of

infants weighing less than 1250 g at birth compared to those weighing greater than 1250 g at birth. However, infants less than 1250 g had significantly smaller heads and shorter lengths (17). Previous work has supported the hypothesis that insufficient protein intake contributes to postnatal growth restriction (18, 19). This was the underlying basis for the randomized controlled study of early protein supplementation in infants less than 1250 g. The primary of aim of the study was to understand the effects of this intervention on growth, metabolic response during the infant's hospital stay, and subsequent effects on long-term neurodevelopmental outcomes. As a secondary analysis, we tested the hypothesis that the brief period of early protein supplementation in very low birth weight (VLBW) infants modulated sympathetic nervous system activity leading to increases in the percentage of time spent in active sleep.

Methods

Study Population

The study population consisted of 71 appropriate for gestational age, VLBW infants (birth weight 500–1250 grams), all of whom were enrolled in a prospective, double blind, randomized controlled study of early, high protein supplementation. The study was approved by the institutional review board at Columbia University Irving Medical Center and written consent was obtained from the parents of all infants. All infants were enrolled within 24 hours of birth. Randomization was computer generated and stratified by three birth weight groups: 500–750 g, 751–1000 g, and 1001–1250 g. A stratified randomization approach was utilized to ensure an equal number of infants from each birth weight range in the two groups. The parents, the physicians and nurses taking care of the infants, and all investigators with the exception of one were blinded to the randomization. The investigators performing the behavioral sleep state coding were also blinded to the randomization. In order to achieve this, a nutritionist and a pharmacist prepared and delivered the TPN, the formula, or the breast milk daily. The protein content listed on the TPN bag was covered so that the healthcare team was unaware of the randomization assignment.

Nutritional Regimens

At the start of the study, infants were randomized to either a high protein nutritional regimen or a conventional protein nutritional regimen. The nutritional regimens were divided into two feeding periods: the parenteral feeding period and the enteral feeding period. The parenteral feeding period included two phases: phase one included the time that the infants were receiving total parenteral nutrition (TPN) as the sole source of nutrition and phase two included the time when TPN was being used to supplement enteral feeds. This period was the time of experimental intervention. As summarized in Figure 1, during phase one of the parenteral period, infants randomized to the high protein regimen received 18% of their gross energy intake as protein (protein to energy ratio: 4.5g:100kcal). When phase two began, the protein intake was fixed 4 g/kg/d (0.004 kcal/kg/d) in this group. Infants randomized to the conventional regimen received 12.5% of their energy intake as protein (protein to energy ratio: 3.1g:100kcal) during phase one of the parenteral period. During phase two, these infants received fixed protein intakes of 3 g/kg/d (0.003 kcal/kg/d). The targeted gross energy intake during this period was 115 kcal/kg/d. Of note, trophamine was

utilized the amino acids. Cysteine was added at a ratio of 30 mg of cysteine per one gram of protein. The amount of cysteine added did not exceed 100 mg.

Enteral feeds were started and advanced at the discretion of the attending physician. In addition, periods of feeding intolerance were managed by the primary healthcare team. The infants whose mothers elected to formula feed were fed a standard preterm formula (Enfamil Premature Formula, Mead Johnson Nutrition, Chicago, IL). Infants whose mothers elected to provide breast milk received expressed breast milk (EBM). The EBM was fortified with Human Milk Fortifier (Mead Johnson Nutrition, Chicago IL) when enteral feedings reached 80–100 mL/kg/d. If the mother was unable to provide an adequate volume of EBM, feedings were supplemented with the standard preterm formula. The enteral feeding period started when 120 milliliters/kilogram/day (mL/kg/d) of enteral feeds were tolerated and total parenteral nutrition was discontinued. Feedings were increased in both groups to full enteral intakes of 165 mL/kg/d. At this intake, the protein and energy intakes of infants was targeted to be 4 g/kg/d (0.004 kcal/kg/d) and 133 kcal/kg/d respectively. The volume of intake was held constant until discharge.

Experimental Design

Infants were studied in the Infant Physiology Laboratory at Morgan Stanley Children's Hospital of New York. In order to participate in the behavioral state coding study, the infants had reached full enteral intake of 165 mL/kg/d, were maintained in room air with no respiratory support, and were free of apnea of prematurity. Furthermore, all infants were studied at 36 weeks post-menstrual age. Infants were excluded from participation if they had a diagnosis of grade three or grade four intraventricular hemorrhage. Infants were not receiving cardiac or respiratory drugs at the time of the state coding. In addition, infants had not recently received sedation medications for procedures.

Each study was comprised of two sequential three-hour periods of behavioral sleep-wake state assignments for each minute of the study. Infants were assigned to the supine position for the first three-hour epoch and the position was then reversed to prone in the second three-hour epoch. They remained in their assigned positions throughout the inter-feeding period, and no further manipulations were performed.

Experimental Protocol

Infants were brought to the laboratory at about 0730 when electrodes for physiological vital sign monitoring were attached. They were then placed in a radiantly warmed, clear plastic incubator and maintained under thermoneutral conditions. No physical constraints such as swaddling were used. Studies began after the 0800 feed and continued until the 1400 feed. The studies were interrupted for the 1100 feed, after which sleeping position was changed. The volume and composition of the two feeds were identical.

Assessment of Sleep States

Behavioral codes were assigned each minute using a scoring system developed and validated in our laboratory (20). The individuals performing sleep state assessments were blinded to the infant's randomization group. Briefly, active sleep was coded if one or more rapid eye

movements were observed during the minute. In addition to the small body movements typical of active sleep, movements of whole extremities and the torso were seen in this state. Quiet sleep was designated when the infant was asleep without rapid eye movements and appeared "rag-doll" floppy and relaxed; movements were limited to startles and non-nutritive sucking or jaw jerks. Indeterminate state was coded when small body movements were observed, without rapid eye movements. Codes were also assigned for awake, crying, and feeding periods.

Data Analysis

Demographic characteristics of the study population were analyzed using t-tests, chi-square tests, and Fisher's exact tests. Data from each study were segregated and analyzed for different sleep states in prone and supine positions. High protein and conventional nutritional regimen group comparisons for various sleep states were made using unpaired t-tests.

Results

178 infants and their families were approached for consent and 115 infants were ultimately included in the study. 63 families declined participation in the study. 59 infants were randomized to the high protein nutritional regimen and 56 infants were randomized to the conventional nutritional regimen. Six infants in each group expired. One infant in the conventional group was transferred to an outside hospital. 71 infants out of the 115 infants included in the primary study met criteria for participation in the behavioral state coding study. 34 infants out of the 71 included infants were in the conventional group while 37 infants out of the 71 included infants were in the high protein group.

T-tests, chi-square, and Fisher's exact test analyses did not reveal differences in the demographic characteristics or morbidities between the conventional nutritional regimen group and the high protein nutritional regimen group (Table 1). Table 2 describes the nutritional intakes of each group. There were no differences in the age at which enteral feeds were started or the age at which phase two concluded between the two groups. There were also no differences between the groups in the energy intake during phase one of the parenteral period, phase two of the parenteral period, or the enteral period. Furthermore, the protein intake during phase two differed between the two groups, reflecting the aim of the study intervention. The groups did not differ in the number of infants receiving breastmilk only, formula only, or both breastmilk and formula.

As shown in Table 3, the overall incidence of quiet sleep was almost double in the prone position when compared to the supine position ($p<0.01$) in both the high protein nutritional regimen group and the conventional nutritional regimen group. In contrast, the probability of being awake ($p<0.01$) or crying ($p<0.01$) was increased in the supine position in both groups.

Infants randomized to the high protein nutritional regimen spent more time in active sleep *(p<0.01)* and less time in quiet sleep *(p<0.01)* when compared to infants randomized to the

conventional nutritional regimen. Group differences were not observed for the indeterminate state, crying state, or awake state. (Table 4, Figure 2)

Discussion

In the present study, VLBW infants were randomized to a high protein nutritional regimen or a conventional protein nutritional regimen. Analyses demonstrate that the experimental intervention was achieved. The effects of both nutritional regimens on overall sleep distribution in supine and prone body positions in this study reinforce the results previously reported for low birth weight infants (21, 22, 23, 24). Furthermore, VLBW infants randomized to the high protein nutritional regimen spent an increased percentage of time in active sleep when compared to infants randomized to a conventional protein regimen. Importantly, the infants in each group did not differ in several variables that are known to influence sleep-wake behavior including the use of caffeine, the development of intraventricular hemorrhage, and the use of formula or breastmilk.

The responses of our VLBW infants to different early protein regimens provide support for the prevailing hypothesis relating diet and sleep. Experimental evidence linking active sleep to protein synthesis has been derived primarily from animal studies. A positive correlation between the amount of active sleep and the rate of protein synthesis has been reported in animals when protein synthesis is spontaneously activated (25), stimulated by parental amino acids (26) or inhibited by chloramphenicol (27). Limited data from humans also support a relationship between protein metabolism and sleep. In adult humans, decreases in active sleep are observed when protein synthesis is inhibited by drugs or starvation (28). The fact that rapidly growing human infants have a disproportionately higher percentage of active sleep has been cited as further evidence for a link between protein metabolism and sleep (29).

It is hypothesized that the amount of protein in a diet is particularly influential on sleep because many amino acids are precursors for neurotransmitter synthesis. Increased dietary protein intake may influence sleep-wake behavior through alterations in ratios of amino acids. We speculate that as protein intake and protein storage increase, the plasma concentration of tryptophan relative to other large neutral amino acids (Trp ratio) changes. Plasma tryptophan competes with other large neutral amino acids (LNAA), i.e. tyrosine, valine, leucine, isoleucine, and phenylalanine for a common transport carrier into the central nervous system, rendering the flux of tryptophan into the brain proportional to the Trp ratio. Upon entry to the brain, tryptophan promotes serotonin synthesis in direct relationship to its tissue concentration (30, 31). Serotonin, in turn, has an acute hypnotic effect on both animals and humans (32) and appears to reduce active sleep preferentially. Changes in sleep latencies and percent time spent in active sleep in term infants have been reported to follow feedings designed to perturb the Trp ratio (33).

Broadly, there is a growing interest in early nutrition as a potential contributor to long-term neurodevelopmental outcomes, especially in VLBW infants. A recent systematic review suggested that some nutrients (i.e. glutamine) might improve neurodevelopment (34). Decreased protein intake in infants born between 32 weeks and 36 weeks is

associated with increased discontinuity on EEG, suggesting decreased cortical maturation (35). Animal models have demonstrated that active sleep is important for neuronal maturation, synaptic plasticity and therefore learning (36, 37). Additional work has found that decreased rapid eye movements per minute are associated with decreased scores on the Bayley Scale at 12 months and 24 months of age (38). Although our prospective randomized controlled trial is one of the first studies to directly link protein intake and behavioral states in VLBW infants, which influence neurodevelopment, it does not specifically evaluate long-term neurodevelopment. Since VLBW infants are at risk for adverse neurodevelopmental outcomes, it is important to consider modifiable variables that may be linked to neurodevelopment including nutrition and sleep-wake behavior.

In conclusion, data obtained from our studies of VLBW infants fed different amounts of proteins support the general hypothesis that nutrition and behavior are interactive. Whether the noradrenergic system is activated and or whether the serotonergic system is suppressed by nutrient intake will take additional studies and additional measurements, including direct measurements of neurotransmitter activity, and whether any change in these systems will prove to be of general importance also remains to be determined.

Statement of Financial Support

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Impact Statement

Infants randomized to an early, high protein nutritional regimen spent an increased percentage of time in active sleep, supporting the hypothesis that nutrition and behavior are interactive. Furthermore, sleep states are an indirect measure of sympathetic nervous system activity, suggesting that dietary intake may influence sympathetic nervous system activity. This study highlights the importance of considering the impact of nutrition during critical periods of development in order to further understand and improve the long-term outcomes of very low birth weight infants.
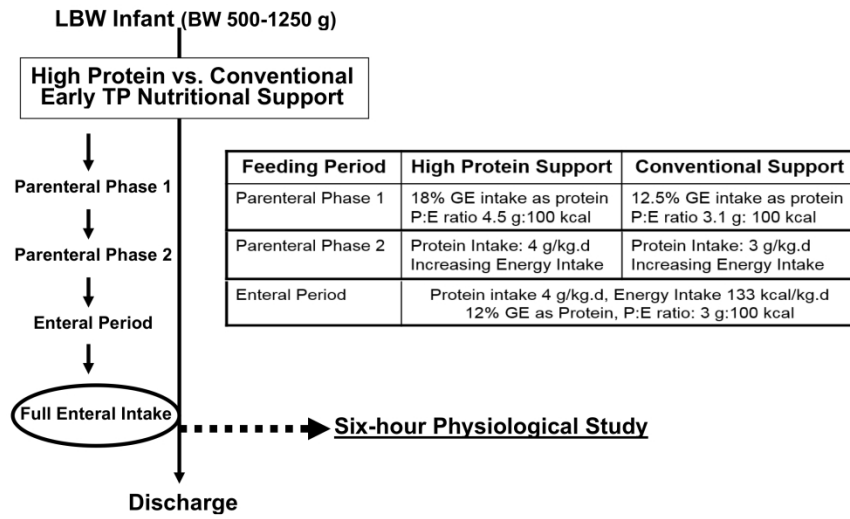
Figure 1.
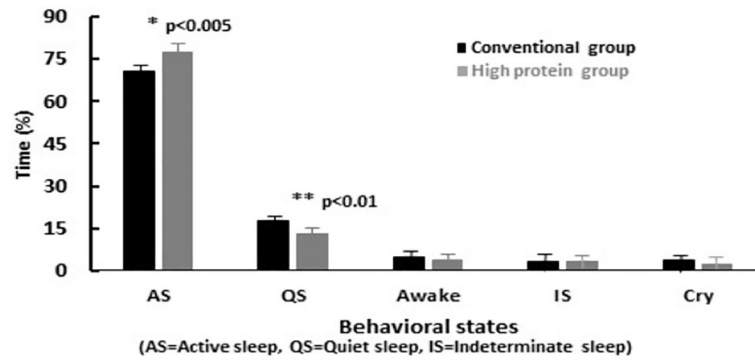Description of Experimental Protocol

Figure 2.
Effects of an Early, High Protein Nutritional Regimen (High Protein Group) and a
Conventional Protein Nutritional Regimen (Conventional Group) on Percent Distribution
of Behavioral Sleep-wake States in Very Low Birth Weight Infants.

Table 1.

Demographic Characteristics of the Study Population

Characteristic	Total Population (*N*=71)	Conventional Group (*N*=34)	High Protein Group (*N*=37)	Statistical Test
Gestational Age (weeks)	26.8 (2.1)	26.8 (1.8)	26.8 (2.1)	t stat: 0.76; p=0.76
Gender				
Male	35	13	22	Chi Square: 3.19; p=0.07
Female	36	21	15	
Ethnicity				
Caucasian	24	11	13	p=0.83
Black	15	6	9	
Hispanic	30	16	14	
Asian	2	1	1	
Birth Weight (grams)	933.4 (223.0)	937.9 (230.4)	938.9 (219.65)	t stat: 1.67; p=0.88
Birth Length (cm)	34.9 (2.9)	35.1 (3.0)	34.8 (2.8)	t stat: 2.0; p=0.69
Birth Head Circumference (cm)	25.1 (2.3)	25.2 (2.3)	24.9 (2.3)	t stat: 2.0; p=0.63
Caffeine Use				
Yes	27	16	11	Chi Square: 2.26; p=0.13
No	44	18	26	
Sepsis [1]				
Yes	27	15	12	Chi Square: 1.03; p=0.31
No	44	19	25	
Necrotizing Enterocolitis [2]				
Yes	7	4	3	p=0.70
No	64	30	34	
Urinary Tract Infection				
Yes	11	5	6	Chi Square: 0.03; p=0.86
No	60	29	31	
Intraventricular Hemorrhage				
None	57	26	31	p=0.81
Grade 1	10	6	4	
Grade 2	4	2	2	
Patent Ductus Arteriosus Treatment				
None	32	15	17	Chi Square: 0.08; p=0.96
Indomethacin	32	16	16	
Ligation	15	7	8	

[1] Sepsis was diagnosed if infant had a positive blood culture and was treated with antibiotics for at least 7 days.

[2] NEC was diagnosed if x-ray findings demonstrated pneumatosis (Bell Stage 2).

Table 2.

Nutrition Intakes of the Study Population

Characteristic	Conventional Group (*N*=34)	High Protein Group (*N*=37)	p-Value
Age, Enteral Feeds Started (Day of Life)	4.97 (4.19)	5.81 (4.55)	p=0.89
Age, End of Phase 2 (Day of Life)	29.1 (19.7)	26.3 (18.6)	p=0.24
Energy Intake, Phase 1 (kilocalories/kilogram/day	39.6 (8.0)	42.1 (13.1)	p=0.37
Energy Intake, Phase 2 (kilocalories/kilogram/day	94.1 (10.7)	91.8 (9.3)	p=0.38
Energy Intake, Enteral Period (kilocalories/kilogram/day	123.9 (4.8)	121.9 (8.6)	p=0.26
Protein Intake, Phase 2 (grams/kilogram/day)	2.9 (0.17)	3.7 (0.23)	p<0.01
Number Infants Receiving Breastmilk Only	2	4	p=0.67
Infants Receiving Formula Only	7	5	p=0.43
Number Infants Receiving Breastmilk and Formula	25	28	p=0.83

Table 3.

Behavioral State Distribution in Supine and Prone Positions in High Protein and Conventional Nutritional Regimens (n=71).

	Supine Position	Prone Position	*p-Value*
Quiet Sleep (%)	10.62 ± 7.7	19.91 ± 10.8	< 0.01
Indeterminate Sleep (%)	2.92 ± 3.4	3.79 ± 5.5	0.28
Active Sleep (%)	74.24 ± 18.5	72.80 ± 11.07	0.15
Awake (%)	7.79 ± 14.7	1.74 ± 3.8	< 0.01
Crying (%)	4.40 ± 7.9	1.75 ± 2.9	< 0.01

Table 4.

Differences in Behavioral Sleep-Wake State Distribution in an Early, High Protein Nutritional Regimen (High Protein Group) and a Conventional Nutritional Regimen (Conventional Group).

	Conventional Group (*N=34*)	High Protein Group (*N=37*)	*p-Value*
Quiet Sleep (%)	12.9 ± 3.4	17.7 ± 7.0	< 0.01
Indeterminate Sleep (%)	3.4 ± 3.5	3.4 ± 3.4	0.31
Active Sleep (%)	77.2 ± 10.5	70.7 ± 11.8	< 0.01
Awake (%)	3.7 ± 5.1	4.7 ± 6.4	0.13
Crying (%)	2.4 ± 3.5	3.7 ± 4.9	0.16