Nucleotides in infant nutrition: an update

ABSTRACT

Infants are at higher risk for morbidity resulting from infectious diseases, due in part to immaturity of their immune system. Immune modulators in human milk explain some of the protective effects of mother milk. One of those is Nucleotides (NT). NT and their related products play key roles in many biological processes. They play a role in the development, maintenance and function of the gastrointestinal and immune systems. Studies of compromised or normal term and preterm babies, fortified by NT, showed beneficial effect on cellular and humoral immunity and morbidity. It seems that there is a clinical dose response effect and mainly the disadvantaged infants benefit the most. NT provide scientific evidence to justify their addition to breast milk substitutes.

INTRODUCTION

Nucleotides (NT) are ubiquitous component in human milk and are crucially important to fundamental cellular metabolism and functions. When the body needs are greater than the amounts of NT synthesized or salvaged, the term semiessential or conditionally essential nutrients can be applied. Rapid growth, certain disease states, low nutrient intake or disturbed endogenous synthesis represent such conditions.

Infant formulas are continuously modified and their addition to breast milk substitutes. In the last few years, several infant manufacturers NT provide scientific evidence to justify their addition to breast milk substitutes.

REFERENCES AND NOTES

added NT to their products. This step was taken due to numerous studies suggesting beneficial effect on animal and human immunity, morbidity and intestinal repair. The current review is based on several updates and reviews, published recently in the nutritional and medical literature (1-10) and some recent original work done in the field. The objectives are to update on NT in infant nutrition and discuss their routine supplement to infant formula.

NUCLEOTIDES-DEFINITION

NT consist of a nitrogenous base, a five-carbon sugar (ribose or deoxyribose) and one of the three phosphate groups. The nitrogen-containing bases are derivatives of two parent heterocyclic compounds, purines (adenine and guanine) and pyrimidines (cytosine, thymine and uracil). The ribonucleotides and deoxyribonucleotides serve as the monomeric precursor units of RNA and DNA, respectively.

NUCLEOTIDE-IN HUMAN MILK

Human milk is the only source of NT for neonates during the first months of life and presents a profile that markedly differs, qualitatively as well as quantitatively, from that of bovine milk. Human milk contains approximately 25-30% of its nitrogen as non-protein nitrogen. NT represent 2-5% of those substances. About 50% of the NT in human milk are present as RNA while formulas are supplemented with the monophosphate forms only. It is estimated that human milk provides about one third of the term baby requirements. There are only few studies collecting quantitative data about the NT levels in human milk. Leach et al. (11) have defined in 1995 the nucleoside content, as the total potentially available nucleosides (TPAN), where all ribonucleotides are converted to nucleosides. The mean content of individual nucleosides in colostrums, transitory and mature human milks were determined, establishing the quantitative frame of NT to be added to infant formulas. After three months of lactation, the overall range of TPAN in human milk is 82-402 μmol/L and the overall mean ±sd is 189±70 μmol/L. There was remarkable similarity in average TPAN in the milk of mothers in the USA (72 mg/L), Europe (68 mg/L), and Asia (69 mg/L). They comprise nucleosides, mono-, di-, and triphosphated, nucleotide adducts (nucleotide-containing compounds such as coenzymes) and free bases.

NUCLEOTIDES-HUMAN METABOLISM

Dietary nucleoproteins and nucleic acids are degraded by proteases and nucleases in the human gastrointestinal tract. Intestinal alkaline phosphatases and nucleotidases cleave the phosphate groups from NT to form nucleosides. The absorbed nucleosides are mainly degraded in the small gut to uric acid and allantoin and some are resynthesized to NT. In the body, NT can be de novo synthesized from elemental components, at a high metabolic cost. The intestine and the haematopoietic system have defined in 1995 the nucleoside requirements. There are only few studies about one third of the term baby population added NT to their products. This step was taken due to numerous studies suggesting beneficial effect on animal and human immunity, morbidity and intestinal repair. The current review is based on several updates and reviews, published recently in the nutritional and medical literature (1-10) and some recent original work done in the field. The objectives are to update on NT in infant nutrition and discuss their routine supplement to infant formula.

EFFECTS

A. In animal

NT have numerous biological effects, most of them were demonstrated in experimental animal models. They can be grouped to: immune, metabolic, gastrointestinal and more recently described, central effects. Immune effects on: graft versus host disease mortality, rejection of allogeneic grafts, delayed cutaneous hypersensitivity, alloantigen-and mitogen-induced lymphoproliferation, reversal of malnutrition and starvation-induced immunosuppression, natural killer cell activity, macrophage activation and phagocytic capacity, resistance to microbial, fungi and Cryptosporidium challenges, spleen and lymph node production of cytokines, up-regulated immune response of type 1 T helper cells in the systemic immunity, reduction of circulating immunoglobulins M, G, E and G-but not of Gc- concentrations, promotion of B lymphocyte proliferation, maturation and differentiation, promoted expression of TCRαβ / TCRγδ in inter-epithelial lymphocytes, splenic IL-2, interferon-γ and antibody production, accelerated maturation and differentiation of intestinal lymphocytes, polypeptide lymph node cytokine secretion, peripheral blood total leukocyte and neutrophil counts following infections(2, 5, 7, 8, 12-13). Metabolic effects on: energy transfer, synthesis of carbohydrates, components of cofactors that play an integral role in protein, carbohydrate, fat and hormone metabolism, increased iron bioavailability, regulation of lipid metabolism, (2, 7, 9, 13) Gastrointestinal effects: weight gain, increased mucosal DNA and protein synthesis, enhanced enterocyte proliferation and maturation, increase in villus cell number and height, increased disaccharidase activity, intestinal wall thickness and weight, accelerated gut recovery after food deprivation, post diarrhoea increased DNA content, disaccharidase activity and improved histology, promoted healing of small bowel ulcers, intestinal bacterial translocation inhibition, reduction of leukocyte accumulation, protein leak and nitrite production after ischemia and reperfusion, prevention of fatty-infiltration of liver, enhance liver regeneration, recovery of thioacetamide-induced liver injury (2, 7, 9, 13).

Central effects: increase potassium-evoked dopamine release, promote neurite outgrowth and increase acetylcholine levels and release in aged rats (14, 15).

B. In premature babies

Based on the animals’ studies, some of the investigations were explored in human babies. Fewer were performed on premature ones. Navarro et al. showed increased IgA and IgM levels in preterms supplemented with 20 mg/L of NT in their formula (16). In the lipid metabolism aspect, NT supplementation may improve lipid tolerance in preterms, by enhancing plasma lectin cholesterol acyltransferase activity, probably as a result of an increase in apoA-IV plasma concentration (17), 3.4 mg/L of 5 monophosphate NT affected preterms serum levels by increasing HDL-C and decreasing LDL-C (18). The long-term clinical relevance of these results remains to be elucidated. More recently, NT supplementation to preterm infant’s meal was demonstrated to increase superior mesenteric artery blood flow velocities (19), thus potentially affecting gut-associated lymphoid tissues, intestinal performance and immune status (20). A new developing research area is in-utero nutrition. In this context, the expression, localization and function of MRP5, a transporter for cyclic NT in the placenta (21) in human placenta were characterized (21). In view of the important role of cGMP for fetal development, MRP5 may play a role in placental development in parallel with embryonic NT specific needs.

C. In term babies

C1. Effect on intestinal flora

Two studies investigated the effect of supplemented NT on infant intestinal microflora. In the first, higher percentage of bifidobacteria were found in the NT.
supplemented group, resulting in a stool microbial pattern that was more like that of breastfed infant (22). On the contrary, in a more recent study, dietary NT were not associated with the enhancement of bifidobacteria in the infant gut (23).

C2. Effect on somatic growth
No growth effect was depicted when term infants were supplemented with NT (24) in at least 13 studies, including a very recent one (25). The only study with a beneficial growth effect was in term infant with severe small for gestational age, showing catch-up in weight, length and head circumference with a follow-up to 6 month of age (26). The long-term benefits persisting into late infancy is unknown.

C3. Immune effects
Table no 1 summarizes the 9 studies evaluating NT supplementation to infant formula and their immune effects:

The above studies are incomparable due to the variable experimental designs and no definitive conclusions can be drawn. Nevertheless, it seems that despite low NT levels in the supplemented formula, the disadvantaged populations like the marasmic preterm, low socioeconomic originated and slams resident infants, have beneficial clinical outcome (36,37). On a higher NT concentration supplemented formulas, the advantage infant populations, coming from developed regions, enjoy a lower incidence of diarrhea (28, 31, 38). The last two studies (25, 34) were designed for immune responses and function and not primarily for morbidity assessment.

It is uncertain if studies conducted among infants from low socio-economic stratum living within a contaminated environment, could be applied to an urban area in developed countries or in industrialized nation.

C4. NT and infant morbidity
Several studies aimed to investigate the effect of NT supplementation to infant formula on infant morbidity (35) (table no 2):

More recently, the beneficial effect of NT supplemented formula on iron status was not confirmed (48).

Side effects of NT
No side effects have been reported in the numerous clinical studies using NT supplemented formulas, until to day. On a theoretical basis and based on animal studies some concerns should be mentioned (2): dietary adenine may be nephrotoxic and adversely affect growth in animals; heat treatment of powdered formula degrades pyrimidine nucleotide to orotate that can interfere with apo-B synthesis, resulting in fatty infiltration of the rat liver, extracellular ATP is a pro-apoptotic molecule that may inhibit cell growth and increase oxidative stress. Finally, one should be aware of several NT metabolic inherited diseases in which some specific NT should be avoided.

C5. Effect on lipid metabolism
An area of controversy in NT nutrition relates to their ability to modulate the accumulation of long-chain PUFA (LCPUFA). Whereas 2 reports from the same laboratory found beneficial effects (39, 40), subsequent studies involving preterms (41), low-birth-weight infants (42), and more recent ones, in terms (43, 44) have been unable to confirm the LCPUFA-enhancing effects of supplemental NT, compared with unsupplemented formula. It was suggested that the beneficial effects on LCPUFA might be related to the influence of dietary NT on desaturation and elongation rates in fatty acid metabolism (45).

C6. Effect on iron absorption
NT contribute to iron absorption in the gut and may present an iron absorption enhancer in human milk (46). It was found that inosine increases intestinal uptake of iron in rats (47).

NUCLEOTIDE-AGENCIES REGULATIONS AND FORMULA CONTENT
Different authoritative societies, in the field of pediatric gastroenterology and nutrition, considered adding NT to infant formulas. The European Union has recognized NT as semi-essential component of initial formula and has drafted norms to regulate NT-supplemented formula (49). The European Commission’s Scientific Committee for Food (ECSFC) was the first to publish guidelines in 1991 and again in 1996 on NT supplementation of infant formula (50). 5 sodium salts of 5’-monophosphate cytidine, uridine, adenosine, guanosine and inosine, in precise concentrations were approved to be added to the formulas. The upper limit was recommended to be 1.2 mg/100 J, as is the order of magnitude of free NT in human milk. The current European regulations are 5 mg/100 kcal total or 33.5 mg/L at standard concentrations (9). In the USA, the FDA recommendations are a maximum of 5mg/ 100 J for term infant formula and 7mg/ 100 J for preterm one. (51). A recent US expert panel restated their recommendations for the allowance of higher levels of NT in infant formula: up to 16 mg/100 kcal, or 107 mg/L at standard concentrations (9). Practically, there are 4 categories of NT supplemented formulas on the market: unsupplemented, low (19.2 mg/L), middle (33.5 mg/L) and higher (72mg/L) NT levels. In summary, in some regions, including the USA, the recommended maximum content of NT and
NT precursors, in term infant formula is 107 mg/L (16mg/100 kcal), a value similar to the upper level reported for human milk. However, in other regions, like Europe and Australia, infant formula is supplemented to 33.5 mg/L (5mg/100kcal), which is equivalent to the free NT concentration in human milk (52).

**NUCLEOTIDES - A MAST OR AN OPTION?**

On the current available data, it is difficult to answer the question. Many of the studies, summarized in the current update are of too low number of participants, use variable amount of supplemented formula, on different infant population and are not comparable. There are no dose response studies, analyzing the effect of NT concentrations in the formula, on the same studied population. Furthermore, even the technique of NT analytical determination is not standartized and on different part of the globe free NT are reported opposed to TPAN. No bioavailability of the NT supplemented formulas is available. Human milk contains numerous compounds that do not exist in the current formulas with the potential to affect NT bioavailability.

Despite the above, several conclusions can be drown:

1. Addition of relatively small amount of NT (~20 mg/L) can be efficacious for gut health, decreasing episodes of diarrhea (37). Higher levels (~30 mg/L), have immune effect on the cellular immunity increasing NK cell and IL-2 activities, and no major effect on humoral response (27). Higher concentrations of ribonucleotides (72mg/L), in the range of human milk levels, have enhanced antibiotic production against common infant vaccines (28, 30, 34), modify immune cell types (33) and reduce diarrhea (28, 31, 38). The benefits conferred by the supplemented NT appear to depend on their concentration (34).

2. It is suggested that the high risk infants benefit the most from NT supplementation (25) as shown for prematures (36), socially disadvantaged (37) and those born small for gestational age (26).

3. The studies showing a positive effect of lower levels of NT fortification(20-33 mg/L) were performed on vulnerable populations (36, 37). One can ask what would have been the results with higher NT fortification of their formula. It can be concluded that NT-enriched formula confers beneficial biological and clinical effects in the early stage of human life. The gastrointestinal and the immune effects are well established mainly in the compromised infants and it is hypothesized that higher NT fortification of infant formula will confer better health.

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Whey protein concentrates from acidic whey#: benefits for use in infant formulas

ABSTRACT
Breast milk is the gold standard for infant formulae. The closer the essential amino acid pattern of an infant formula matches that of breast milk, the lower the protein content of such a formula can be. This minimizes the risk of renal overload in the infant. The application of whey protein concentrates low in or free from the casein derived glycomacropeptide provides two additional benefits: closer resemblance to plasma amino acid patterns from breast milk fed infants (lower threonine, higher tryptophan and cysteine) and the possibility to lower protein levels in the infant formula towards recommended levels considered to be adequate and safe for infant growth and development.

INTRODUCTION
The composition of infant formulae is under continuous development following the latest scientific insights. Recently, the Codex Committee on Nutrition and Foods for Special Dietary Uses requested and received an opinion of an international expert group, coordinated by ESPGHAN (The European Society for Pediatric Gastroenterology, Hepatology and Nutrition), on recommended levels of nutrients in infant formulae, based on scientific analysis and taking into account existing scientific reports on the subject. The Nov 2005 report (1) of this consultation with experts from Germany, France, US, Canada, Japan, Australia, India, Brazil, Singapore, Thailand, Mexico, Israel and China, proposes compositional requirements for energy, protein, lipid, carbohydrates, vitamins, minerals and trace elements and other substances / optional ingredients. Nutrient contents are generally given per 100 kcal basis for ready to use formulas, as this is considered as physiologically meaningful. Although the acceptable protein content covers the full range between 1.8 and 3.0

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49. Directiva 96/4 CE de la Comisión por la que se modifica la Directiva 91/321/CEE relativa a los preparados para lactantes y preparados de continuación. DOCE 28-2-96.