# Improved circadian sleep-wake cycle in infants fed a day/night dissociated formula milk

**J. Cubero [1], D. Narciso[1], S. Aparicio [2], C. Garau [2], V. Valero [1], M. Rivero [3], S. Esteban [2], R. Rial [2], A.B. Rodríguez [1] & C. Barriga [1]**

[1] Department of Physiology, Faculty of Science, Extremadura University, Badajoz, Spain
[2] Department of Fundamental Biology and Health Sciences, University of Islas Baleares, Spain
[3] Scientific Division, Laboratorios Ordesa S.L. Barcelona, Spain.

*Correspondence to:* Carmen Barriga Ibars,
Department of Physiology, Faculty of Science,
University of Extremadura, Avda de Elvas s/n. 06071 Badajoz, SPAIN.
PHONE/FAX: +34 924289388.
EMAIL: cibars@unex.es

*Submitted: May 5, 2006* *Accepted: May 20, 2006*

*Key words:* **tryptophan; adenosine; uridine; formula milk; sleep; newborn.**

Neuroendocrinol Lett 2006; **27**(3):373–380 **PMID:** 16816833 NEL270306A05  **www.nel.edu**

## Abstract

On the basis of the circadian nutritional variations present in breast milk, and of the implications for the sleep/wake cycle of the nutrients present in infant formula milks, we designed a formula milk nutritionally dissociated into a Day/Night composition. The goal was to improve the bottle-fed infant's sleep/wake circadian rhythm. A total of 21 infants aged 4–20 weeks with sleeping difficulties were enrolled in the three-week duration study. The sleep analysis was performed using an actimeter (Actiwatch) placed on an ankle of each infant to uninterruptedly record movements during the three weeks. The dissociated Day milk, designed to be administered from 06:00 to 18:00, contained low levels of tryptophan (1.5g/100g protein) and carbohydrates, high levels of proteins, and the nucleotides Cytidine 5´monophosphate, Guanosine 5´monophosphate and Inosine 5´monophosphate. The dissociated Night milk, designed to be administered from 18.00 to 06.00, contained high levels of tryptophan (3.4g/100g protein) and carbohydrates, low levels of protein, and the nucleotides Adenosine 5´monophosphate and Uridine 5´monophosphate. Three different milk-feeding experiments were performed in a double-blind procedure covering three weeks. In week 1 (control), the infants received both by day and by night a standard formula milk; in week 2 (inverse control), they received the dissociated milk inversely (Night/Day instead of Day/Night); and in week 3, they received the Day/Night dissociated formula concordant with the formula design. When the infants were receiving the Day/Night dissociated milk in concordance with their environment, they showed improvement in all the nocturnal sleep parameters analyzed: total hours of sleep, sleep efficiency, minutes of nocturnal immobility, nocturnal awakenings, and sleep latency. In conclusion, the use of a chronobiologically adjusted infant formula milk seems to be effective in improving the consolidation of the circadian sleep/wake cycle in bottle-fed infants.

ORIGINAL ARTICLE

## Introduction

Of the physiological functions that are under circadian control, especially important is the sleep/wake cycle. Before birth, sleep/activity cycles are entrained to those of the mother's body [25, 45]. After birth, these systems take at least three months to attain the sufficient maturity to be able to adapt to the normal 24-hour rhythm determined by the rotation of the Earth [4].

Melatonin, a hormone secreted mainly by the pineal gland, has a circadian rhythm with levels that are high at night and low during the day, and is responsible for regulating the sleep/wake cycle. Since the newborn presents no clear circadian rhythms until 3 months in age, the rhythmic secretion of melatonin and the sleep/wake cycle do not become consolidated until this age [1].

Many of the components of breast milk present circadian oscillations, so that breast-feeding has an influence on the regulation of the infant's sleep/wake behaviour. Indeed, breast-fed infants have better sleep patterns and a better entrained sleep/wake cycle than bottle-fed babies [13].

Tryptophan is one of the most important amino acids in infant nutrition, since it is the precursor of the neurotransmitter serotonin and the neurosecretory hormone melatonin. This amino acid has a circadian rhythm in breast milk, with the acrophase at 03:00 [3]. These oscillations in the tryptophan concentrations of the milk occur in phase with oscillations in levels of 6-sulfatoxymelatonin excreted in the infant's urine [3].

The oral administration of tryptophan modifies the circulating levels of melatonin and serotonin [14], which are key substances in the regulation and quality of sleep. The synthesis of the hormone and the neurotransmitter depend on the percentage of carbohydrates and of proteins in the diet. Changes in these macronutrients affect the tryptophan/LNAA (large neutral amino acid) ratio and the circulating serotonin levels, parameters which influence both the absorption of tryptophan and its passage across the blood-brain barrier [18, 47]. The Adenosine is an important molecule in the initiation of sleep, as it excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral pre-optic nucleus [11]. Uridine is another sleep-promoting nucleoside, its action apparently being through uridine receptors in the Central Nervous System (CNS), and its interaction with gamma aminobutyric acid (GABA) receptors [26]. Such macronutrients as the medium-chain triglyceride (MCT) lipids act to improve the infant's sleep/wake cycle thanks to their ease of digestion and their thermogenic character [44]. Finally, micronutrients such as vitamin B12 can also act on nocturnal rest [43].

Given this background, a new bottle-feeding formula was developed to facilitate the consolidation of sleep/wake rhythms in the newborn. The components of the milk formula were distributed into two preparations according to their value as facilitating either sleep or wakefulness. The sleep-promoting milk contained higher levels of L-tryptophan and carbohydrates, lower levels of proteins, and MCT and the nucleotides Adenosine 5´monophosphate and Uridine 5´monophosphate. The activity-promoting milk contained lower levels of L-tryptophan and carbohydrates, higher levels of proteins, and the nucleotides Cytidine 5´monophosphate, Guanosine 5´monophosphate and Inosine 5´monophosphate. To carry out the study, the Day/Night dissociated milk was administered to infants of up to 5 months old, who preferentially presented sleep problems. Their sleep was monitored by recording their activity/inactivity on an actimeter (Actiwatch) placed on an ankle throughout the duration of the study (3 weeks). The actimetry measurements were complemented with a sleep diary in which the parents conscientiously noted how long the baby slept as well as any observation that they considered pertinent.

## Materials and Methods

**Dissociated Day/Night formulas.** A new formula milk has been developed for bottle-fee ding to facilitate the consolidation of the sleep/wake cycle in infants. The components of a commercial infant formula milk were dissociated into two formulas according to their components which promote either wakefulness or sleep. The Night formula milk has MCTs, the nucleotides Adenosine 5´monophosphate and Uridine 5´monophosphate, and higher L-tryptophan and carbohydrate content. The Day formula milk has lower tryptophan and carbohydrate content, higher protein, and the nucleotides Cytidine 5´monophosphate, Guanosine 5´monophosphate and Inosine 5´monophosphate. Both satisfy the 1996/49/CE and 2003/14/CE directives for infant milk formulas. Table I lists the nutritional composition of the Blemil Plus Day and Night formula milks, elaborated by Laboratorios Ordesa S.L.

**Subjects.** Healthy infants (n=21) of both sexes, fed with artificial milk before the beginning of their recruitment were selected in collaboration with the pædiatric service of the primary medical care of the local Social Security organization, and private clinics. To be selected, the infants had to be in good health at the beginning of the study and at most 20 weeks old. (It is known that the first signs of circadian rhythms begin to be present at 12 weeks, and in many infants with sleeping difficulties are well established at 20 weeks.) In addition, no relationships existed within the group of infants, so that the families were unable to exchange experiences about the intermediate results of the experiments. The parents gave their informed consent, and the study was approved by the Ethical Research Committee of Extremadura University, Badajoz (Spain).

**Study protocol.** The different formula milks were administered to each infant in a double blind procedure, each formula being administered for one week. The day period was taken to be from 06:00 to 18:00, and the night period from 18:00 to 06:00. In one week (control), the infants always received the same standard milk (Blemil Plus I Forte, Ordesa S.L.) both by day and at night. In another week (inverse control), they

**Table I**. Nutrient composition of the ***BLEMIL 1 PLUS NIGHT*** and ***BLEMIL 1 PLUS 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 |

received Blemil Plus I Night milk (Ordesa S.L.) during the day, and Blemil Plus I Day milk (Ordesa S.L.) during the night. In the experimental week, they received Blemil Plus I Day milk (Ordesa S.L.) during the day, and Blemil Plus I Night milk (Ordesa S.L) during the night. Hence, each infant acted as its own control.

**Measurement.** Non-invasive actigraphic monitoring was used to record and display the temporal patterns of the infant's activity and rest. All infants had a programmed wrist actimeter (Actiwatch, Cambridge Neurotechnology Ltd., U.K.) which was uninterruptedly (excepting for bath time) attached to the ankle to record motor activity. These actimeters weigh 22 g and measure 37 mm × 27 mm x 9 mm each. An internal acceleration sensor records movements and accumulates them over a pre-set 2-minute interval. These sums are saved on an internal microchip. The activity logged between lights-off and waking next morning was analyzed to measure the different sleep parameters.

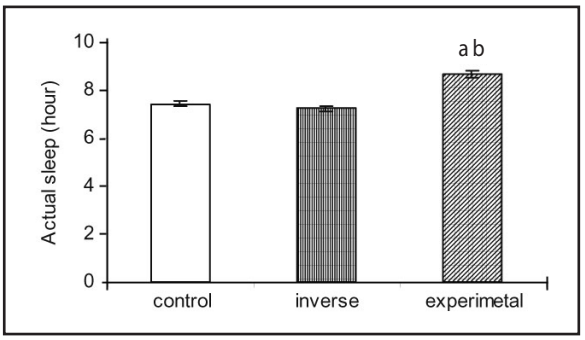


**Figure 1**. Hours of real nocturnal sleep (X±SD) of each day, in infants less than 20 weeks old, fed during the first week with the control combination (06:00–18:00 Standard Formula Milk, 18:00–06:00 Standard Formula Milk), the second week with the inverse control combination (06:00–18:00 Night Formula Milk, 18:00–06:00 Day Formula Milk), and the third week with the experimental dissociated combination (06:00–18:00 Day Formula Milk, 18:00–06:00 Night Formula Milk). **a.** ($p<0.001$) with respect to the week of the control combination. **b.** ($p<0.001$) with respect to the week with the inverse control combination. (n=21)

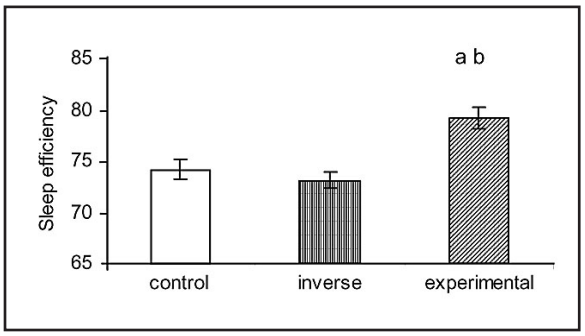


**Figure 2**. Nocturnal sleep efficiency (X±SD) of each day, in infants less than 20 weeks old, fed during the first week with the control combination (06:00–18:00 Standard Formula Milk, 18:00–06:00 Standard Formula Milk), the second week with the inverse control combination (06:00–18:00 Night Formula Milk, 18:00–06:00 Day Formula Milk), and the third week with the experimental dissociated combination (06:00–18:00 Day Formula Milk, 18:00–06:00 Night Formula Milk). **a.** ($p<0.001$) with respect to the week of the control combination. **b.** ($p<0.001$) with respect to the week with the inverse control combination. (n=21)

Before the study, the parents were instructed on how to maintain a daily sleep diary, recording the hours asleep, as well as the timing and the amount of milk ingested.

**Sleep analysis.** The dark-period sleep patterns were recorded actigraphically (Actiwatch, Cambridge Neurotechnology Ltd., U.K.) for 7-day periods, with the device on the infant's ankle. Sleep was automatically registered based on the activity data, and analyzed with the software package Sleep Analysis (Cambridge Neurotechnology Ltd., U.K.).

After retrieving the logs of the activity for a week, we proceeded to the study of the following sleep parameters:

- **Actual sleep**: The time of the sleep period in which activity is below a threshold denominated sensitivity, which was established automatically by the Sleep Analysis software package for each individual.
- **Sleep efficiency**: Percentage of time asleep relative to the time in the crib.
- **Minutes of immobility**: Minutes during which there was an unbroken period of inactivity.
- **Waking episodes**: Number of episodes of increased activity during the period of sleep.
- **Sleep latency:** The time lapse from laying the baby in the crib until sleep.

Also, the parents maintained at home a sleep diary which consisted in recording the infant's periods of sleep over 24 hours, together with the number of bottle feeds and any observations or incidences that might have affected the baby's rest. At the end of the study, the parents were asked as to which week they had observed improvement in their child's sleep.

**Statistical analysis.** The results were analyzed for significance of the differences between weeks using a proprietary statistical package (Statitiscas). ANOVAs followed by *post hoc* LSD tests were used to determine differences between specific weeks. A value of $p<0.05$ was considered statistically significant. The actual type of milk administered remained unknown by both parents and experimenters until the experiments and the corresponding statistical analyses had been finished for all infants.

## Results

The results obtained from the Actiwatch activity recordings are shown in the following figures.

Figure 1 shows the daily mean for each week of the hours of actual sleep during the nocturnal rest. There was a clear and significant increase ($p<0.001$) for the week in which the infants were fed the dissociated Day/Night formula milk relative to the control and the inverse control weeks. Also, there were no differences between the control and the inverse control.

Figure 2 shows the results for the sleep efficiency — the ratio between the hours of actual nocturnal sleep and the hours that the child is in its crib. Of the three weeks studied, the greatest value was 79.19 ± 1.09 corresponding to the week during which the infants were fed the Day/Night formula. The difference was significant ($p<0.01$) with respect to the other two weeks.

The results for the minutes of nocturnal immobility per night are shown in Fig. 3. The mean for the week in which the infants were fed the dissociated Day/Night formula was 411.52 ± 14 minutes. This was significantly greater ($p<0.001$) than the control (327 ± 9 minutes), and than the inverse control (306.5 ± 6.5 minutes).

The following two parameters also showed the same trends.

Figure 4 displays the results for the nocturnal awakenings. These were significantly fewer ($p<0.001$)

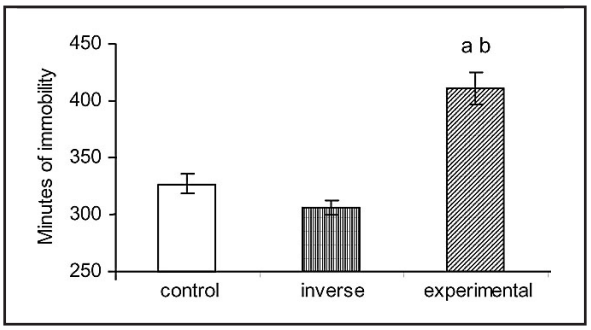


**Figure 3**. Minutes of nocturnal immobility (X±SD) of each day, in infants less than 20 weeks old, fed during the first week with the control combination (06:00–18:00 Standard Formula Milk, 18:00–06:00 Standard Formula Milk), the second week with the inverse control combination (06:00–18:00 Night Formula Milk, 18:00–06:00 Day Formula Milk), and the third week with the experimental dissociated combination (06:00–18:00 Day Formula Milk, 18:00–06:00 Night Formula Milk). **a.** ($p<0.001$) with respect to the week of the control combination. **b.** ($p<0.001$) with respect to the week with the inverse control combination. (n=21)

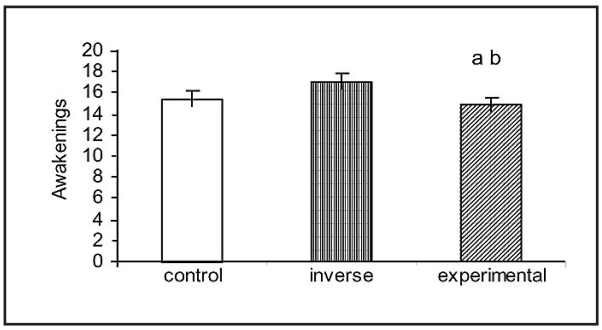


**Figure 4**. Percentage of nocturnal waking episodes (X±SD) of each day, in infants less than 20 weeks old, fed during the first week with the control combination (06:00–18:00 Standard Formula Milk, 18:00–06:00 Standard Formula Milk), the second week with the inverse control combination (06:00–18:00 Night Formula Milk, 18:00–06:00 Day Formula Milk), and the third week with the experimental dissociated combination (06:00–18:00 Day Formula Milk, 18:00–06:00 Night Formula Milk). **a.** ($p<0.001$) with respect to the week of the control combination. **b.** ($p<0.001$) with respect to the week with the inverse control combination. (n=21)

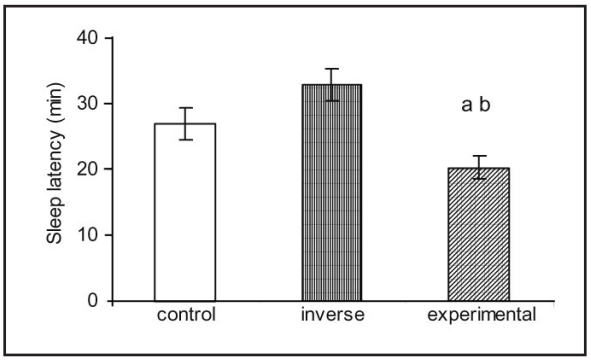


**Figure 5**. Nocturnal sleep latency (X±SD) of each day, in infants less than 20 weeks old, fed during the first week with the control combination (06:00–18:00 Standard Formula Milk, 18:00–06:00 Standard Formula Milk), the second week with the inverse control combination (06:00–18:00 Night Formula Milk, 18:00–06:00 Day Formula Milk), and the third week with the experimental dissociated combination (06:00–18:00 Day Formula Milk, 18:00–06:00 Night Formula Milk). **a.** ($p<0.05$) with respect to the week of the control combination. **b.** ($p<0.05$) with respect to the week with the inverse control combination. (n=21)

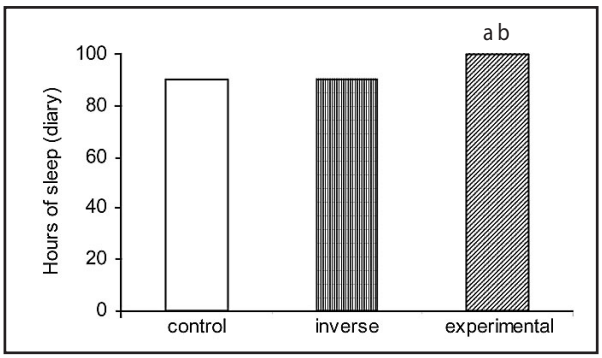


**Figure 6**. Percentage of Hours of nocturnal sleep (X±SD) recorded by the parents in the daily sleep diaries, in infants each week with one of the three combinations of initiation formula milk: control, inverse control, and experimental dissociated Day/Night formula. **a.** ($p<0.05$) with respect to the control week. **b.** ($p<0.05$) with respect to the inverse control week. (n= 21)

in the week in which the infants were fed the dissociated Day/Night formula than in the control and inverse control weeks.

The last sleep parameter (Fig. 5) determined by actimetry was the sleep latency (the time from the child's being put into the crib for the night until it falls asleep). The shortest sleep latency (20.5 ± 2 minutes) was for the week in which the infants were fed the dissociated Day/Night formula. This was significantly less ($p<0.05$) than the control and inverse control weeks.

Finally, Fig. 6 shows the hours of sleep noted by the parents in the sleep diaries analyzed by week. The number was greater ($p<0.05$) in the Day/Night experimental week than in the control and the inverse control weeks, confirming the results obtained by actimetry. In their responses to the question as to which of the three weeks of the study their child had slept better, 75% of the parents reported that they had noted improvement in their child's sleep during the third week.

## Discussion

The makers of infant formula milk have taken pains to achieve formulas as similar as possible to the breast-milk gold standard. However, complete equality is impossible due, amongst other causes, simply to the proteins or cell elements of the milk of each animal species being essentially different. But differences have also been documented in relatively simple components, such as the high uridine and tryptophan content of breast milk. Up to now, these have not been reflected in commercial infant formula milks. Indeed,

it has been shown that the tryptophan in breast milk is around 2.5% of the total protein content, while there are many registered formula milks with only 1–1.5% of tryptophan [15].

There are marked circadian variations in the composition of human milk that must have a key functional importance in the development of the infant. However, no infant formula milks at all have been developed that take these aspects into account, in spite of the circadian variability of human milk being known for decades [35]. For example, there exist numerous literature references demonstrating differing values of the acrophase and nadir according to the component in question. Thus the amino acid tryptophan is at maximum levels in breast milk during the night [3], and maximum levels are reached at dusk for the sleep-inducing peptide [13], cortisol, sodium and potassium [24], and folates and lipids [20,21,42]. The maximum of cytoplasm organelles dispersed in the milk occurs after nightfall, while the maximum concentrations of copper, zinc, iron [7, 23, 35], and lactose [46] occur in the morning, this last being inversely correlated with other oligosaccharides.

Sleep perturbations have been the object of growing attention on the part of health professionals because of their importance with respect to the general state of health. One of the groups suffering such perturbations is that of people with very young children. The sleep disorders of babies not only affect the development of the infant's nervous system, since the abundant amount of sleep in neonatal life serves an important function in brain maturation and plasticity [32], but also affects the rhythm of work and the mood of the parents [10].

The development of the sleep circadian rhythm has its origin in the fœtal period. Both quiet (NREM) and active (REM) sleep are distinguishable during the last 10 weeks of gestation [32]. The circadian rhythms have an endogenous character, being generated by a central clock located in the hypothalamic suprachiasmatic nucleus (SCN) which is present by mid-gestation in the human [37]. The ultradian rhythm to which the newborn is subject has to evolve to a circadian rhythm in synchrony with external time, which is not achieved until approximately 12 weeks in age [1]. This age, however, varies widely, and it is currently reported that over 30% of infants suffer some kind of sleep perturbation in the first year of life [6,30]. Despite the endogenous nature of the rhythm, certain environmental periodic factors, such as the light/dark cycle or feeding patterns, can act as synchronizers (zeitgebers) of the circadian rhythms [31]. 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 [28].

The essential amino acid tryptophan is a precursor of the neurotransmitter serotonin and the hormone melatonin in the brain [19]. The exogenous administration of tryptophan increases the levels of serotonin and melatonin in the brain [5, 14, 16, 19]. Both substances —serotonin and melatonin— have important actions involved in sleep. Serotonin promotes the quantity of slow wave sleep [48, 49], and melatonin sets the sleep/wake cycle [29]. There have been attempts to improve the quantity and quality of sleep by administering the amino acid tryptophan rhythmically, and indeed today it is frequently used in the treatment of depression and sleep disorders [5, 36].

Another factor to bear in mind with respect to tryptophan is the quantity of LNAAs that are administered at the same time [8, 9], because these compete with tryptophan for blood-brain barrier transport [34]. Since dietary carbohydrates and proteins affect the plasma concentration of tryptophan and the other LNAAs, these macronutrients also effect brain tryptophan concentrations, and thereby serotonin synthesis and release [38, 39, 47]. Dietary carbohydrates produce major, insulin-mediated decreases in the branched-chain amino acids but lesser reductions in plasma tryptophan, thus raising the plasma tryptophan ratio and facilitating tryptophan's entry into the brain [8]. Proteins, in contrast, lower the plasma tryptophan ratio because they contribute less tryptophan than do other LNAAs to the circulation.

In breast milk, the levels of tryptophan are high during the night, and low during the day [3], and formula milks have lower tryptophan content than breast milk [15, 40]. Also, the hormone melatonin – the key in the regulation of the sleep/wake cycle, and whose precursor is the amino acid tryptophan— is present in breast milk [22]. In both the mother and the breast-fed infant, the urine levels of its excretion metabolite – 6-sulfatoxymelatonin – follow a circadian rhythm which parallels that of the tryptophan concentration in the milk [3]. There is evidence that, compared with the breast-fed infant, the formula-fed infant has poorer arousability [17], and greater anxiety and more irregular intervals of activity-inactivity.

Adenosine and uridine are two nucleosides present in breast milk [41]. They both are clearly involved in the regulation of sleep [26, 11]. Indeed, in monophasic, bihemispheric animals (similar to man) such as the ringdove (*Streptopelia roseogrisea*), it has been found that in young animals the oral administration of adenosine increases nocturnal rest [12].

Other nutrients present in infant-formula milks have an influence on sleep. The MCTs have a rapid thermogenic action which is reflected in an improvement of sleep in the infant [44]. Vitamin B12 may act in synergy with other nutrients as a hypnotic [43, 27].

Given this context, the Day/Night dissociated formula milk was designed by considering the nutritional components that are involved in the infant's sleep/wakefulness. The Day formula contained the nucleotides Cytidine 5´monophosphate, Guanosine 5´monophosphate and Inosine 5´monophosphate, high levels of proteins, and low levels of tryptophan and carbohydrates. The Night formula contained Adenosine 5´monophosphate, Uridine 5´monophosphate, MCTs, high levels of tryptophan, carbohydrates, and low levels of proteins. The proportions of both formulas were

within the limits of the 1996/49/CE and 2003/14/CE directives for infant formula milks. For the study, 21 infants were enrolled who presented sleep problems (more than three nocturnal awakenings), under the supervision of their pædiatricians. They presented no health problem at the time of introduction into the study. They wore an Actiwatch on an ankle which logged their 24-hour activity/inactivity for the three weeks' duration of the study. On the basis of previous trials carried out by our team, and given the time lapse for the absorption and transformation of tryptophan into serotonin and melatonin [2, 47], it was decided that the Night dissociated formula milk should be administered in the period 18:00–06:00, and the Day dissociated formula milk in the period 06:00–18:00. The amounts were those recommended by their pædiatricians, and were recorded by the parents daily in the sleep diaries.

The results were very clear with respect to the improvement in the quality and quantity of the infants' sleep. The Day/Night dissociated formula milks were administered during the third week of the study. With respect to the two previous weeks, during that week there were significant increases in the hours of sleep as quantified by the real sleep, the sleep efficiency, and the minutes of immobility in the crib, and a significant reduction in sleep latency. In sum, therefore, during this third week the infants showed a significant improvement in their nocturnal sleep.

Another interesting finding was that there were no differences in the sleep parameters between the first two weeks, i.e., that corresponding to when the infants were fed with control formula milk (Blemil Plus I Forte) both by day and at night, and that corresponding to when they were fed the dissociated formula milk in the inverse order (inverse control).

The actimetry results for the week in which the nocturnal rest improved were confirmed both by the parents' subjective evaluation of an improvement and by the data that they recorded daily in the sleep diaries. Nonetheless, it has to be noted that the subjectivity of their assessment of an improvement could lead to a bias in their recording the sleep diary data, so that these must be seen as having less reliability than the more technical sleep data.

Given everything discussed above, it would seem that in the first months of life the ideal feed at each moment of the day could act as a zeitgeber, helping in the maturation and consolidation of the sleep/wake cycle and immune system [33]. Our results open a door to Chrononutrition based on the understanding that feeding must not only be considered from a nutritional standpoint, but that it should also be in harmony with the environment and in concordance with intestinal motility, intestinal secretions, hormone levels, liver metabolism, metabolic rate, etc. 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 chronobiologically adjusted infant formula milk seems to be effective in improving the consolidation of the circadian sleep/wake cycle in bottle-fed infants.

## Acknowledgments

This work was financed by Laboratories Ordesa S.L. through project 03–2003. The authors wish to express their gratitude to the pædiatricians: Drs Galan, Cardesa, Durán, Ortiz, Botana. Bermejo, Jimenez, Escobar, Cabañes, Corrales, Arce, Benito, Lema, Alverez, and to the M.E.C. for the research grant FPU reference AP2003-3894 given to David Narciso Repilado, and our laboratory technician Elena Circujano.

REFERENCES

1 Ardura J, Gutierrez R, Andres J, Agapito T. Emergence and evolution of the circadian rhythm of melatonin in children. Horm Res 2003; **59**(2):66–72.
2 Arnulf I, Quintin P, Alvarez JC, Vigil L, Touitou Y, Lèbre A, Varaguaux O, Derenne JP, Allilaire JF, Benkelfat Ch, Leboyer M. Midmorning tryptophan depletion delays REM sleep onset in healthy subjects. Neuropsychopharmacology 2002; **27**(5):843–851.
3 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.
4 De Vries JIP, Visser GHA, Prechtl HFR. The emergence of fœtal behaviour. Qualitative aspects. Early Hum Dev 1982; **7**(4): 301–322.
5 Esteban S, Nicolau MC, Gamundi A, Rial RV, Rodríguez AB, Ortega E, Barriga C. Effect of orally administered L-tryptophan on serotonin, melatonin and the innate immune response in the rat. Mol Cell Biochem 2004; **267**(1–2):39–46.
6 Estivill E. Childhood insomnia due to disorderly habits. Rev Neurol 2000; **30**(2):188–191.
7 Feeley RM, Eitenmiller RR, Jones JB Jr, Barnhart H. Copper, iron, and zinc contents of human milk at early stages of lactation. Am J Clin Nutr 1983; **37**(3):443–8.
8 Fernstrom J, Wurtman R. Brain serotonin content: physiological dependence on plasma tryptophan levels. Science 1971; **173**(992):149–52.
9 Fernstrom J, Faller DV. Neutral amino acids in the brain: changes in response to food ingestion. J Neurochem 1978; **30(6):**1531–8.
10 Fukumizu M, Kaga M, Kohyama J, Hayes MJ. Sleep-related night time crying **(***Yonaki***)** in Japan: A community-based study. Pediatrics 2005; **115**(1): 217–224.
11 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.
12 Garau C, Aparicio S, Esteban S, Nicolau C, Barriga C, Rivero M, Rial R. Adenosine increases rest in young rats but not in adult ones. Sleep Biol Rhythms 2005; **3**(1):58.
13 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.
14 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**:17–21.
15 Heine WE. The significance of tryptophan in infant nutrition. Adv Exp Med Biol 1999; **467**: 705–710.
16 Herichova I, Zeman M, Vaselovky J. Effect of tryptophan administration on melatonin concentrations in the pineal gland, plasma and gastrointestinal tract of chickens. Acta Vet Brno 1999; **67**: 89–95.

17 Horne RS, Parslow PM, Ferens D, Watts AM, Adamson TM. Comparison of evoked arousability in breast and formula fed infants. Arch Dis Child 2004; **89** (1):22–5.
18 Hudson C, Hudson SP, Hecht T, MacKenzie J. Protein source tryptophan versus pharmaceutical grade tryptophan as an efficacious treatment for chronic insomnia. Nutr Neurosci 2005; **8**(2):121–7.
19 Huether G, Poeggeler B, Reimer A, George A. Effect of tryptophan administration on circulating melatonin levels in chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Science 1999; **12** (51): 945–953.
20 Hurgoiu V, Caseanu E. Mineral composition of the milk of mothers of premature infants during early lactation. Pediatrie 1986; **41**(6):469–73. In French.
21Hurgoiu V, Marcu A, Sopon E, Olariu M. Dynamics of the composition of lipids in human milk during lactation. Pediatrie 1985; **40**(3):201–5. In French.
22 Illnerova H, Buresova M, Presl J. Melatonin rhythm in human milk. J Clin Endocrinol Metab 1993; **77**(3):838–41.
23 Karra MV, Kirksey A. Variation in zinc, calcium, and magnesium concentrations of human milk within a 24-hour period from 1 to 6 months of lactation. J Pediatr Gastroenterol Nutr 1988; **7** (1):100–6.
24 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.
25 Kennaway DJ. Programming of the fetal suprachiasmatic nucleus and subsequent adult rhythmicity. Trends Endocrinol Metab 2002; **13**(9):398–402.
26 Kimura T, Ho IK, Yamamoto I. Uridine receptor: discovery and its involvement in sleep mechanism. Sleep 2001; **24**(3):251–60.
27 Kohsaka M. Non-24-hour sleep-wake syndrome. Nippon Rinsho 1998; **56**(2):410–5.
28 Lohr B, Siegmund R. Ultradian and circadian rhythms of sleep-wake and food-intake behavior during early infancy. Chronobiol Int 1999; **16**(2):129–48.
29 Luboshitsky R, Lavie P. Sleep-inducing effects of exogenous melatonin administration. Sleep Med Rev 1998; **2**(3):191–202.
30 Lucas A, ST James-Roberts I. Crying, fussing and colic behaviour in breast and bottle-fed infants. Earl Hum Develop 1998; 53 (1):9–18.
31 Madrid JA, Rol de Lama MA, Sanchez-Vazquez FJ, Zamora S. La importancia de la cronobiología en la nutrición (In Spanish). Nutr Hosp 2003; **18** (5): 269–324.
32 Mirmiran M, Maas YGH, Ariafno RL. Development of fetal and neonatal sleep and circadian rhythms. Sleep Med Rev 2003; **7**(4):321–34.
33 Moldofsky H. Sleep and the immune system. Int J Immunopharmacol 1995; **17**(8):649–54.
34 Pardridge WM, Oldendorf WH. Kinetic analysis of blood-brain barrier transport of amino acids. Biochim Biophys Acta 1975; **401** (1):128–36.
35 Picciano MF, Guthrie HA. Copper, iron, and zinc contents of mature human milk. Am J Clin Nutr 1976; **29** (3):242–54.
36 Riemann D, Vorderholzer U. Treatment of depression and sleep disorders. Significance of serotonin and L-tryptophan in pathophysiology and therapy. Fortschr Med 1998; **116** (32):40–2.
37 Riukees SA, Hao H. Developing circadian rhythmicity. Semin Perinatal 2000; **24** (4):232–42.
38 Schaechter JD, Wurtman RJ. Tryptophan availability modulates serotonin release from rat hypothalamic slices. J Neurochem 1989; **53**(6):1925–33.
39 Schaechter JD, Wurtman RJ. Serotonin release varies with brain tryptophan levels. Brain Res 1990; **532**(1–2):203–10.
40 Shubat PJ, Parker D, Huxtable RJ. The effect of suckling and diurnal influences on the concentrations of free amino acids in milk. Proc West Pharmacol Soc 1989; **32**:73–8.
41 Skala JP, Koldovky O, Hahn P. Cyclic nucleotides in breast milk. Am J Clin Nutr 1981; **34**(3): 343–50.
42 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.
43 Takahashi K, Okawa M, Matsumoto M, Mishima K, Yamadera H, Sasaki M, Ishizuka Y. Double-blind test on the efficacy of methylcobalamin on sleep-wake rhythm disorders. Psychiatry Clin Neurosci 1999; **53**(2):211–3.
44 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.
45 Thomas L, Drew JE, Abramovich DR, Willians LM. The role of melatonin in the human fetus. Int J Mol Med 1998; **1** (3):539–43.
46 Viverge D, Grimmonprez L, Cassanas G, Bardet L, Solere M. Diurnal variations and within the feed in lactose and oligosaccharides of human milk. Ann Nutr Metab 1986; **30** (3):196–209.
47 Wurtman RJ, Wurtman JJ, Regan MM, McDermott JM, Tsay RH, Breu JJ. Effects of normal meals rich in carbohydrates or proteins on plasma tryptophan and tyrosine ratios. Am J Clin Nutr 2003; **77**(1):128 –32.
48 Wyatt RJ, Engelman K, Kupfer DJ, Fram DH, Sjoerdsma A, Snyder F. Effects of L-tryptophan (a natural sedative) on human sleep. Lancet 1970; **2**(7678):842–6.
49 Yogman MW, Zeisel SH, Roberts C. Assessing effects of serotonin precursors on newborn behavior. J Psychiatr Res 1982–83; **17**(2):123–33.