ORIGINAL ARTICLE

Chrononutrition applied to formula milks to consolidate infants' sleep/wake cycle

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Submitted: March 13, 2007 *Accepted: March 30, 2007*

Key words: **formula fed; tryptophan; nucleotides; sleep; newborn**

Neuroendocrinol Lett 2007; **28**(4):360–366 **PMID:** 17693960 NEL280407A33 © 2007 Neuroendocrinology Letters • **www.nel.edu**

Abstract

Some 30% of pre-weaning infants present problems of sleep during the night, especially those who are bottle-fed. The solution is for them to be breast-fed for as long as possible, or, if this is not possible, for the formula milk to reproduce breast-milk's natural circadian variations in the concentrations of tryptophan and those nucleotides which have a beneficial effect in consolidating the circadian sleep–wake cycle. **OBJECTIVE**: To study in pre-weaning infants the effect on nocturnal sleep of the administration of formula milk dissociated into its day/night components. **MATERIALS AND METHODS**: A prospective study was carried out on 30 pre-weaning infants of 4–20 weeks in age who preferentially showed sleep problems. The day dissociated formula, administered from 06:00–18:00, had lower levels of tryptophan and carbohydrates, and higher levels of proteins together with cytosine-5P, guanosine-5P, and inosine-5P. The night dissociated formula, administered from 18:00–06:00, had lower levels of proteins and medium-chain triglycerides, higher levels of tryptophan and carbohydrates, together with adenosine-5P and uridine-5P. In a random, double-blind, design, three one-week diets were administered: Diet A (Control): normal initiation milk; Diet B: 06:00–18:00 normal initiation milk, 18:00–06:00 dissociated night formula; and Diet C: day/night formulas with the schedule given above. The sleep patterns were analyzed by means of actimeters (Actiwatch®). Statistical analysis consisted of an ANOVA with a Scheffe F-test, taking a value of $p<0.05$ to be statistically significant. **RESULTS**: The children receiving the week of Diet C (with the day/night formulas in synchrony with the environment) showed increased hours of actual sleep (7.68 ± 0.54 h vs. 6.77 ± 0.12 h for the Diet A control) and improved sleep latency (0.44 ± 0.04 h vs. 0.60 ± 0.08 h for the Diet A control). The same children receiving the Diet B in another different week showed an improvement in sleep efficiency (76.43 ± 3.4% vs. the Diet A control 69.86 ± 0.94%) and sleep latency (0.45 ± 0.04 h vs. the Diet A control 0.60 ± 0.08h) The parents also reported, in response to follow-up questions, an improvement in the sleep of their infants during the Diet C week. **CONCLUSION**: Day/night infant formula milks designed according to the principles of chrononutrition help to consolidate the sleep/wake rhythm in bottle-fed infants.

INTRODUCTION

Certain physiological functions, among which the activity/rest rhythm is especially important, are under circadian control. During the perinatal period, the sleep/wake cycles are adapted to those of the mother's body [12]. Immediately after birth, these systems have yet to reach the sufficient maturity to adapt to the normal 24-hour rhythm imposed by the Earth's rotation, and the infant needs at least three months for these rhythms to begin to appear [4].

Melatonin, a hormone secreted principally by the pineal gland, presents a circadian rhythm with high nocturnal and low diurnal levels, and is responsible for regulating the circadian sleep/wake cycle. In the newborn, this circadian rhythm, like that of the sleep/wake cycle, also does not appear until after 12 weeks of age [1].

The mother's milk, however, presents circadian oscillations in many of its nutritional components that participate in the regulation of the circadian rhythm, and thus influences the breast-feeding infant's sleep/wake regulation. Indeed, the breast-fed infant shows a greater development and a better circadian sleep/wake rhythm, and therefore more advantageous patterns of sleep than the bottle-fed child [8].

Tryptophan is an essential amino acid in infant nutrition. It is a precursor of the neurotransmitter serotonin and of the hormone melatonin [9]. This amino acid has a circadian rhythm in breast-milk with maximum levels at 03:00 h (acrophase), and it is also observed that the acrophase and nadir (time of minimum levels in the cycle) of the infant's melatonin circadian rhythm coincide with those in the mother's milk [2]. Oral administration of tryptophan modifies the circulating levels of serotonin and melatonin (Hajak, 1999) [9] which are key substances in the regulation and quality of sleep. As the mechanism for tryptophan's absorption and transport through the blood-brain barrier is insulin-dependent, if the feed is rich in carbohydrates and tryptophan, it will be easier for this amino acid to be absorbed and penetrate into the brain [12].

The nucleoside adenosine is a molecule that acts on the A2A receptors located in the neurons of the ventrolateral nuclei, signaling for sleep to begin [7]. The nucleoside uridine, interacting with the gamma aminobutyric acid (GABA) receptors in the central nervous system, acts as a sleep promoting substance [13]. Finally, other nutrients such as the lipids of the medium-chain triglyceride (MCT) class act to improve the newborn's sleep/wake cycle thanks to their ease of digestion and their biotransformation into energy [20].

Given this context, an infant formula was designed that was dissociated chronobiologically into its macro- and micro-nutrients with the aim of facilitating the consolidation of the sleep/wake cycle in the milk-fed infant. In particular, the nutritional components of an infant formula milk (Blemil Plus 1 Forte, Ordesa S.L.) were distributed into two separate preparations according to whether their nutritional components facilitated sleep or wakefulness. The sleep-promoting nocturnal milk (Blemil Plus 1 Night) contained high levels of L-tryptophan and carbohydrates, low protein levels (to guarantee optimal tryptophan absorption), high concentrations of MCTs, and the nucleotides uridine 5'-monophosphate and adenosine 5'-monophosphate. The wakefulness activating milk, Blemil Plus 1 Day, contained high protein levels and low tryptophan levels, vitamins A, C, and E which have antioxidant capacity and stimulate wakefulness, and the nucleotides cytidine 5'-monophosphate, guanosine 5'-monophosphate, and inosine 5'-monophosphate. In sum, we had divided the nutritional components of standard milk into two milks that were complementary: a day formula to be administered from 06:00 h to 18:00 h, and a night formula to be administered from 18:00 h to 06:00 h. Nothing had been increased or eliminated from the standard milk (Blemil Plus 1 Forte, Ordesa S.L.). Its components had merely been dissociated according to their effects on sleep or wakefulness, fulfilling the infant milk directives 1996/49/CE and 2003/14/CE.

To carry out the present study, the dissociated milks Blemil Plus 1 Day/Night were administered to infants of less than 5 months, who preferentially presented sleep problems. The infant's sleep was monitored by means of an actimeter (Actiwatch®) placed around the child's ankle that recorded its activity/inactivity throughout the study period (3 weeks).

MATERIALS AND METHODS

Trial protocol

The study was conducted with infants (n=30) from the Extremadura Autonomous Community who had preferentially presented sleep problems (more than three nocturnal awakenings) in a prior prospective test. In a double blind, random design, each infant was given different formula milk combinations over a period of three weeks (Table 1). The nutritional composition of the Day/Night dissociated milks is given in Table 2. The day period was considered as being from 06:00 to 18:00, and the night period from 18:00 to 06:00. In one of the three weeks, the infants received Diet A consisting of standard milk (Blemil Plus 1 Forte, Ordesa S.L.) both by day and night. In another week, they received Diet B consisting of standard milk Blemil Plus 1 Forte during the day (06:00–18:00), and Blemil Plus 1 Night during the night (18:00–06.00). And in another week, they received Diet C consisting of Blemil Plus 1 Day during the day (06:00–18:00), and Blemil Plus 1 Night (Ordesa S.L.) during the night (18:00–06:00).

In this way each infant was its own control. The actual type of milk that was administered to the infants each week was not known either by the parents or by the researchers administering the trial until all the experiments and statistical analyses had been completed. The company, Ordesa S.L., then revealed which milk had been administered to the infants each week.

Table 1. Combinations used in the second clinical trial of milk formulas dissociated into their nutritional components in infants with sleep problems.

Infant no.	Week 1st	Week 2nd	Week 3rd
1	A	C	B
2	B	A	C
3	A	C	B
4	A	B	C
5	C	A	B
6	C	B	A
7	B	A	C
8	A	C	B
9	C	A	B
10	C	B	A
11	A	B	C
12	B	C	A
13	A	C	B
14	C	B	A
15	C	B	A
16	B	A	C
17	A	C	B
18	C	B	A
19	B	A	C
20	A	B	C
21	B	A	C
22	A	B	C
23	C	A	B
24	B	C	A
25	B	A	C
26	C	A	B
27	B	C	A
28	A	C	B
29	B	A	C
30	C	A	B

Diet A: Day formula (06:00–18:00), Blemil Plus 1 Forte; night formula (18:00–06:00), Blemil Plus 1 Forte. Diet A was taken as the Control.
Diet B: Day formula (06:00 -18:00): Blemil Plus 1 Forte; night formula (18:00–06:00): Blemil Plus 1 Night: formula dissociated into its nutritional components to consolidate sleep.
Diet C: Day formula (06:00–18:00): Blemil Plus 1 Day, formula dissociated into its nutritional components that consolidate wakefulness. Night formula (18:00–06:00): Blemil Plus 1 Night, formula dissociated into its nutritional components that consolidate sleep.
Number of children used: n=30.

Table 2. Nutrient composition of the BLEMIL PLUS 1 NIGHT and BLEMIL PLUS 1 DAY formulas, both of which satisfy the 1996/49/CE and 2003/14/CE directives for infant milk formulas.

NUTRIENTS (per 100 g milk powder)	BLEMIL 1 PLUS NIGHT (3.4 g tryptophan/ 100 g protein)	BLEMIL 1 PLUS DAY (1.5 g tryptophan/ 100 g protein)
Macronutrients		
Proteins	10.7 g	12 g
Tryptophan total	0.40 g	0.18 g
Fats	26 g	26 g
Vegetable	16.4 g (63%)	25.7 g (98.75%)
MCT	9.6 g (37%)	
Formulaid		0.3 g (1.25%)
Carbohydrates	59.3 g	58 g
Lactose	44.8 g	44.8 g
Maltodextrin	14.5 g	13.2 g
Taurine	32 mg	32 mg
L-carnitine	17 mg	17 mg
Minerals		
Minerals	2.5 g	2.5 g
Sodium	175 mg	175 mg
Potassium	535 mg	535 mg
Chlorine	290 mg	290 mg
Calcium	420 mg	420 mg
Phosphorus	230 mg	230 mg
Iron	6 mg	6 mg
Magnesium	42 mg	42 mg
Zinc	4.4 mg	4.4 mg
Copper	300 mcg	300 mcg
Iodine	70 mcg	70 mcg
Manganese	50 mcg	50 mcg
Selenium	10.7 mcg	10.7 mcg
Ca/P ratio	1.8	1.8
Vitamins		
Vitamin A	500 mcg/1756 IU	640 mcg/2133 IU
Vitamin D	10.3 mcg/412 IU	10.3 mcg/412 IU
Vitamin E	12.2 mg	25 mg
Vitamin K	42 mcg	42 mcg
Vitamin B1	520 mcg	520 mcg
Vitamin B2	620 mcg	620 mcg
Vitamin B6	825 mcg	825 mcg
Vitamin B12	2 mcg	2.5 mcg
Vitamin C	50 mg	60 mg
Folic acid	42 mcg	42 mcg
Calcium pantothenate	3.2 mg	3.2 mg
Nicotinamide	6 mg	6 mg
Biotin	16 mcg	16 mcg
Nucleotides		
Cytidine 5´monophosphate		7.9 mg
Uridine 5´monophosphate	5.3 mg	
Adenosine 5´monophosphate	2.7 mg	
Guanosine 5´monophosphate		1.6 mg
Inosine 5´monophosphate		1.6 mg

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The study was approved by the Research Ethics Committee of the University of Extremadura, in accordance with the Helsinki Declaration. The parents consented in writing, and the children were under the continuous monitoring of their paediatricians of the Extremadura Health Service (S.E.S).

Measurement of sleep parameters

In order to analyze the evolution of the infants' sleep with the different adapted formulas, non-invasive actimeters were used to record activity/inactivity. The children wore the actimeter (Actiwatch®, Cambridge Neurotechnology Ltd., U.K.) on their ankle, except at bath-time. The actimeters weigh 22 g and measure 37 mm × 27 mm × 9 mm, and consist of an internal accelerometer that quantifies the movements and a sensor that accumulates them every 2 minutes. Before the study, the parents of the children were instructed on a series of norms to follow to try to obtain the same ambient conditions of temperature, light, and sound, and the same time in the evening for putting the infant in the cradle. The parents kept at home a daily sleep diary, which consisted of noting down the child's periods of sleep over 24 hours, and the number of bottle feeds, as well as any observations or incidences that occurred during that day which could have had an influence on the infant's rest. This diary helped us to better understand, interpret, and evaluate the results given by the Actiwatch® system.

Analysis of sleep of the milk-fed infants

Once the actimeter was removed from the child's ankle, it was immediately analyzed by computer using the "Sleep Analysis" software package (Cambridge Neurotechnology Ltd., U.K.). This gives measurements of the following parameters: (1) actual time of nocturnal sleep; (2) minutes of immobility of the infant in its cradle during the night; (3) sleep latency, the time that the infant takes to fall asleep from when it is placed in the cradle; (4) percentage of nocturnal awakenings; (5) sleep efficiency, the time the infant is asleep out of the total time it is in the cradle.

At the end of the study, the parents were asked in which week they had observed improvement in their infant's sleep.

The statistical study of the results was carried out using an ANOVA (analysis of variance) with a Scheffe F-test, with $p<0.05$ being taken as the level of significance for differences between groups.

RESULTS

The analysis of the Actiwatch data indicated an improvement in the infants' nocturnal sleep in both the week with Diet B (Control/Night) and that with Diet C (dissociated Day/Night milk). Figure 1 shows how in the weeks with Diets C the children showed a significant increase ($p<0.05$) in the hours of actual sleep with respect to Diet A (7.68 ± 0.54 h with Diet C vs. 6.77 ± 0.12 h with Diet A=control).

With Diet C (Day/Night milks), as is shown in Figure 2, there was a significant reduction ($p<0.05$) in sleep latency (the time the infant takes to fall asleep after being placed in the cradle) (0.44 ± 0.04 h vs. Diet A=control 0.60 ± 0.08 h, $p<0.05$)). This significant reduction in latency was also observed with Diet B (Day/Night milks 0.45 ± 0.04 h vs. Diet A=control 0.60 ± 0.08 h.

There were no significant differences between the diets in the technical parameters of Minutes of Immobility (Figure 3) or Percentage of Awakenings (Figure 4).

Figure 5 shows the Percentage Nocturnal Sleep Efficiency. There was a significant increase ($p<0.05$) in this parameter with Diet B (76.43 ± 3.4% vs. Diet A=control 69.86 ± 0.94%), i.e., there was an increase in the percentage of time that the infant was really asleep when it was in the cradle.

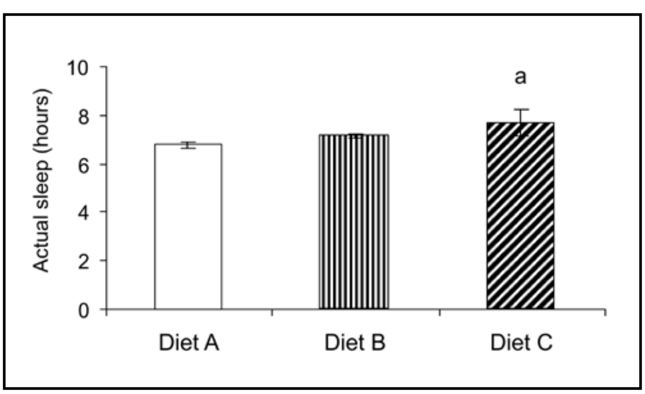

Figure 1. Daily hours of actual nocturnal sleep (X±SD) of infants of 4–20 weeks of age, fed for 3 weeks with three different formula diets: Diet A (Control), Normal Initiation Milk; Diet B, 06:00–18:00 Normal Initiation Milk and 18:00–06:00 dissociated Night formula and Diet C, Day/night formulas at the schedule described above; **a**: statistically significant with respect to the week with Diet A ($p<0.05$). (n=30).

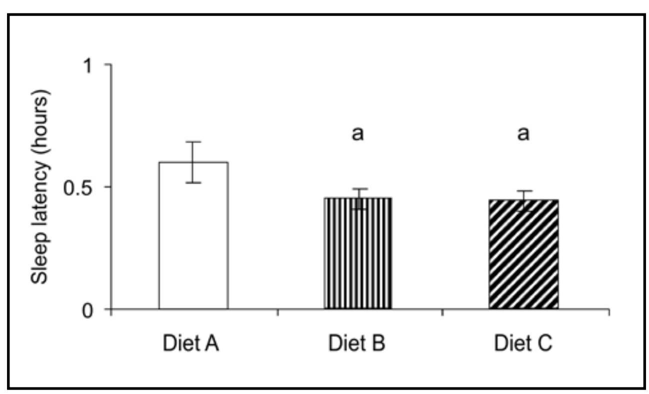

Figure 2. Nocturnal sleep latency (X±SD) of infants of 4–20 weeks of age, fed for 3 weeks with three different formula diets: Diet A (Control), Normal Initiation Milk; Diet B, 06:00–18:00 Normal Initiation Milk and 18:00–06:00 dissociated Night formula; and Diet C, Day/night formulas at the schedule described above. **a**: statistically significant with respect to the week with Diet A ($p<0.05$). (n=30).

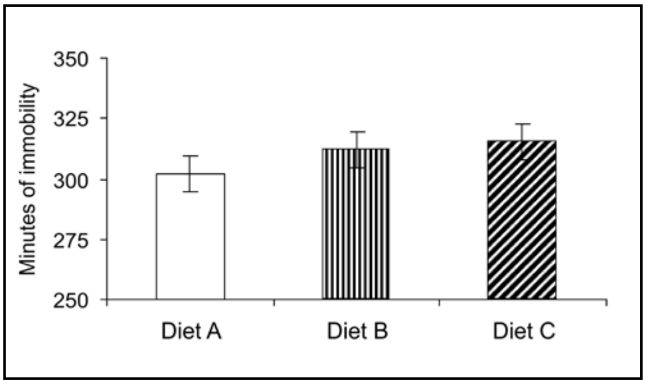

Figure 3. Minutes per day of nocturnal immobility (X±SD) of infants 4–20 weeks of age, fed for 3 weeks with three different formula diets: Diet A (Control): Normal Initiation Milk, Diet B: of 06:00–18:00 Normal Initiation Milk and 18:00–06:00 dissociated Night formula and Diet C: Day/night formulas in the scheduled times previously. (n=30).

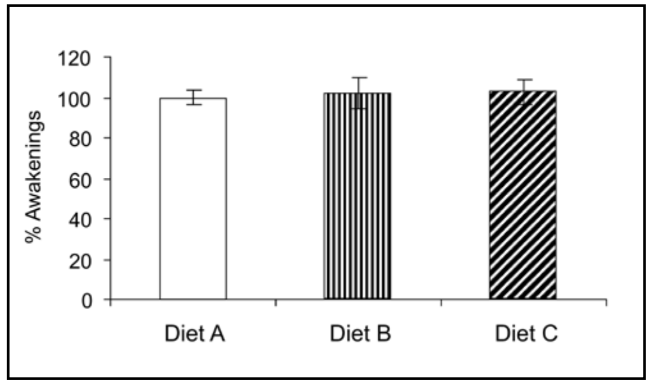

Figure 4. Number of nocturnal awakenings (X±SD) per day of infants 4–20 weeks of age, fed for 3 weeks with three different formula diets: Diet A (Control): Normal Initiation Milk, Diet B: of 06:00–18:00 Normal Initiation Milk and 18:00–06:00 dissociated Night formula and Diet C: Day/night formulas in the scheduled times previously. (n=30).

Most of the parents (53%), in response to post-trial survey questions, gave a subjective evaluation of an improvement in their infant's sleep during the week of Diet C (Figure 6), with this percentage being lower for the week with Diet B (35%) and even lower with Diet A (12%). Both Diets C and B used Blemil Plus 1 Night from 18:00–06:00.

DISCUSSION

Infant food manufacturers have concerned themselves with obtaining milk formulas that approximate breast-milk as closely as possible. Nevertheless, complete equality is impossible due, among other causes, to the proteins or cellular elements of the milk of each animal species being essentially different. But in addition there are documented differences in relatively simple components such as the high uridine and tryptophan content of breast-milk which, until now, has not been reflected in commercial infant milk formulas. Indeed, the tryptophan concentration of breast-milk is 2.5% of the total proteins, whereas in most commercial formulas the corresponding measured tryptophan content is 1.5% [10].

There are marked circadian variations in the composition of human milk that must have a key functional importance in the development of pre-weaning infants. Nevertheless, until now no infant food formulas have been developed that take these aspects into consideration, although the information on the circadian variability of milk has been known for decades [17]. For example, several studies have demonstrated that the acrophase and nadir of the circadian variability depend on the component in question: the amino acid tryptophan has maximum levels in breast-milk during the night [2],

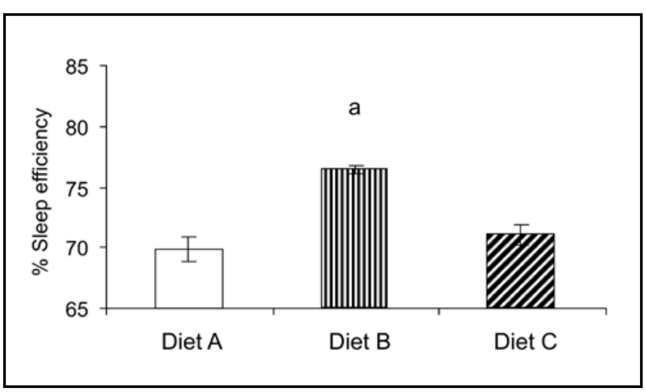

Figure 5. Nocturnal sleep efficiency % (X±SD) of infants 4–20 weeks of age, fed for 3 weeks with three different formula diets: Diet A (Control): Normal Initiation Milk, Diet B: of 06:00–18:00 Normal Initiation Milk and 18:00–06:00 dissociated Night formula and Diet C: Day/night formulas in the scheduled times previously. **a**: statistically significant with respect to the week with Diet A ($p<0.05$). (n=30).

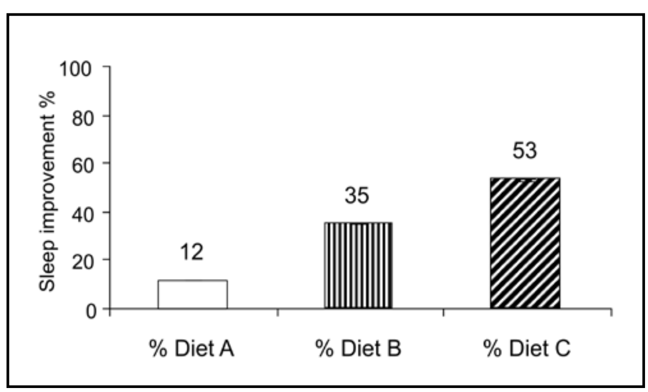

Figure 6. Sleep improvement % as reflected in the responses to the questionnaire given by the parents of infants 4–20 weeks of age, fed for 3 weeks with three different formula diets: Diet A (Control): Normal Initiation Milk, and Diet B: of 06:00–18:00 Normal Initiation Milk and 18:00–06:00 dissociated Night formula and Diet C: Day/night formulas in the scheduled times previously. (n=30).

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while the maximum values are at dusk for the sleep-inducing peptide [8], sodium, potassium, and cortisol [11], and folates and lipids [19].

Sleep disturbances are receiving increasing attention on the part of health professionals, given the importance they present in relation to the general state of health. One of the groups that present these disturbances is that of parents of young children. Infants' sleep problems not only affect their own nervous system development, but also affect the work rhythm and mood of the parents [6].

The development of the circadian rhythm of sleep begins in foetal life, since periods of wakefulness are followed by NREM and REM activity [16]. The circadian rhythm has an endogenous character, and is generated by a central clock located in the hypothalamic suprachiasmatic nucleus (SNC) which is present by mid-gestation in the human [18]. The newborn's ultradian rhythm has to evolve to a circadian rhythm in synchrony with the external environment, which is not achieved until approximately 12 weeks in age [1]. Nevertheless, not all infants achieve this transformation since 30% of pre-weaning infants present sleep problems [5]. Despite the endogenous nature of the rhythm, certain periodic environmental factors, such as the light/dark cycle or feeding patterns, can act as synchronizers (zeitgebers) of the circadian rhythms. Indeed, in the first moments of life, the interactions of the mother and the baby are fundamental for the optimal development of the circadian rhythm.

Given these facts, and the involvement of the components present in breast-milk in the development of the sleep/wake rhythm, dissociated Day/Night formula milks for pre-weaning infants were designed to take account of the function of the substances that intervene in the infant's sleep/wake behaviour. To this end, the night milk contained the sleep neuromodulating nucleotides uracil and adenine [13,7], MCTs that because of their easy digestion facilitate sleep in pre-weaning infants [20], high levels of tryptophan [9] and of carbohydrates to raise circulating insulin levels and facilitate transport through the blood-brain barrier [21], and low levels of proteins in order to reduce the competition of tryptophan with other neutral amino acids both in intestinal absorption and passage through the blood-brain barrier [14,15]. The day milk contained the nucleotides thymine, cytosine, and guanine, and, to compensate the nutrient composition of the night milk, high levels of proteins and vitamins A, C, and E, and low levels of tryptophan and carbohydrates.

The results obtained by our group in a first trail already published [3] showed us that dissociated Day/Night milks help to consolidate the circadian sleep/wake cycle. Bearing in mind that these Day/Night milks were administered during the last of the three weeks that this first test lasted, and in order to evaluate whether those results might have been due to the fact that, after three weeks, the pre-weaning infants were naturally more mature in the consolidation of the sleep/wake cycle, in this second test the day/night milks were randomly administered over the three weeks, using a double blind protocol.

In this second study, we recruited 30 children under the supervision of their paediatricians, and who presented no health problems when they began the trial. For three weeks, the children wore an Actiwatch® round their ankle which recorded their daily activity/inactivity during the time the study lasted. On the basis of previous trials carried out by our team, and given the time that must elapse for the absorption and transformation of tryptophan into serotonin and melatonin [21], it was considered that the dissociated Night milk should be administered from 18:00 to 06:00, and the dissociated Day milk from 06:00 to 18:00, in the quantities recommended by the paediatricians that the parents noted daily in the sleep diaries.

The actimetry results for the week with Diet B and the week with Diet C, both with Blemil Plus 1 Night administered from 18:00–06:00, showed an increase in nocturnal rest. This was corroborated by the opinions that the parents had noted in the diaries. Nevertheless, it has to be noted that the there was a greater bias in the results obtained through the parents' opinions due to their subjectivity in collecting the data. This data is therefore less reliable than the results of the more technical sleep analysis represented by the Actiwatch system.

Although both Diets C and B showed an improvement in night rest, the optimal week chrononutritionally was the week with Diet C (Day/Night milks), since the two formulas complement their nutritional components in harmony with breast-milk.

Artificial milk dissociated into its day/night components administered during each child's week with Diet C led to a significant increase with respect to the Diet A week (control) in the quantity and quality of sleep during the time the child was in the cradle, as was quantified in the actual nocturnal sleep parameter. The results of this second study therefore confirmed our initial hypothesis, since the best values of the sleep parameters were always obtained during the week in which the infants took the Blemil Plus 1 Day/Night combination, in synchrony with their environment.

With this second trial, we have again shown that, in the first months of life, a feed that is suited to that time of the day can act as a zeitgeber (time giver or synchronizer), helping maturation in consolidating the sleep/wake cycle. Our results lend further support to the principle underlying chrononutrition research that it is not only necessary to consider feeding from a nutritional standpoint, but that it should also be in harmony with the environment and in concordance with intestinal motility, digestive secretions, hormone levels, liver metabolism, metabolic rate, and variations in the sensitivity of target cells. Knowledge of the proper rhythm of feeding in synchrony with our internal rhythms is of great importance both for healthy individuals and for subjects with certain pathologies such as sleep disorders, obesity, and diabetes.

In sum, the use of infant milk formulas with chronobiologically adapted nutritional components improves the consolidation of the sleep/wake cycle in bottle-fed infants.

ACKNOWLEDGMENTS

This work was financed by Laboratories Ordesa S.L. through project 003-2003, and to the M.E.C. for the research grant FPU reference AP2003-3894 given to David Narciso, and our laboratory technician Elena Circujano.

REFERENCES

1 Ardura J, Gutierrez R, Andres J, Agapito T. Emergence and evolution of the circadianrhythm of melatonin in children. Horm Res. 2003; **59**(2): 66–72.
2 Cubero J, Valero V, Sanchez J, Rivero M, Parvez H, Rodriguez AB, Barriga C. The circadian rhythm of tryptophan in breast milk affects the rhythms of 6-sulfatoxymelatonin and sleep in newborn. Neuro Endocrinol Lett. 2005; **26**(6): 657–661.
3 Cubero J, Narciso D, Aparicio S, Garau C, Valero V, Rivero M, Esteban S, Rial R , Rodríguez AB, Barriga C. Improved Circadian sleep-wake cycle in infants fed a day/night dissociated formula milk. Neuro Endocrinol Lett. 2006; **27** (3): 373–380.
4 De Vries JIP, Visser GHA, Prechtl HFR. The emergence of fśtal behaviour. Qualitative aspects. Early Hum Dev.1982; **7** (4): 301–322.
5 Estivill E. Childhood insomnia due to disorderly habits. Rev Neurol. 2000; **30**(2): 188–191.
6 Fukumizu M, Kaga M, Kohyama J, Hayes MJ. Sleep-related night time crying (Yonaki) in Japan: A community-based study. Pediatrics. 2005; 115: 217–224
7 Gallopin T, Luppi PH, Cauli B, Urade Y, Rossier J, Hayaishi O, Lambolez B, Fort P. The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral preoptic nucleus. Neuroscience. 2005; **134**(4): 1377–90.
8 Graf MV, Hunter CA, Kastin AJ. Presence of delta-sleep-inducing peptide-like material in human milk. J Clin Endocrinol Metab. 1984; **59**(1): 127–32.
9 Hajak G, Huether G, B.lanke J, Freyer B, Poeggeler P, Reimer A, Rodenbeck A, Schulz-Varszegi A, Ruether M. The influence of intravenous L-tryptophan on plasma melatonin and sleep in men. Pharmacopsychiat. 1999; **24**(1): 17–21.
10 Heine WE. The significance of tryptophan in infant nutrition. Adv Exp Med Biol. 1999; 467 : 705–710.
11 Keenan BS, Buzek SW, Garza C. Cortisol and its possible role in regulation of sodium and potassium in human milk. Am J Physiol. 1983; **244**(3): 253–61.
12 Kennaway DJ. Programming of the fetal suprachiasmatic nucleus and subsequent adult rhythmicity. Trends Endocrinol Metab. 2002; **13**(9): 398–402.
13 Kimura T, Ho IK, Yamamoto I. Uridine receptor: discovery and its involvement in sleep mechanism. Sleep. 2001; **24**(3): 251–60.
14 Markus R, Olivier B, HF de Haan E. Whey protein rich in a-lactoalbumine increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. Am J Clin Nutr. 2002; **75**: 1051–6.
15 Millward J, Fereday A, Gibson N, Pacy J. Human adult amino acid requirements: 1-^{13}C leucine balance evaluation of the efficiency of utilization and apparent requirements for wheat protein and lysine compared with those for milk protein in healthy adults. Am J Clin Nutr. 2000; **72**(1): 112–21.
16 Mirmiran M, Maas YGH, Ariafno RL. Development of fetal and neonatal sleep and circadian rhythms. Sleep Med Rev. 2003; **7**(4): 321–34.
17 Picciano MF, Guthrie HA. Copper, iron, and zinc contents of mature human milk. Am J Clin Nutr.1976; **29**(3): 242–54.
18 Riukees SA, Hao H. Developing circadian rhythmicity. Semin Perinatal. 2000; **24**(4): 232–42.
19 Stafford J, Villalpando S, Urquieta Aguila B. Circadian variation and changes after a meal in volume and lipid production of human milk from rural Mexican women. Ann Nutr Metab. 1994; **38**(4): 232–7.
20 Telliez F, Bach V, Leke A, Chardon K, Libert JP. Feeding behavior in neonates whose diet contained medium-chain triacylglycerols: short-term effects on thermoregulation and sleep. Am J Clin Nutr. 2002; **76**(5): 1091–5.
21 Wurtman RJ, Wurtman JJ, Regan MM, McDermott JM, Tsay RH, JJ Breu. Effects of normal meals rich in carbohydrates or proteins on plasma tryptophan and tyrosine ratios. Am J Clin Nutr. 2003; **77**(1): 128–132.