Clinics in Mother and Child Health
ISSN: 2090-7214

Bar-Yoseph et al., Clinics Mother Child Health
2017, 14:2
DOI: 10.4172/2090-7214.1000263

Research Article OMICS International

SN2-Palmitate Improves Crying and Sleep in Infants Fed Formula with Prebiotics: A Double-Blind Randomized Clinical Trial

Fabiana Bar-Yoseph[1*], Yael Lifshitz[1], Tzafra Cohen[1], Patrice Malard[2], Zailing Li[3], Hong Cui[4], Aimin Zhang[5], Jing-Lan Wu[6] and Chundi Xu[6]

[1]*Nutrition R&D, Enzymotec Ltd., Migdal HaEmeq, Israel*

[2]*R&D, Biostime, Guangzhou, China*

[3]*Department of Pediatrics, Peking University Third Hospital, Beijing, China*

[4]*Pediatric Ward, Beijing Friendship Hospital Affiliated with Capital University of Medical Science, Beijing, China*

[5]*Department of Pediatrics, The People's Hospital of Hunan Province, Changsha, China*

[6]*Department of Pediatrics, Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China*

[*]**Corresponding author:** Fabiana Bar-Yoseph, Nutrition R&D, Enzymotec Ltd., Migdal Haemeq, Israel, Tel: +972747177177; E-mail: fabiana@enzymotec.com

Received date: June 02, 2017; **Accepted date:** June 15, 2017; **Published date:** June 25, 2017

Abstract

Background and Aim: Palmitic acid (PA, C16:0), one of the major saturated fatty acids in human milk fat being 17-25% of the fatty acids, is esterified mainly at the SN2-position (SN2-palmitate). Contrary in vegetable oils, which are commonly used as fat source in infant formulas, PA is esterified mainly at the outer positions, i.e, SN1 and SN3 positions, resulting in reduced fat absorption and harder stools. SN2-palmitate and prebiotics have been shown to improve digestion and reduce stool hardness. Our aim was to study the potential effects of SN2-palmitate in addition to prebiotics in formula-fed Chinese infants.

Methods: 171 healthy term infants were included (within 14 days from birth) in the study. Formula-fed infants were randomly assigned to receive either SN2-palmitate containing formula (INFAT®, Advanced Lipids), (n=57) or a Control formula (n=57). The two study formulas (Biostime, China) differed only in the ratio of PA at the SN2-position (43% *vs.* 13%). A similar group of breastfed infants (n=57) was included as a reference.

Results: The pattern of crying and sleep differed between the formula-fed groups. Fewer infants in the SN2 group cried at 12 weeks (23.2% *vs.* 45.5%, $p<0.05$); they had fewer crying episodes (2.0 *vs.* 3.6, at 6 weeks, $p<0.05$ and 1.0 *vs.* 2.2 at 12 weeks, $p<0.02$) and the duration of crying was lower (25.1 *vs.* 41.3 min at 6 weeks, $p<0.05$ and 11.2 *vs.* 21.2 min at 12 weeks, $p<0.01$) similar to the crying pattern of breastfed infants. Moreover, the infants in the SN2 group had longer daily sleep duration.

Conclusions: SN2-palmitate formula improves crying and sleeps patterns in addition to prebiotics in the first weeks of life. Thereby, SN2-palmitate improves the well-being of formula-fed infants and consequently the quality of life of their parents, further emphasizing the importance of SN2-palmitate for infant nutrition.

Keywords: Sn2-palmitate; Palmitic acid; Prebiotics; Infant formula; Crying; Sleep

Abbreviation:

PA: Palmitic Acid; BF: Breastfed; TG: Triglycerides; GOS: Galacto Oligo Saccharides.

Introduction

Infant sleep and excessive crying are of major parental concern [1,2]. Infant crying is usually believed to be related to general or abdominal discomfort [3], disease, hunger, temperament, etc. The biological nature of excessive infant crying is debated [4]. Most infants follow a universal crying pattern during the first few months of life, in which crying peaks at 6 weeks and then declines until 3 months of age [1,5-9] with a typical diurnal pattern wherein ~40% of crying occurs during afternoon-evening hours. The regulation of crying develops with the circadian rhythm and this coincidence suggests that excessive crying in infancy may be associated with disturbances in the developing sleep structure or sleep-wake rhythm [10].

SN2-palmitate structured triglycerides and oligosaccharides, are bioactive ingredients used in infant formulas. Clinical studies suggest SN2-palmitate triglycerides have beneficial effects on fatty acids and calcium absorption [11-16], infant bone strength [17], intestinal flora [18] and reduced crying [19] (for review see [20]). Other studies suggest oligosaccharides benefit neonatal digestion, intestinal development, including protection against infection and nutrient absorption [21] and crying reduction in formula-fed colicky infants [22]. This study aimed to examine the effect of SN2-palmitate on infant comfort and fat absorption in Chinese healthy term infants fed infant formulas that already include prebiotics.

Materials and Methods

Study design and participants

As described earlier [11], 171 healthy term infants were enrolled to this multi-centre, randomized double-blind study at five clinical centers located in four cities in China (Beijing, Shanghai, Changsha and Chengdu) between June 2011 and April 2012. Inclusion criteria were <14 days of age, gestational age at birth 37-42 week, birth weight 2,500-4,500 g. Infants in the formula groups were exclusively formula-fed at inclusion, and infants in the breastfed group were exclusively breast-fed at inclusion. Formula fed infants were randomly assigned to receive an infant formula with SN2-palmitate (INFAT, Advanced Lipids AB, the SN2-palmitate group), in which 43% of the PA was esterified to the SN2 position of the glycerol backbone, or an infant formula containing a standard vegetable oil mixture, in which 13% of the PA was esterified to the SN2 position of the glycerol backbone (the control group). The study formulas (Biostime, Guangzhou, China) were produced as described elsewhere [11] and differed primarily in their FA structural distributions. This study was conducted according to the principles of the Declaration of Helsinki and good clinical practices. The protocol was approved by the Ethics Committees of each medical center, and parents gave written informed consent prior to inclusion.

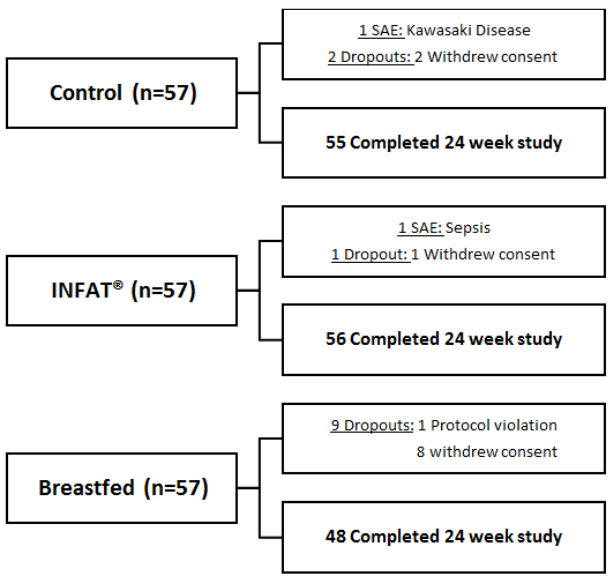

Figure 1: Study participant flow diagram.

Parents' questionnaires

Parents used a 24 h behaviour diaries for 3-5 days before each visit to report: (1) the formula volume consumption of each feeding during 3 days, (2) stool characteristics, including colour (yellow, green, brown, and black), frequency and consistency (hard, hard-formed, soft-formed, soft, or watery) of each stool passed during 5 days, (3) all crying episodes of more than 5 min during 3 days and (4) all sleep episodes during 3 days.

Statistical analysis

The main aim of the study was comparing the formula fed groups, thus the statistical analysis was performed between those two groups. Breastfed infants served as the reference group; therefore, all parameters were additionally compared to this group.

The crying was assessed using three outcomes: whether the infant cried for more than 5 min per day, the daily crying duration and the number of crying episodes. The crying was pairwise compared with categorical crying outcome using Pearson chi-square test over the visit. The pairwise analyses of crying duration and number of crying episodes were performed using a generalized linear model with Negative binomial distribution and log link. This analysis was conducted separately for each visit and time of day (00:00-06:00, 06:00-12:00, 12:00-18:00, and 18:00-00:00). At age of 24 weeks, crying events were rare, and the model was not reliable; therefore, the data is not presented. Finally, to examine extreme crying, category outcome variables were defined; 1-no recorded crying (for more than 5 min), 2-Crying <75% percentile of crying duration, and 3-crying >75% percentile of crying duration. The categorical outcome was analysed in an ordinal regression using generalized estimating equations with multinomial distribution and cumulative-log link.

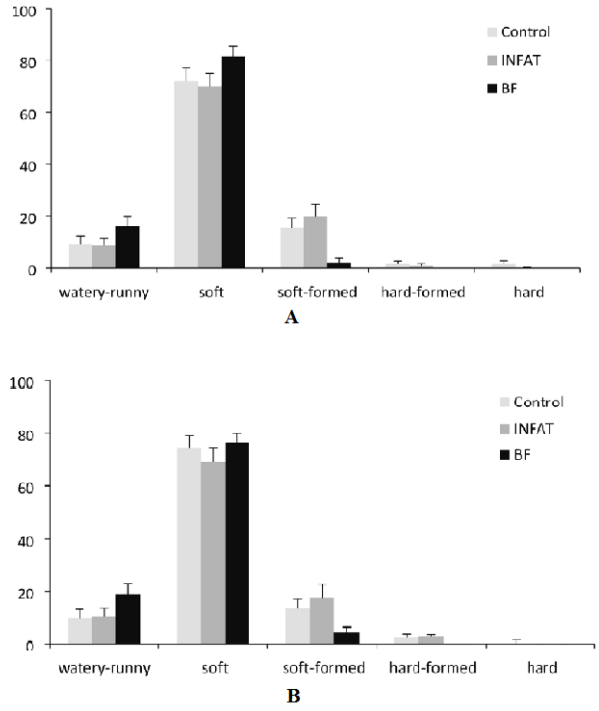

Figure 2: Stool characteristics as reported during each period at 6 (upper panel) and 12 weeks postnatal (lower panel). Data are percentage of infants with each stool category.

Repeated measures analysis of variance (RM-ANOVA) was used to model the group effect on sleep parameters. In this analysis, the within effect was analysed at the 6 and 12-week time points and the between effect was analysed with the formula. Time*group interaction was also

included in the model. In all the above analyses, $p<0.05$ was considered statistically significant.

Results

Of 171 included infants, 111 formulas fed and 48 breastfed infants (93%) remained in the study by the end of the intervention period (Figure 1). No significant differences were observed in growth and formula consumption between the groups [11].

Stool characteristics

Stool characteristics were evaluated by calculating frequency (mean daily number of stools) and consistency score (1=hard, 2=hard-formed, 3=soft-formed, 4=soft, 5=watery).

The BF infants had significantly higher stool frequency and consistency scores compared to the formula fed infants at 6 weeks postnatal and 12 weeks postnatal. No significant differences in stool frequencies or consistencies were observed between the two formula groups. At 6 and 12 weeks, no hard stools were reported for any infant in the study (Figure 2A and B).

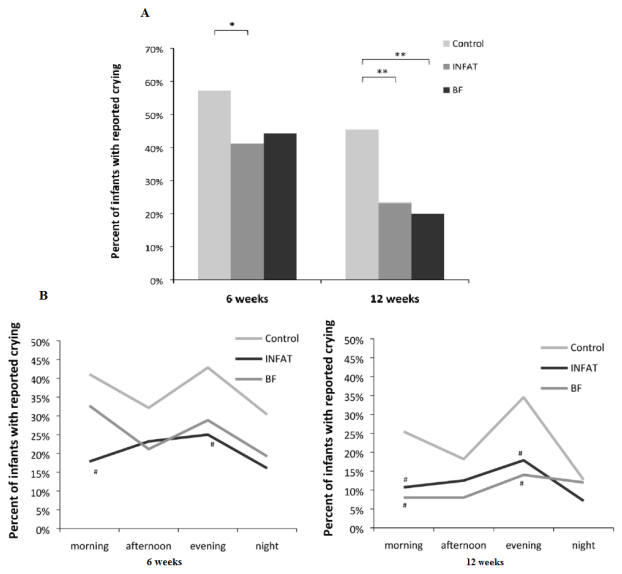

Figure 3: Percentage of infants with crying reported during each period of the day and at 6 (upper panel) and 12 weeks postnatal (lower panel). Data are percentage of infants with reported crying for each 6 hour period of day (morning 06:00-11:59, afternoon 12:00-17:59, evening 18:00-23:59, night 00:00-05:59). $^{**}p<0.05$, $^{*}p<0.1$, $^{\#}$significantly different from control, $p<0.05$.

Crying characteristics

The daily crying frequency and duration were calculated for weeks 6 and 12. The 24 h were divided into 4 parts, each 6 h, in which morning=06:00-12:00, afternoon=12:00-18:00, evening=18:00-00:00, and night=00:00-06:00. The pattern of crying was analysed by calculating the percentage of crying infants and the total duration of crying per part of the day. Analysing the difference in the number of crying babies, the frequency and duration, revealed the superiority of the sn2-palmitate and breastfeeding groups compared to the control group.

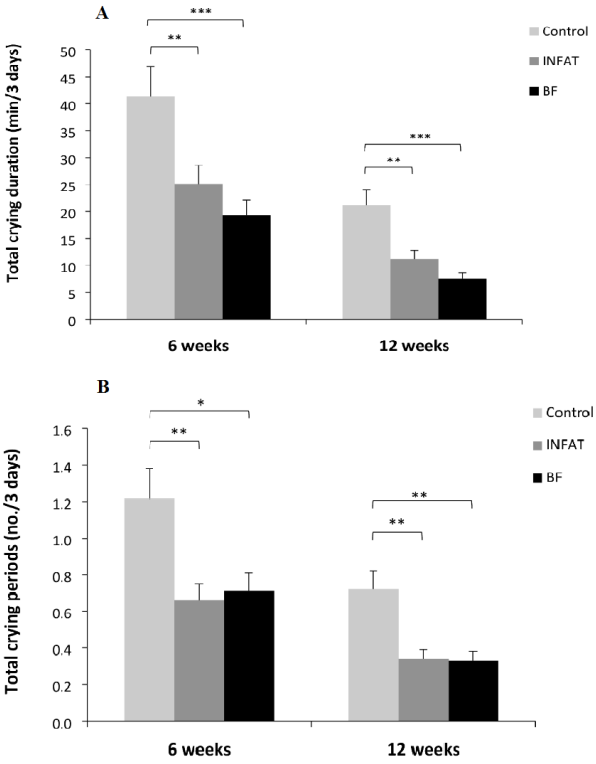

Figure 4: Crying at 6 and 12 weeks. Parents completed 3 diaries in which each crying period more than 5 min was recorded. The A panel represents the total crying duration, and the B panel represents the number of crying episodes. Data are presented as the means ± standard error, $^{***}p<0.01$, $^{**}<0.05$, $^{*}p<0.1$.

Fewer infants in the sn2-palmitate and breastfed groups cried compared to the control group at 12 weeks (23.2% and 20% *vs.* 45.5%, respectively, $p<0.05$) (Figure 3A), mainly during evening hours (Figure 3B). The duration of crying in a period of 3 days (72 h) in the sn2-palmitate group and breastfed groups was lower compared that in the control group (25.1 and 19.4 *vs.* 41.3 min at 6 weeks, $p<0.05$ and 11.2 and 7.5 *vs.* 21.2 min at 12 weeks for sn2-palmitate, BF and control groups, respectively, $p<0.01$) (Figure 4A). The crying frequency was also lower for the sn2-palmitate and breastfed groups compared to that in the control group (2.0 and 2.1 *vs.* 3.6 episodes at 6 weeks, and 1.0 and 1.0 *vs.* 2.2 episodes at 12 weeks respectively, $p<0.05$), (Figure 4B). The crying patterns the control group was significantly different from that of the SN2-palmitate and the BF groups (Figure 5).

Sleep characteristics

The mean daily number of sleep periods, sleep duration, and the pattern of sleep during 24 h and night hours only (20:00-06:00) were calculated. Analysing the difference between the three groups by repeated measures at 6 and 12 weeks, revealed an advantage for the sn2-palmitate group (Figure 6), which demonstrated higher sleep duration during 24 h and during night hours compared to the control

and BF groups (15.4 *vs.* 14.5 and 13.6 h at 6 weeks and 14.7 *vs.* 13.7 and 14.2 h at 12 weeks, respectively, p<0.05).

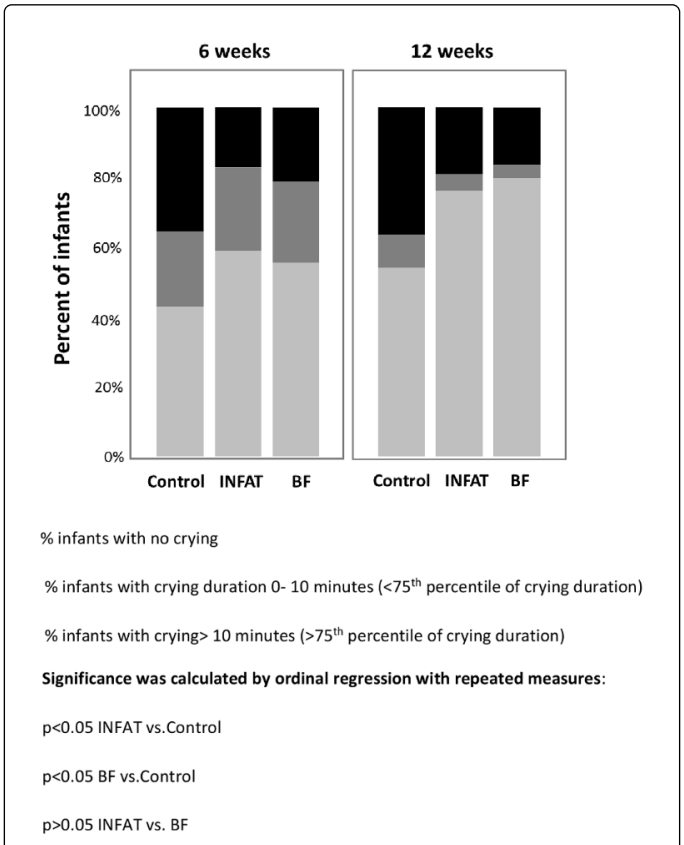

Figure 5: Crying patterns at 6 and 12 weeks. Ordinal regression on the category outcome variables defined for each visit; 1-no recorded crying (for more than 5 min), 2-Crying <75% percentile of crying duration and 3-crying >75% percentile of crying duration.

A difference was also observed in the duration of sleep during night hours between the sn2-palmitate and control groups (6.9 *vs.* 6.5 h at 6 weeks and 7.5 *vs.* 6.9 h at 12 weeks, respectively, p=0.078). No significant difference in night sleep duration was observed between the BF and formula-fed groups. No significant differences in the sleep patterns between groups were observed at 24 weeks postnatal.

Discussion

This is the first randomized controlled study to investigate the effect of a formula containing SN2-palmitate in Chinese babies and demonstrate that adding SN2-palmitate to a formula already containing prebiotics has an additive effect on baby comfort. This study showed that term infants who consumed formula with high sn2-palmitate cried significantly less than infants who consumed the control formula with the same level of palmitic acid but in a different chemical position.

While these results are in contrast to Kennedy et al. [14], who did not find differences in crying between the beta-palmitate and the control groups but also did not mention the method of crying assessment, they further strengthen Litmanovitz et al. [19] who described a reduction in crying by sn2-palmitate. Contrary to Litamanovitz et al. where the reduction of crying was observed with reduction in stool hardness, no hard stools were observed in this study probably due to the presence of oligosaccharides in both study formulas. Though the crying reduction cannot be attributed to stool characteristics, the effect on the comfort should not be excluded.

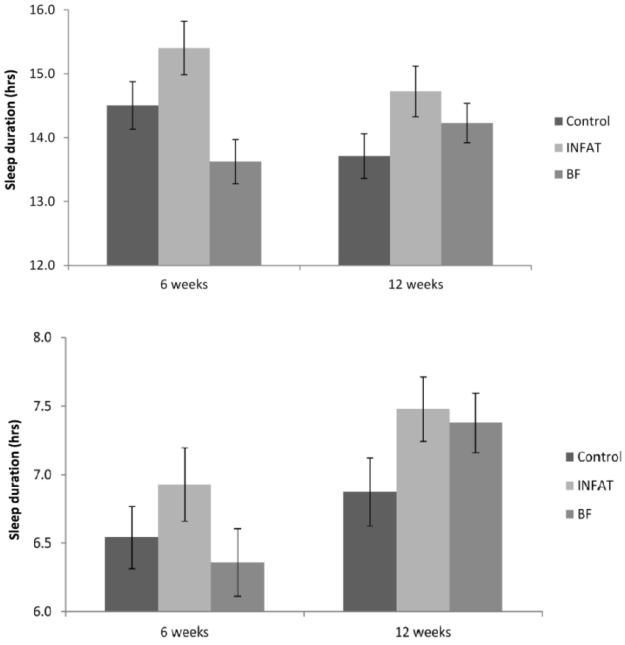

Figure 6: Sleep duration at 6 and 12 weeks postnatal. Parents completed 3 diaries in which each sleep period was recorded. Data are presented as the means ± standard error.

Similar to Litmanovitz et al. [19] and Harrison et al. [23], our study showed a pattern in which the crying peaked at age 6 weeks and then decreased at 12 weeks, probably a result of general maturation.

The current study demonstrates that this effect of reducing crying is persistent even if the infant formula contains prebiotics that were shown to reduce infant crying and are considered an important component in infant formulas in recent years, with potential beneficial effects on neonatal intestinal development, including protection against infection and facilitation of nutrient absorption as well as reduced crying [22] and improved infant comfort [21].

Similar to others reports [19,24,25], a peak in the number of crying infants was observed during the evening hours in all three groups when both breastfed and formula-fed infants exhibit their most distressed behaviour [4]; however, fewer infants in the sn2-palmitate group cried during those hours compared to the two other groups.

Interestingly, when comparing to Litmanovitz et al. [19] report, although the same ingredient was examined with a similar protocol, in this study, less infants cried. Part of the difference can be attributed to the presence of oligosaccharides in both study formulas, but this difference cannot be the only reason, as this dissimilarity is also observed in breastfed infants. Thus, another basis can be a cultural difference as suggested by Lee et al. [26], who reported shorter crying duration in a study in South Korea compared with Western

counterparts and by Zhong et al., [27] who reported lower crying in Chinese infants. Lee related this observation to the caretaking behaviour of Korean mothers, where there is longer contact between the mother and baby and co-sleeping is more common. Another interesting difference, which might be related to culture, is the relatively lower dropout rate in this study compared to our previous studies [17-19], but similar to other published clinical infant studies in the East Asia [28].

The effect of reduced crying is interesting, and the mechanism of action underlying the observed effects can be due to easy digestion and better comfort at a neurobiological/neuroendocrine basis. Additional studies are required to reveal the possible mechanism of action.

Another important finding of our study is that the infants in the sn2-palmitate group had longer daily sleep duration compared with the control and with breastfed infants until 12 weeks postnatal. Importantly, although differences are observed, the durations reported are within the normal range for infants [29]. Interestingly, whereas the total sleep duration per 24 h decreased with time in the formula-fed groups, it was increased in the breastfed group, especially between the ages of 6 to 12 weeks, and the differences between the groups were smaller. We observed in our study that the mean sleep episode increased with time. Furthermore, sleep duration during night hours increased as part of the process of sleep maturation and development of the circadian rhythm of new-borns, and a step to consolidated nocturnal sleep thereafter [30], which is a highly important developmental milestone in infancy.

Since sleep is one of the major parenteral concerns, improvement in infant sleep quality is of interest. Though few studies have examined the possible relationship between the type of feeding and infant sleep, to the best of our knowledge, there is no published study examining the effect of SN2-palmitate on infants' sleep cycle. Lee et al. [26] observed that the total sleep duration in the breast-fed group was longer, although individual sleep episodes were shorter compared to formula-fed infants at age 2-4 months. Cohen Engler et al. [31] reported a trend towards longer nocturnal sleep in breast-fed infants with a more fragmented sleep compared to formula-fed infants. They attributed this pattern to the presence of melatonin in breast milk affecting the circadian pattern. Steinberg et al. [32] and Yogman et al. [33] demonstrated a beneficial effect of tryptophan supplementation in infant formulas on sleep latency.

Although our study has some limitations since data was based on parental reports, it was a multicentre, randomized, double-blind study (for the formula fed groups) and thus represents a heterogenic population that differs in culture and socioeconomic status.

Thus, we believe our results indicate SN2-palmitate is associated with less crying and better sleeping. These findings emphasize the importance of fat structure in infant formulas and suggest that part of the effect of human milk on infant comfort may reside in the lipid structure of human milk, although more research is needed to fully understand the mechanism underlying these effects.

These results represent the effect of structured lipids that are closer to human milk fat composition and emphasize the importance of fat structure in infant formulas. Our study indicates that high beta-palmitate (SN2-palmitate) formulas affects infant crying and sleep patterns during the first weeks of life. High SN2-palmitate reduced crying duration, primarily during the evening hours, and improved sleep duration, thereby improving the well-being of formula-fed infants and their parents.

Acknowledgements

We thank Prof. Raanan Shamir from the Institute of Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Center of Israel for scientific support.

We thank Diklah Geva from IntegriStat, Biostatistics Services, Tel Aviv, for the statistical support.

We also thank American Journal Experts for English editing.

Financial Support

This study was sponsored by Enzymotec Ltd. and study formulas were provided by Biostime.

Conflict of Interest

Fabiana Bar-Yoseph, Yael Lifshitz and Tzafra Cohen are Enzymotec employees, and Patrice Malard is a Biostime employee, and therefore, they declare a relevant financial interest in this work. All other authors declare no competing interest.

Authors employed by the sponsors contributed to study design, data analysis, interpretation of findings, and preparation of the manuscript.

References

1. Brazelton TB (1962) Crying in infancy. Pediatrics 29: 579-588.
2. Sadeh A, Andres TF (1993) Infant sleep problems: Origins, assessment, interventions. Inf Mental Hlth J 14.
3. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, et al. (2006) Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology 130: 1527-1537.
4. Lucas A, St James-Roberts I (1998) Crying, fussing and colic behaviour in breast-and bottle-fed infants. Early Hum Dev 53: 9-18.
5. Baildam EM, Hillier VF, Ward BS, Bannister RP, Bamford FN, et al. (1995) Duration and pattern of crying in the first year of life. Dev Med Child Neurol 37: 345-353.
6. Barr RG, Kramer MS, Pless IB, Boisjoly C, Leduc D (1989) Feeding and temperament as determinants of early infant crying/fussing behavior. Pediatrics 84: 514-521.
7. Evanoo G (2007) Infant crying: a clinical conundrum. J Pediatr Health Care 21: 333-338.
8. St James-Roberts I, Halil T (1991) Infant crying patterns in the first year: Normal community and clinical findings. J Child Psychol Psychiatry 32: 951-68.
9. St James-Roberts I, Hurry J, Bowyer J (1993) Objective confirmation of crying durations in infants referred for excessive crying. Arch Dis Child 68: 82-84.
10. Kirjavainen J, Lehtonen L, Kirjavainen T, Kero P; 24-Hour Ambulatory Sleep Polygraphy study (2004) Sleep of excessively crying infants: a 24-Hour Ambulatory Sleep Polygraphy study. Pediatrics 114: 592-600.
11. Bar-Yoseph F, Lifshitz Y, Cohen T, Malard P, Xu C (2016) Sn2-palmitate reduces fatty acid excretion in chinese formula-fed infants. J Pediatr Gastr Nutr 62: 341-347.
12. Carnielli VP, Luijendijk IH, van Goudoever JB, Sulkers EJ, Boerlage AA, et al. (1995) Feeding premature newborn infants palmitic acid in amounts and stereoisomeric position similar to that of human milk: effects on fat and mineral balance. Am J Clin Nutr 61: 1037-1042.
13. Carnielli VP, Luijendijk IH, Van Goudoever JB, Sulkers EJ, Boerlage AA, et al. (1996) Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance. J Pediatr Gastroenterol Nutr 23: 553-560.

14. Kennedy K, Fewtrell MS, Morley R, Abbott R, Quinlan PT, et al. (1999) Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization. Am J Clin Nutr 70: 920-927.
15. López-López A, Castellote-Bargalló AI, Campoy-Folgoso C, Rivero-Urgël M, Tormo-Carnicé R, et al. (2001) The influence of dietary palmitic acid triacylglyceride position on the fatty acid, calcium and magnesium contents of at term newborn faeces. Early Hum Dev 65: 83-94.
16. Lucas A, Quinlan P, Abrams S, Ryan S, Meah S, et al. (1997) Randomised controlled trial of a synthetic triglyceride milk formula for preterm infants. Arch Dis Child Fetal Neonatal Ed 77: 178-184.
17. Litmanovitz I, Davidson K, Eliakim A, Regev RH, Dolfin T, et al. (2013) High beta-palmitate formula and bone strength in term infants: A randomized, double-blind, controlled trial. Calcified Tissue Int 92: 35-41.
18. Yaron S, Shachar D, Abramas L, Riskin A, Bader D, et al. (2013) Effect of high beta-palmitate content in infant formula on the intestinal microbiota of term infants. J Pediatr Gastr Nutr 56: 376-381.
19. Litmanovitz I, Bar-Yoseph F, Lifshitz Y, Davidson K, Eliakim A, et al. (2014) Reduced crying in term infants fed high beta-palmitate formula: A double-blind randomized clinical trial. BMC Pediatrics 14: 152.
20. Bar-Yoseph F, Lifshitz Y, Cohen T (2013) Review of sn-2 palmitate oil implications for infant health. Prostaglandins Leukot Essent Fatty Acids 89: 139-143.
21. Calder PC, Krauss-Etschmann S, de Jong EC, Dupont C, Frick JS, et al. (2006) Early nutrition and immunity - progress and perspectives. Br J Nutr 96: 774-790.
22. Savino F, Palumeri E, Castagno E, Cresi F, Dalmasso P, et al. (2006) Reduction of crying episodes owing to infantile colic: A randomized controlled study on the efficacy of a new infant formula. Eur J Clin Nutr 60: 1304-1310.
23. Newman JD (2007) Neural circuits underlying crying and cry responding in mammals. Behav Brain Res 182: 155-165.
24. James-Roberts IS, Conroy S, Hurry J (1997) Links between infant crying and sleep-waking at six weeks of age. Early Hum Dev 48: 143-152.
25. St James-Roberts I (1991) Persistent infant crying. Arch Dis Child 66: 653-655.
26. Lee K (2000) Crying and behavior pattern in breast- and formula-fed infants. Early Hum Dev 58: 133-140.
27. Zhong W, Tang X, Hou H, Levi L, Lifshitz Y, et al. (2016) Tolerance and efficacy of infant formula with high sn-2 palmitate in formula-fed chinese term infants: An open label, controlled trial. J Nutr Health Food Eng 4.
28. Yao M, Lien EL, Capeding MR, Fitzgerald M, Ramanujam K, et al. (2014) Effects of term infant formulas containing high sn-2 palmitate with and without oligofructose on stool composition, stool characteristics, and bifidogenicity. J Pediatr Gastroenterol Nutr 59: 440-448.
29. Galland BC, Taylor BJ, Elder DE, Herbison P (2012) Normal sleep patterns in infants and children: A systematic review of observational studies. Sleep Med Rev 16: 213-222.
30. Rosen LA (2008) Infant sleep and feeding. J Obstet Gynecol Neonatal Nurs 37: 706-714.
31. Engler AC, Hadash A, Shehadeh N, Pillar G (2012) Breastfeeding may improve nocturnal sleep and reduce infantile colic: Potential role of breast milk melatonin. Eur J Pediatr 171: 729-732.
32. Steinberg LA, O'Connell NC, Hatch TF, Picciano MF, Birch LL (1992) Tryptophan intake influences infants' sleep latency. J Nutr 122: 1781-1791.
33. Yogman MW, Zeisel SH (1983) Diet and sleep patterns in newborn infants. N Engl J Med 309: 1147-1149.