

Immune response to nucleotide-supplemented infant formulae: systematic review and meta-analysis

Pedro Gutiérrez-Castrellón, Ignacio Mora-Magaña, Luisa Díaz-García, Carlos Jiménez-Gutiérrez, Jaime Ramirez-Mayans and Guillermo A Solomon-Santibáñez*

Paediatric Health Evidence Analysis Centre, National Institute, Mexico

Human milk is recommended as the only alimentary source for the first six months of life. Additionally there is a medical and social need for safe and effective alternative forms of nutrition for infants who cannot be fed with breast milk. Recently the safety and efficacy of some ingredients in infant formulae, such as nucleotides have been discussed. This systematic review analyzed the available evidence to establish the efficacy, safety and dose-response effect of ribonucleotide-supplemented infant formulae (RSIFs). Randomised controlled clinical trials (RCTs) comparing RSIFs to formulae without nucleotides or breast milk were considered in this review. Outcome measures were: antibody titres to common paediatric vaccinations, total lymphocytes, lymphocyte subclasses and NK-cells, episodes of diarrhoea and acute respiratory infection. Publication quality was determined using Jadad and CONSORT guidelines. Results were combined using a random effects model and reported through standardised mean differences (WMD) or risk ratio (RR). Systematic review and meta-analysis showed that RSIFs were associated with a better antibody response to immunisation with *Haemophilus influenzae* vaccine [SMD 1.74 (99%CI 1.43–2.05), $P = 0.001$], diphtheria toxoid [SMD 0.94 (0.75–1.12), $P = 0.001$], oral polio vaccine [SMD 0.73 (0.51–0.95), $P = 0.001$], and fewer episodes of diarrhoea [RR 0.67 (0.58–0.76), $P = 0.02$]. We did not find a major risk of upper respiratory infections [RR 1.11 (0.90–1.36), $P = 0.50$]. Available evidence suggests a positive benefit of RSIFs on infant health without any risk. These benefits begin with nucleotide addition of 1.9 mg/418.4 kJ and are maintained or increased with 10.78 mg/418.4 kJ.

Nucleotides: Infant formulae: Meta-analysis

Background

Human milk is considered the gold standard of paediatric feeding during the first six months of life. However it is known there is a medical and social need to provide safe and effective alternative forms of nutrition for infants who cannot be fed with breast milk. As our knowledge and understanding of infant nutrition and physiology has increased, and formulae development technologies have become more sophisticated, recent efforts have focused on improving growth and development parameters in areas such as the immune, gastrointestinal or nervous systems. Nucleotides and nucleosides have an indispensable role in the structure of DNA and RNA. Various forms of ribonucleic acid found in cells and biological fluids are required intermediaries in energy metabolism, glycoconjugate synthesis and signal transduction, among other functions¹. Additionally, nucleotides have been identified as conditionally essential nutrients, since their *de novo* presence in rapidly growing tissues is both a functional and structural requirement for proper cell function. Animal studies show that the addition of nucleotides to experimental diets improves intestinal function and reduces the risk of *Candida albicans* and *Staphylococcus aureus* infections through modulation of the immune response^{2–4}. These animal studies supported the supplementation of milk-based infant formulae with ribonucleotides. Initially, supplementation was based on the “apparent” concentrations of nucleotides in

breast milk (determined with analytical methods available at the time) and ranged from 12 to 33 mg/l (1.9 to 4.9 mg/418.4 kJ). Studies conducted with formulae supplemented at these levels showed statistically significant effects on weight gain, decrease in the risk of diarrhoea and improvement in certain immunological parameters^{5–10}. In 1995, Leach et al.¹¹ pre-treated human milk samples by hydrolyzing the polymeric forms of ribonucleotides and ribonucleic acid adducts present in the sample to the corresponding ribonucleosides. The released total potentially available ribonucleoside content was subsequently quantified via HPLC. By conducting this analysis it became apparent that the biologically relevant concentrations of Total Potentially Available Nucleosides (TPAN) in human milk were higher than previously thought, at levels of 72 ± 24 mg/l (10.78 mg/418.4 kJ)¹¹. Studies carried out with formulae supplemented with nucleosides at these TPAN levels showed similar results to those reported in previous publications regarding reduction of diarrhoea episodes but demonstrated higher antibody titres after vaccination^{12–15,19–21}. No significant adverse events were observed in these studies. In November 2004 the Codex Committee on Nutrition and Foods for Special Delivery Uses (CCNFSDU), asked the Federation of International Societies of Paediatric Gastroenterology Hepatology and Nutrition (FISPGHAN) and the European Society (ESPHGAN) to join an International Expert Group (IEG) in the area of nutrition to discuss the optimum content of nutrients

*Corresponding author: Guillermo A. Solomon Santibáñez, fax +5255 1084 0906, email gsantibanez@salud.gob.mx

in infant formulae including ribonucleotide levels¹⁶. The aim of this study is to systematically review the available evidence pertaining to formulae supplementation with ribonucleotides with the purpose of determining if the data support notions and positions on the biological activity and safety of these nutrients.

Objective

To review the available evidence to establish the efficacy, safety and dose-response effect of ribonucleotide-supplemented infant formulae.

Criteria for considering studies for this review

Types of studies. Only randomised controlled clinical trials (RCTs) comparing infant formulae containing ribonucleotides with formulae without nucleotides or breast milk were considered in this review.

Types of participants. Healthy children under 2 years of age, fed with formulae containing ribonucleotides vs. formulae without ribonucleotides or breast milk for at least one month. Studies with formulae that contained additional novel ingredients, other than nucleotides, were not included in this review.

Type of intervention. Groups of infants fed formulae containing nucleotides compared with groups fed with identical formulae without nucleotides, or groups fed with breast milk.

Outcome measures. The relevant outcome measures were humoral immune response (antibody titres) to common paediatric vaccinations, cellular immune response (total lymphocytes and lymphocyte subclasses and NK-cells), episodes of diarrhoea, and acute respiratory infections.

Search methods for identification of studies

A highly sensitive search strategy for the identification of controlled clinical trials¹⁸ was used, in addition to using keywords (nucleotides OR nucleosides OR ribonucleotides OR ribonucleosides) AND [nutrition OR formulae]), limiting subjects to infants

0 to 24 months old. No limits for language or country of publication were set. The following databases were searched: PubMed (1966 to October 2005), Embase (1988 to October 2005), LILACS (1990 to October 2005), ARTEMISA (review of the 9th edition to December 2004), Cochrane Controlled Trials Register, Bandolier and DARE. Direct contact with the lead authors of detected publications was established to increase the chances of identifying sources in the so-called 'grey literature'.

Methods of the review

Four evaluators working together extracted relevant data and carried out the methodological quality assessment for each of the studies. Disagreements were resolved by consensus. Standardised methods described by the Ibero American Cochrane Collaborator for preparing protocols were used, applying inclusion criteria, assessing the quality of publications and extracting information. Publication quality was determined using a point system described by Jadad using the CONSORT status as a guideline¹⁹. When information was deemed qualitatively and quantitatively relevant for the study, it was determined whether or not bio-statistical homogeneity criteria were met for inclusion in the study. Risk ratio for binary results and standardized mean differences for continuous findings were calculated. Results were combined using a model of random effects based on the weighted average of the results with proportional weights inverse to variance. For all estimates, a 99% confidence interval (CI) was calculated. As necessary a heterogeneity test using Pearson's chi square test was performed with a *p* value of < 0.05 indicating significance and the possibility of publication bias was assessed using a funnel plot.

Results

Quantitative Synthesis of the Results

Fifteen studies were considered suitable for inclusion in the analysis^{6-10,12-15,19-24}. A total of six studies were excluded

Table 1. Quantitative results of the randomised controlled trials included in the meta-analysis

Author	Year	Outcome	Breast Milk group	Nucleotide Fortified group	Control Formula group	<i>P</i>
Carver ⁶	1991	NK [¥] cells (%)	41 ± 4.7	32 ± 3.4	22 ± 2.2	<0.05
		IL-2 [≠] (U/ml)	1.84 ± 0.35	1.52 ± 0.21	0.75 ± 0.21	<0.05
Brunser ⁷	1994	Episodes of diarrhoea	**	109	140	<0.05
		Children with first diarrheic episode	**	74	102	<0.05
		Children free of diarrhea (%)	**	45	31	NS
Navarro ⁹	1996	IgA (mg/dl) at 20–30 days	**	22 ± 4	10 ± 1	<0.05
		IgA (mg/dl) at three months	**	28 ± 5	20 ± 1	NS
Pickering ¹²	1998	Hib-Abs (mg/ml) at 7 months	4.1 ± 1.4	7.2 ± 2.4	4.0 ± 1.5	<0.05
		Diphtheria-Abs (mg/ml) at 7 months	1.3 ± 0.3	1.8 ± 0.3	1.4 ± 0.2	<0.05
		Children with diarrhea (%)	13	15	41	<0.05
Ostrom ¹⁵	2002	Hib Abs (mg/ml) at 7 months	3.02 ± 1.8	7.0 ± 3.8	5.6 ± 2.0	<0.05
		Children with URI* reported (%)	65	64	66	
Yau ¹⁴	2003	Children with URI* reported (%)	**	22	20	<0.05
		Children without diarrhea at 24m (%)	**	60	50	NS
Schaler ¹⁹	2004	Oral Polio Virus VN1 Abs at 7 months	886 ± 269	834 ± 343	643 ± 249	<0.05
		Hib Abs (mg/ml) at 7 months	1.87 ± 0.6	1.89 ± 0.51	1.62 ± 0.58	NS
Buck ²⁰	2004	R0 + T-helper cells at 7 months of age (%)	4.7 ± 1.2	4.8 ± 0.5	4.4 ± 0.7	NS
Hawkes ²¹	2005	Diphtheria Abs (mg/ml)	**	0.36 ± 0.08	0.27 ± 0.13	<0.05

*URI = Upper respiratory infection reported (include otitis), ** Data non available, ¥ = Natural Killer cells, ≠ = Interleukin-2.

(three because ingredients other than nucleotides were not present at equal concentrations in experimental and control formulae^{8,10,13} and the remaining three because they were cohort or open label studies²²⁻²⁴). It was possible to identify important differences in immune function and clinical variables between groups. (Table 1)

Meta-analysis results

Meta-analysis showed that nucleotide-fortified formulae compared to breast milk or control formulae showed a better antibody response to immunisation with Haemophilus influenza vaccine [SMD 1.74 (99%CI 1.43, 2.05), *P*=0.001], diphtheria toxin [SMD 0.94 (0.75, 1.12), *P*=0.001] and oral polio vaccine [SMD 0.73 (0.51, 0.95), *P*=0.001]; and fewer episodes of diarrhoea [RR 0.67 (0.58, 0.76), *P*=0.02]. Regarding safety, we did not find differences in relation to the risk of upper respiratory infections [RR 1.11 (0.90, 1.36), *P*=0.50]. (Figs. 1 & 4)

Recommendations

There is sufficient evidence to support the addition of nucleotides to infant formulae based on health benefit related findings, independently of the compositional evidence of their presence in human milk. Available evidence suggests that these benefits are conferred by the addition

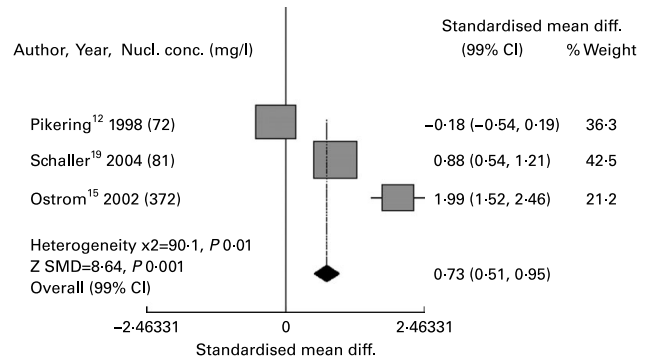


Fig. 3. Nucleotides and Antibody Response to Oral Polio Vaccine.

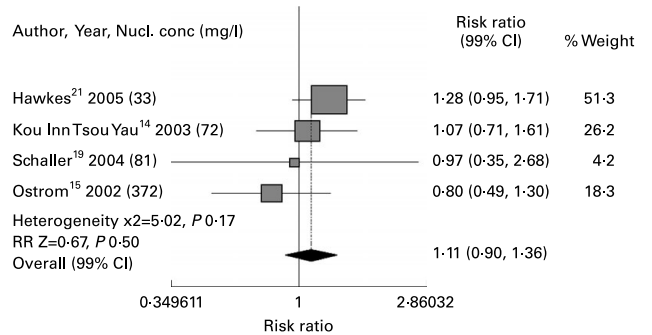


Fig. 4. Nucleotides and respiratory tract infections (Include otitis).

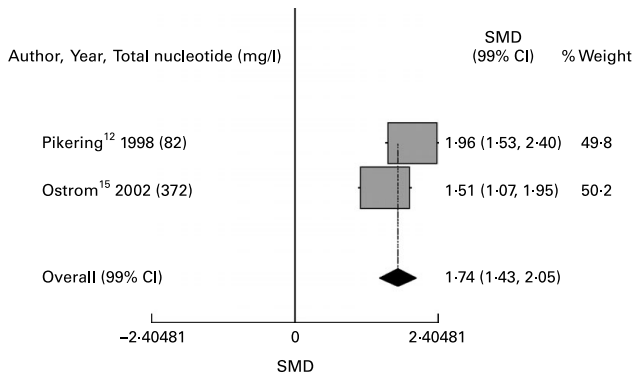


Fig. 1. Nucleotides and Hib-Abs production.

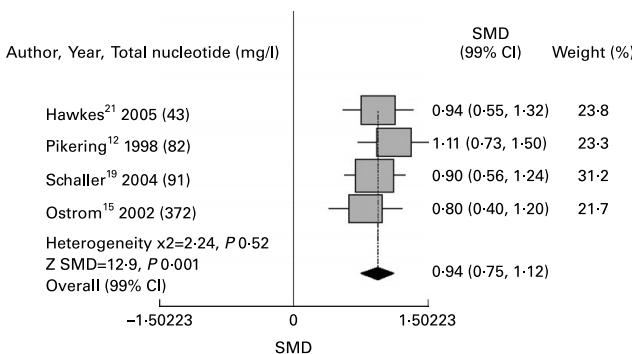


Fig. 2. Nucleotides and antibody response to diphtheria toxoid.

of 1.9 mg/418.4 kJ and are maintained or increased in studies with supplementation at 10.78 mg/418.4 kJ. Considerably greater effects for the selected endpoints and significantly larger clinical and bio-statistical differences among groups were observed at the higher levels of supplementation. The two main benefits supported by the studies considered in this analysis are improved maturation of the immune system and decrease in the incidence of diarrhoea. The evidence considered for the present analysis supports the supplementation of formulae at levels of at least 5 mg/418.4 kJ (33 mg/l) and with higher levels of 10.78 mg/418.4 kJ (72 mg/l), however, it is important to note that 50% of the evidence from studies with formulae supplemented with 10.78 mg/418.4 kJ shows greater effects on endpoint variables. Other research groups have tested formulae with total nucleotide concentrations of 16 mg/418.4 kJ, which is recommended by the Panel of Experts of the Science Research Office. These concentrations have proven to be safe since there is no evidence of increase in the risk of infections or other related adverse events. Given the need to continue generating evidence and the potential beneficial impact on the health of infants, we recommend the continuing development of clinical trials of high methodological rigor testing nucleotide concentrations similar to those identified in breast milk through the TPAN analytical method. These studies would aim to establish the point at which the supplementation with higher concentrations of nucleotides does not represent a significant gain in health benefits. As in all trials involving infants and sole sources of nutrition, close surveillance of the occurrence of potential adverse events should be a high priority.

Conflict of interest statement

The authors have no conflict of interest to report. The work presented was supported by funds from the Pediatric Evidence Based Center COCHRANE-INP, Mexico. PG-C & GS-S participated by assembling the protocol, CJ & LD did the MEDLINE, EMBASE, LILACS and ARTEMISA search, CJ & LD built the data base and PG-C, JRM & GS-S did the statistical analysis & wrote the article.

References

- Aggett P, Leach JL, Rueda R & MacLean WC Jr (2003) Innovation in infant formula development: a reassessment of ribonucleotides in 2002. *Nutrition* **19**, 375–384.
- Ortega MA, Nuñez MC, Gil A & Sanchez-Pozo A (1995) Dietary nucleotides accelerate intestinal recovery after food deprivation in old rats. *J Nutr* **125**, 1413–1418.
- Kulkarni AD, Fanslow WC, Rudolph FB & Van Buren CT (1986) Effect of dietary nucleotides on response to bacterial infections. *JPEN* **10**, 169–171.
- Uauy R, Stringel G, Thomas R & Quan R (1990) Effect of dietary nucleotides on growth and maturation of the developing gut in the rat. *J Pediatr Gastroenterol Nutr* **10**, 497–499.
- Gil A, Corral E, Martinez A & Molina JA (1986) Effects of dietary nucleotides on the microbial pattern of feces of at term newborn infants. *J Clin Nutr Gastroenterol* **1**, 34–38.
- Carver JD, Pimentel B, Cox WI & Barness LA (1991) Dietary nucleotide effects upon immune function in infants. *Pediatrics* **88**, 359–363.
- Brunser O, Espinoza J, Araya M, *et al.* (1994) Effect of dietary nucleotide supplementation on diarrhoeal disease in infants. *Acta Paediatr* **83**, 188–191.
- Martinez Agustin O, Boza J, Del Pino JI, *et al.* (1997) Dietary nucleotides might influence the humoral response against cow's milk proteins in preterm neonates. *Biol Neonate* **71**, 215–223.
- Navarro J, Martinez O, Schlessinger L & Gil A (1996) New insights in immune modulation mediated by dietary nucleotides. In *Nutritional and Biological Significance of Dietary Nucleotides and Nucleic Acids*, pp. 123–146 [A Gil and R Uauy, editors].
- Cosgrove M, Davies DP & Jenkins HR (1996) Nucleotide supplementation and the growth of term small for gestational age infants. *Arch Dis Child* **74**, F122–F125.
- Leach JL, Baxter JH, Molitor BE, Ramstack MB & Masor ML (1995) Total potentially available nucleosides of human milk by stage of lactation. *Am J Clin Nutr* **61**, 1224–1230.
- Pickering LK, Granoff DM, Erickson JR, *et al.* (1998) Modulation of the immune system by human milk and infant formulae containing nucleotides. *Pediatrics* **101**, 242–249.
- Lama More Ra & Gonzalez GA (1998) Nucleotidos en las formulas infantiles. *Ann Esp Pediatr* **48**, 371–375.
- Kuo-Inn Tsou Yau & Chung-Bin Huang (2003) Effect of nucleotides on diarrhea and immune responses in healthy term infants in Taiwan. *J Pediatr Gastr Nutr* **36**, 37–43.
- Ostrom KM, Cordle CT, Schaller JP, *et al.* (2002) Immune status of infants fed soy-based formulae with or without added nucleotides for 1 year: part 1: vaccine responses, and morbidity. *J Pediatr Gastroenterol Nutr* **34**, 137–144.
- Koletzko B, Baker S, Cleghorn G, Neto UF, Gopalan S, Hernell O, Hock QS, Jirapinyo P, Lonnerdal B, Pencharz P, Pzyrembel H, Ramirez-Mayans J, Shamir R, Turck D, Yamashiro Y & Zong-Yi D (2005) Global standard for the composition of infant formulae: recommendations of an ESPGHAN coordinated international expert group. *J Pediatr Gastroenterol Nutr* **41**, 584–599.
- Karen A & Robinson (2002) Development of a high sensible search strategy for the retrieval of reports of controlled trials using Pubmed. *Int J Clin Epidemiol* **31**, 150–153.
- Moher D, Schulz KF & Altman D (2001) The CONSORT Statement: Revised recommendations for Improving the Quality of Reports of Parallel Group Randomized Trials. *Ann Intern Med* **134**, 657–662.
- Schaller JP, Kuchan MJ, Thomas DL, Chordle CT, Winship TR, Buck RH, Baggs GE & Wheeler JG (2004) Effects of dietary ribonucleotides on infant immune status. Part 1: Humoral Responses. *Paediatric Research* **56**, 883–890.
- Buck RH, Thomas DL, Winship TR, Chordle CT, Kuchan MJ, Baggs GE, Schaller JP & Wheeler JG (2004) Effect of dietary ribonucleotides on infant immune status. Part 2: Immune cell development. *Pediatr Research* **56**, 891–899.
- Hawkes JS, Gibson RA, Roberton D & Makrides M (2005) Effect of dietary nucleotide supplementation on growth and immune function in term infants: a randomized controlled trial. *Eur J Clin Nutr* **40**, 631–634.
- Kuchan K, Winship T & Masor M (1999) Nucleotides in infant nutrition: effects on immune function. *Paediatric Nutrition* **8**, 80–94.
- Alarcón PA, Tressler RL, Mulvaney A, *et al.* (2002) Gastrointestinal tolerance of a new infant milk formulae in healthy babies. An international study conducted in 17 countries. *Nutrition* **18**, 484–489.
- Vazquez-Garibay E, Mendez-Estrada C, Romero-Velarde E, *et al.* (2004) Nutritional support with nucleotides addition favours immune response in severe malnourished infants. *Arch Med Research* **35**, 284–288.