Effects of Lactoferrin on Sleep Conditions in Children Aged 12–32 Months: A Preliminary, Randomized, Double-Blind, Placebo-Controlled Trial

This article was published in the following Dove Press journal:
Nature and Science of Sleep

Momoko Miyakawa¹
Shutaro Kubo¹
Hirotugu Oda¹
Noriko Motoki²
Masaru Mizuki²
Teruomi Tsukahara²
Miyuki Tanaka¹
Koji Yamauchi¹
Fumiaki Abe¹
Tetsuo Nomiya²

¹Food Ingredients and Technology Institute, R&D Division, Morinaga Milk Industry Co., Ltd, Zama, Kanagawa, Japan; ²Department of Preventive Medicine and Public Health, Shinshu University School of Medicine, Matsumoto, Nagano, Japan

Purpose: To investigate preliminarily the effect of lactoferrin (LF)-fortified formula on sleep conditions in children.

Study Design: A preliminary, randomized, double-blind, placebo-controlled trial.

Methods: Healthy children between the ages of 12 and 32 months who attended nursery schools in Japan were divided into two groups and assigned a placebo or LF (48 mg/day)-fortified formula. Children's sleep conditions were investigated before and after the 13-week intervention using the Japanese Sleep Questionnaire for Preschoolers (JSQ-P).

Results: Altogether, 109 participants were randomized. Eight participants were eliminated due to lost to follow-up, withdrawal of consent, and ineligibility, with 101 participants (placebo, n = 48; LF, n = 53) included in the full analysis set (FAS) and used for analysis. Wake-up time, bedtime, and nighttime sleep were comparable between the two groups before and after intervention. The change in total JSQ-P T scores tended to improve in the LF group (placebo vs LF: 0.5 ± 6.5 vs -1.9 ± 6.1 , $p = 0.074$), in particular, morning symptoms significantly improved (grumpy in the morning, hard to wake-up, and hard to get out of bed) (placebo vs LF: 0.8 ± 6.2 vs -1.9 ± 6.2 , $p = 0.028$). A better trend was also observed in the LF group regarding restless legs syndrome (RLS)-motor (rubs feet at night and touches feet at night) (placebo vs LF: 2.3 ± 10.7 vs -0.6 ± 13.5 , $p = 0.083$) and insufficient sleep (stays up more than one hour later the day before a holiday and wakes up more than one hour later on a holiday) (placebo vs LF: 0.1 ± 9.8 vs -1.7 ± 8.8 , $p = 0.095$). No adverse drug reactions were found.

Conclusion: LF intake may improve sleep condition, especially morning symptoms in children above one year of age.

Keywords: lactoferrin, sleep, growing-up formula, children

Introduction

Adequate sleep is essential for normal growth and development in children.¹ Pediatric sleep research has shown that insufficient sleep and sleep disturbance have negative impacts on neurobehavioral and cognitive functions, health, and well-being.^{2–4} According to a cross-cultural study, children from Asian countries have significantly later bedtimes and shorter nighttime sleep as compared to those from Caucasian countries, and more parents have considerable concern regarding sleep problems of their child.⁵

Correspondence: Momoko Miyakawa
Food Ingredients and Technology
Institute, R&D Division, Morinaga Milk
Industry Co., Ltd., 1-83, 5-Chome,
Higashihara, Zama-City, Kanagawa-Pref.
252-8583, Japan
Tel +81 46252 3045
Fax +81 46252 3017
Email mo-miyakawa@morinagamilk.co.jp

Lactoferrin (LF) is an 80 kDa iron-binding glycoprotein present in most mammalian milk and has been reported to exhibit various beneficial effects in a wide range of ages. Several benefits of LF have been demonstrated in clinical research involving children, including a protective effect against infection,^{6–9} and improvement of iron metabolism.¹⁰ In an animal study using rats, administration of LF with prebiotics and milk fat globule membranes early in development enhanced non-REM (NREM) sleep and alleviated stress-induced disruption of rapid eye movement (REM) sleep and diurnal rhythms.¹¹ However, the effect of LF on children's sleep has not yet been investigated. Therefore, we preliminarily investigated the effect of LF on sleep conditions as a part of another study, which investigated the impact of an LF-fortified formula on acute gastrointestinal and respiratory symptoms in children aged 12–32 months.⁹

Materials and Methods

Trial Design and Ethical Approval

This was a preliminary randomized (1:1), double-blind, placebo-controlled, parallel-group, comparative trial conducted by the Department of Preventive Medicine and Public Health of Shinshu University School of Medicine in Matsumoto, Japan. The research was conducted in accordance with the current revision of the Declaration of Helsinki¹² and Ethical Guidelines for Medical and Health Research Involving Human Subjects¹³ and the study's protocol and informed consent form were approved by the Institutional Review Board (IRB) of Shinshu University School of Medicine on October 17, 2017 (approval number: 3847). The parents of all child participants provided written informed consent. This study was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan on October 17, 2017 (registration number: UMIN000029685).

Subjects, Eligibility, and Exclusion Criteria

This study was performed between November 27, 2017, and April 1, 2018. Eligible subjects were apparently healthy children between the ages of 12 and 32 months who attended nursery schools in Matsumoto, Japan. Exclusion criteria included possessing allergies to milk or soybeans, ingestion of breast milk, habitual consumption of LF, a history of serious disorders of the liver, kidney, heart, lung, gastrointestinal tract, blood, endocrine system, or metabolic system,

and those otherwise deemed inappropriate by the principal investigator.

Intervention

The participants were randomly assigned to a placebo or LF group. All parents were instructed to give their children one sachet containing either placebo or LF-fortified (48 mg) formula by reconstituting it with water or boiled water cooled to 50°C each day during the 13-week intervention period. Sachets contained powdered formula which was a modified commercial growing-up formula for children aged 12–36 months manufactured by Morinaga Milk Industry Co., Ltd., Japan. The placebo and the LF formulas were identical, with the exception of LF in the LF formula. LF was replaced by dextrin in the placebo formula. Both test formulas were similar in appearance, texture, smell, and packaging. Test formulas were ingested in addition to the usual meal by the child. During the intervention period, parents used diaries to record formula intake and physical changes in children. Formula intake on each day was recorded as 100%, 50%, or 0% of one sachet in the diary. All participants began and completed the administration of the test formula and diary records on the same day. Every 4 weeks, parents were contacted by well-trained public health workers to verify the intake of the test formulas and to ensure diary records were completed.

Sleep Questionnaire

All parents completed the Japanese Sleep Questionnaire for Preschoolers (JSQ-P) survey¹⁴ prior to and after the intervention period. The JSQ-P was developed for Japanese preschool children based on the Children's Sleep Habit Questionnaire¹⁵ which is commonly used in Western countries. The questionnaire consists of 39 items classified into 10 domains according to psychometric conditions, namely: obstructive sleep apnea syndrome, restless legs syndrome (RLS)-sensory, RLS-motor, morning symptoms, sleep habits, parasomnias, insufficient sleep, daytime excessive sleepiness, daytime behaviors, and insomnia or circadian rhythm disorders. Parents answered the questionnaire by rating on a 6-point intensity Likert scale, where a score of 6 referred to strongly agree/true/applicable, and 1 referred to strongly disagree/false/inapplicable. The JSQ-P T scores were calculated in total and for each domain according to the instructions.

Safety Assessment

Any unfavorable or unintended signs, symptoms, or diseases were defined as adverse events. Adverse events were assessed using the Revised National Cancer Institute – Common Toxicity Criteria Version 4.0, and those events, where a causal relationship with the ingestion of the test formula was identified, were designated as adverse drug reactions.

Sample Size

The present study was a part of another study that investigated the effect of an LF-fortified formula on acute gastrointestinal and respiratory symptoms in children. Therefore, sample size was determined referring to the previous study.¹⁶ Based on an estimated incidence of acute illness of 95%, the ability of LF to reduce incidence to 75%, a type 1 error of 0.05, and a power of 80%, we calculated a required sample size of 98 (49 in each group) and set a target sample size of 110 (55 in each group) to account for possible dropouts.

Randomization, Allocation, and Blinding

An independent allocation manager at a different university produced a computer-generated allocation table using the permuted block method (block size of 4 (1:1 ratio)) according to nursery school. The allocation tables were kept sealed in an opaque envelope until key breaking. The investigators, subjects, and public health workers were blind to all information during this period. The investigators enrolled the subjects and assigned them to test formula numbers. Key breaking was performed after locking the database and statistical analysis plan.

Statistical Analysis

Data are expressed as the mean $\pm$ standard deviation. The Mann–Whitney *U*-test was employed to evaluate wake-up time, bedtime, and nighttime sleep. An analysis of covariance adjusted for values at 0 weeks was used to analyze changes in the JSQ-P T scores. Statistical analyses were performed using IBM SPSS Statistics version 25 software (SPSS, Chicago, IL) and *p* values <0.05 were considered statistically significant.

Results

Among 115 participants who provided informed consent, six participants were excluded due to ingestion of breast milk and a total of 109 participants were randomized and allocated the test formula (placebo, *n* = 49; LF, *n* = 60). After

randomization, eight participants were lost: three participants were lost to follow-up with no data available, four withdrew consent, and one was stopped by the principal investigator because of skin eczema and turned out not to satisfy the eligibility. Therefore, the resulting full analysis set (FAS) of data included 101 participants for the primary analysis (placebo, *n* = 48; LF, *n* = 53) (Figure 1). The median (interquartile range) intake rates of the test formula for the placebo and LF group were 84.1% (66.6%, 92.9%), and 75.3% (46.3%, 95.1%), respectively (*p* = 0.265).

Baseline demographics are shown in Table 1. There were no significant differences in the sex, age, body height, weight, weight-for-age, height-for-age, weight-for-height, siblings, number of family members, and frequency of attending nursery schools between the two groups.

Wake-up time, bedtime, and nighttime sleep were comparable between the two groups and maintained during the intervention period (Table 2).

JSQ-P T scores in the LF group showed overall relatively low values (Supplementary Table 1). The change in total JSQ-P T scores tended to improve in the LF group (placebo vs LF: 0.5 ± 6.5 vs -1.9 ± 6.1 , *p* = 0.074) (Table 3). In particular, the change for morning symptoms (grumpy in the morning, hard to wake-up, and hard to get out of bed) showed significant improvement in the LF group compared to the placebo group (placebo vs LF: 0.8 ± 6.2 vs -1.9 ± 6.2 , *p* = 0.028). Moreover, T scores of the LF group showed an improved trend in two further domains as compared to the placebo group; RLS-motor (rubs feet at night and touches feet at night) (placebo vs LF: 2.3 ± 10.7 vs -0.6 ± 13.5 , *p* = 0.083), and insufficient sleep (stays up more than one hour later the day before a holiday and wakes up more than one hour later on a holiday) (placebo vs LF: 0.1 ± 9.8 vs -1.7 ± 8.8 , *p* = 0.095).

Figure 1 Flow diagram of subjects.

Table 1 Baseline Demographics

	Total	Placebo	LF	p
Number	109	49	60	
Male, n (%)	56 (51.4)	28 (57.1)	28 (46.7)	0.337
Age, months	25.8 ± 4.9	25.7 ± 5.2	25.9 ± 4.6	0.973
Body height, cm	84.2 ± 5.0	84.2 ± 5.7	84.1 ± 4.4	0.975
Body weight, kg	11.5 ± 1.4	11.5 ± 1.5	11.6 ± 1.4	0.664
Weight-for-age, z-score	-0.11 ± 0.98	-0.16 ± 1.00	-0.06 ± 0.98	0.697
Height-for-age, z-score	-0.57 ± 1.18	-0.56 ± 1.15	-0.58 ± 1.21	0.793
Weight-for-height, z-score	0.07 ± 0.23	0.05 ± 0.22	0.08 ± 0.23	0.391
Siblings, n	0.87 ± 0.78	0.84 ± 0.80	0.90 ± 0.76	0.576
No. of family members in the household, n	3.07 ± 1.08	2.98 ± 1.05	3.15 ± 1.11	0.401
Frequency of attending nursery schools, times/week	5.06 ± 0.36	5.08 ± 0.28	5.03 ± 0.42	0.658

Note: Values represent the mean ± standard deviation.

Table 2 Sleep Pattern Before and After Intervention

	Placebo	LF	p
Wake-up time (hrs:min)			
Before intervention	6:40 ± 0:27	6:42 ± 0:40	0.892
After intervention	6:42 ± 0:26	6:46 ± 0:34	0.568
Bedtime (hrs:min)			
Before intervention	21:03 ± 0:35	20:57 ± 0:44	0.521
After intervention	21:01 ± 0:32	21:00 ± 0:30	0.960
Nighttime sleep (hrs:min)			
Before intervention	09:37 ± 0:35	09:45 ± 0:36	0.381
After intervention	09:40 ± 0:36	09:46 ± 0:30	0.429

Notes: Values represent the mean ± standard deviation. Nighttime sleep duration was calculated from wake-up time and bedtime.

With respect to sleep setting, all children slept with parents or grandparents in the same room and there was no difference observed between the groups.

Altogether, 24 participants experienced adverse events during the intervention period (13 in the placebo group, 11 in the LF group, $p = 0.357$). Major adverse events were otitis media, eye discharge, asthma, and eczema. No adverse drug reactions were found.

Discussion

The exploratory investigations on sleeping conditions revealed that the administration of LF-fortified growing-

Table 3 Changes in JSQ-P T Scores

Subscales	Placebo	LF	p
Total score	0.5 ± 6.5	-1.9 ± 6.1	0.074
Obstructive sleep apnea syndrome	0.2 ± 6.7	-0.3 ± 6.5	0.575
RLS-sensory	1.6 ± 11.0	0.1 ± 11.0	0.210
RLS-motor	2.3 ± 10.7	-0.6 ± 13.5	0.083
Morning symptoms	0.8 ± 6.2	-1.9 ± 6.2	0.028
Sleep habits	0.1 ± 7.2	-1.5 ± 5.0	0.350
Parasomnias	-0.8 ± 7.8	-1.4 ± 9.0	0.763
Insufficient sleep	0.1 ± 9.8	-1.7 ± 8.8	0.095
Daytime excessive sleepiness	0.1 ± 8.9	-0.3 ± 7.9	0.997
Daytime behaviors	-0.5 ± 8.8	-2.5 ± 10.2	0.439
Insomnia or circadian rhythm disorders	0.4 ± 7.4	-1.3 ± 7.1	0.279

Note: Values represent the mean ± standard deviation.

Abbreviation: RLS, restless legs syndrome.

up formula significantly improved morning symptoms (grumpy in the morning, hard to wake-up, and hard to get out of bed) as assessed by the JSQ-P. LF ingestion also tended to improve RLS-motor (rubs feet at night and touches feet at night) and insufficient sleep (stays up more than one hour later the day before a holiday and wakes up more than one hour later on a holiday). This is the first clinical finding suggesting an amelioration of sleeping conditions in children over the age of one because of LF ingestion.

In this study, the administration of LF improved morning symptoms and somewhat ameliorated insufficient sleep without any changes in wake-up time, bedtime, and nighttime sleep suggesting the possibility that LF improved sleep quality in children.

In an animal study using rats, LF with prebiotics and milk fat globule membranes administered early in development, prolonged NREM episode duration with changes in microbiota and alleviated the impact of stress on sleep and diurnal rhythms.¹¹ The intake of LF modulated microbiota and enhanced NREM sleep, and might have resulted in better sleep conditions.

In this study, among the 10 domains composing JSQ-P, the score in morning symptoms, which included being grumpy in the morning, and having difficulty waking up and getting out of bed, significantly improved in the LF group compared to the placebo group. In a previous clinical study, higher a concentration of cortisol in the morning was linked to LF ingestion.¹⁷ Cortisol plays a role in the regulation of the sleep-wake cycle via diurnal secretion rhythms characterized by an increase upon awakening and a subsequent decline over the course of the day.¹⁸ This process has been demonstrated to be stable in the first year

of life.¹⁹ Considering that low cortisol concentrations in the morning are reported to be associated with high levels of sleepiness upon awakening, and anxiety and exhaustion the day before,²⁰ LF ingestion may improve alertness in the morning via modulation of the sleep-wake cycle.

Some studies have suggested a potential involvement of LF in neural functions. LF promoted early neurodevelopment and cognition in postnatal piglets by upregulating the transcriptional and posttranslational levels of brain-derived neurotrophic factor (BDNF) in the hippocampus and its signaling transduction pathway.²¹ Also, LF has been shown to exert a suppressive effect on psychological distress via an opioid-mediated mechanism involving nitric oxide synthase (NOS) activation in brain.²² While not fully understood, wakefulness and sleep are dynamic physiologic processes regulated through complex neurologic pathways including the brainstem and hypothalamus.¹ Further research is needed to reveal whether LF has an effect on neural pathways and neurotransmitters related to wakefulness and sleep.

Additionally, the LF group exhibited an improved trend on RLS-motor subscales (rubs feet at night and touches feet at night) compared to the placebo group, although no differences were observed in RLS-sensor domains. Iron insufficiency has been reported as one of the causes of RLS,²³ and a clinical study has demonstrated significant improvement in RLS symptoms using iron treatment.²⁴ As LF has been widely shown to improve iron metabolism,^{10,25} an alleviation of iron deficiency through LF ingestion may be associated with the improved trend in RLS-motor observed.

The JSQ-P was originally developed for Japanese preschool children between the ages of 2 and 6 years. In this study, similar trends as the main results in JSQ-P T scores for morning symptoms (placebo vs LF: 0.4 ± 5.5 vs -1.6 ± 5.8), RLS-motor (placebo vs LF: 0.4 ± 8.8 vs -0.8 ± 13.8), and insufficient sleep (placebo vs LF: -1.7 ± 7.8 vs -2.2 ± 9.1) were also observed in children over 24 months which was the target age for JSQ-P. Similar tendency was also true for morning symptoms (placebo vs LF: 1.4 ± 7.2 vs -2.6 ± 7.3), RLS-motor (placebo vs LF: 5.3 ± 12.9 vs 0.0 ± 13.0), and insufficient sleep (placebo vs LF: 3.1 ± 12.2 vs -0.2 ± 8.0) in children less than 24 months. This investigation was conducted at a preliminary level and we adopted JSQ-P by which multiple items on child's sleep conditions could be investigated. LF has a possibility to improve sleep conditions as above in both 1- and 2-year-old children, but further investigation using appropriate method for targets' (children's ages) are needed to verify the efficacy of LF on sleep conditions in children.

In this trial, the effect of LF on children's sleep was investigated by parents' subjective ratings on sleep quality of their child. Although we adopted JSQ-P as a validated and reliable measure to evaluate sleep conditions in children, the opinion of sleep specialists and objective sleep quality measures, such as actinography or electroencephalograph were lacked in this study.

Moreover, sleep habits may also be influenced by environmental and familial factors. Potential factors such as parental affect (depression and anxiety), daytime sleep, food intake other than test product, activities, bedtime routine, wake after sleep onset and use of electronic media devices should also be examined for their effects as background information in such studies.

In addition to the aforementioned limitations, in this study, a beneficial effect of LF on sleep conditions was preliminarily investigated apart from the original purpose. Therefore, the results observed in this exploratory investigation should be validated by further studies on sleep quality as primary endpoints with sufficient sample size.

Conclusion

The intake of LF-fortified growing-up formula improved sleep conditions especially morning symptoms (grumpy in the morning, hard to wake-up, and hard to get out of bed) in children over the age of one. The present study revealed the potential of LF supplementation as a method for improving sleep hygiene, though further investigation is needed to determine the effect of LF on sleep conditions.

Data Sharing Statement

The dataset in the current study is available from the corresponding author on reasonable request.

Acknowledgments

Authors deeply appreciate all individuals involved in this study for their valuable help with data collection: Matsumoto City Medical Association, nursery schools in Matsumoto city (Agata, Azusagawa-higashi, Azusagawa-nishi, Fukashi, Futago, Hata-chuo, Hata-higashi, Hirata, Horigome, Imai, Kanda, Kiri, Kotobuki-higashi, Komiya, Kotobuki, Minami-matsumoto, Mitsuba, Miyata, Murai, Nagisa, Nakajo, Nakayama, Nango, Niimura, Nishikibe, Nobara, Nomizo, Ohisama, Okada, Sakura, Satoyamabe, Shimadachi-chuo, Shimauchi, Shiraita, and Uchida), Mr. Shinji Komatsu and Mr. Keigo Takano of the Matsumoto City Commerce and Industry Tourism Department, and Mr. Katsuya Furihata,

Mr. Akira Shimamura, and the public health nurses at Matsumoto Health Laboratory. We also sincerely thank parents and children for participating in this study.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding

Morinaga Milk Industry provided research funds and the test formulas.

Disclosure

Momoko Miyakawa, Shutaro Kubo, Hirotugu Oda, Miyuki Tanaka, Koji Yamauchi, and Fumiaki Abe were employed by Morinaga Milk Industry Co., Ltd., report a patent 2019-085091 pending, and report no other potential conflicts of interest for this work. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest and report they have no conflicts of interest for this work.

References

- Bathory E, Tomopoulos S. Sleep regulation, physiology and development, sleep duration and patterns, and sleep hygiene in infants, toddlers, and preschool-age children. *Curr Probl Pediatr Adolesc Health Care*. 2017;47(2):29–42. doi:10.1016/j.cppeds.2016.12.001
- Dahl RE. The impact of inadequate sleep on children's daytime cognitive function. *Semin Pediatr Neurol*. 1996;3(1):44–50. doi:10.1016/S1071-9091(96)80028-3
- Corkum P, Tannock R, Moldofsky H. Sleep disturbances in children with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry*. 1998;37(6):637–646. doi:10.1097/00004583-199806000-00014
- Ivanenko A, Johnson K. Sleep disturbances in children with psychiatric disorders. *Semin Pediatr Neurol*. 2008;15(2):70–78. doi:10.1016/j.spen.2008.03.008
- Mindell JA, Sadeh A, Wiegand B, How TH, Goh DYT. Cross-cultural differences in infant and toddler sleep. *Sleep Med*. 2010;11(3):274–280. doi:10.1016/j.sleep.2009.04.012
- Egashira M, Takayanagi T, Moriuchi M, Moriuchi H. Does daily intake of bovine lactoferrin-containing products ameliorate rotaviral gastroenteritis? *Acta Paediatr*. 2007;96(8):1242–1244. doi:10.1111/j.1651-2227.2007.00393.x
- Ochoa TJ, Chea-Woo E, Baiocchi N, et al. Randomized double-blind controlled trial of bovine lactoferrin for prevention of diarrhea in children. *J Pediatr*. 2013;162(2):349–356. doi:10.1016/j.jpeds.2012.07.043
- Chen K, Chai L, Li H, et al. Effect of bovine lactoferrin from iron-fortified formulas on diarrhea and respiratory tract infections of weaned infants in a randomized controlled trial. *Nutrition*. 2016;32(2):222–227. doi:10.1016/j.nut.2015.08.010
- Motoki N, Mizuki M, Tsukahara T, et al. Effects of lactoferrin-fortified formula on acute gastrointestinal symptoms in children aged 12–32 months: a randomized, double-blind, placebo-controlled trial. *Front Pediatr*. 2020;8. doi:10.3389/fped.2020.00233
- Ke C, Lan Z, Hua L, et al. Iron metabolism in infants: influence of bovine lactoferrin from iron-fortified formula. *Nutrition*. 2015;31(2):304–309. doi:10.1016/j.nut.2014.07.006
- Thompson RS, Roller R, Mika A, et al. Dietary prebiotics and bioactive milk fractions improve NREM sleep, enhance REM sleep rebound and attenuate the stress-induced decrease in diurnal temperature and gut microbial alpha diversity. *Front Behav Neurosci*. 2016;10:240. doi:10.3389/fnbeh.2016.00240
- World Medical Association. World Medical Association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191–2194. doi:10.1001/jama.2013.281053
- Sone S. Ethical guidelines for clinical trials in medical research involving human subjects. *Gan to Kagaku Ryoho*. 2015;42(8):893–902.
- Shimizu S, Kato-Nishimura K, Mohri I, et al. Psychometric properties and population-based score distributions of the Japanese sleep questionnaire for preschoolers. *Sleep Med*. 2014;15(4):451–458. doi:10.1016/j.sleep.2013.05.020
- Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. *Sleep*. 2000;23(8):1043–1051. doi:10.1093/sleep/23.8.1d
- Hatakka K, Savilahti E, Pönkä A, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *BMJ*. 2001;322(7298):1327. doi:10.1136/bmj.322.7298.1327
- Oda H, Wakabayashi H, Tanaka M, et al. Effects of lactoferrin on infectious diseases in Japanese summer: a randomized, double-blinded, placebo-controlled trial. *J Microbiol Immunol Infect*. 2020. doi:10.1016/j.jmii.2020.02.010
- Weitzman ED, Fukushima D, Nogeire C, Roffwarg H, Gallagher TF, Hellman L. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J Clin Endocrinol Metab*. 1971;33(1):14–22. doi:10.1210/jcem-33-1-14
- Kiess W, Meidert A, Dressendörfer RA, et al. Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight. *Pediatr Res*. 1995;37(4):502–506. doi:10.1203/00006450-199504000-00020
- Dahlgren A, Kecklund G, Theorell T, Åkerstedt T. Day-to-day variation in saliva cortisol—relation with sleep, stress and self-rated health. *Biol Psychol*. 2009;82(2):149–155. doi:10.1016/j.biopsycho.2009.07.001
- Chen Y, Zheng Z, Zhu X, et al. Lactoferrin promotes early neurodevelopment and cognition in postnatal piglets by upregulating the BDNF signaling pathway and polysialylation. *Mol Neurobiol*. 2015;52(1):256–269. doi:10.1007/s12035-014-8856-9
- Takeuchi T, Hayashida K, Inagaki H, Kuwahara M, Tsubone H, Harada E. Opioid mediated suppressive effect of milk-derived lactoferrin on distress induced by maternal separation in rat pups. *Brain Res*. 2003;979(1):216–224. doi:10.1016/S0006-8993(03)02941-X
- Allen R. Dopamine and iron in the pathophysiology of restless legs syndrome (RLS). *Sleep Med*. 2004;5(4):385–391. doi:10.1016/j.sleep.2004.01.012
- Wang J, O'Reilly B, Venkataraman R, Mysliwiec V, Mysliwiec A. Efficacy of oral iron in patients with restless legs syndrome and a low-normal ferritin: a randomized, double-blind, placebo-controlled study. *Sleep Med*. 2009;10(9):973–975. doi:10.1016/j.sleep.2008.11.003
- Rosa L, Cutone A, Lepanto MS, Paesano R, Valenti P. Lactoferrin: a natural glycoprotein involved in iron and inflammatory homeostasis. *Int J Mol Sci*. 2017;18(9):1985. doi:10.3390/ijms18091985

Nature and Science of Sleep**Dovepress****Publish your work in this journal**

Nature and Science of Sleep is an international, peer-reviewed, open access journal covering all aspects of sleep science and sleep medicine, including the neurophysiology and functions of sleep, the genetics of sleep, sleep and society, biological rhythms, dreaming, sleep disorders and therapy, and strategies to optimize healthy sleep.

The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/nature-and-science-of-sleep-journal>