Nucleotides in Infant Nutrition: A Must or an Option

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Nucleotides are compounds that play a key role in numerous intracellular biochemical processes. Synthesized *de novo* by the body utilizing amino acid precursors or salvaged from degraded nucleic acids and nucleotides, they cannot be considered essential nutrients. However, the term semi or conditionally essential nutrients can be applied in certain conditions where the body's needs are greater than the amounts of nucleotides synthesized or salvaged. Rapid growth, certain disease states, limited nutrient intake or disturbed endogenous synthesis of nucleotides represent such conditions.

Nucleotides participate in several biochemical processes that are essential to the function of the living body [1].

- As nucleic acids: being the monomeric units they carry the genetic code as DNA and RNA
- In biosynthesis: for example, UDP-galactose in the synthesis of lactose or UDP-glucose in the process of glycogenesis
- As components of co-enzymes: NAD, FAD and co-enzyme A
- As biological regulators: cyclic AMP initiates second-messenger cascades and is ubiquitous in all forms of life, playing a key role in regulating biological processes
- As an energy source: ATP is a universal currency of energy in biological systems.

Recently, several infant formula manufacturers announced the addition of nucleotides to their product. This step was taken following the results of studies suggesting potential benefits to intestinal flora, immunity, iron absorption, lipid metabolism and gut development. The question of whether infants require nucleotide supplementation for optimal nutrition cannot be answered categorically until other related questions are resolved. For example: Is there sufficient clinical evidence as to their beneficial effects? Which nucleotides should be added and in what concentration and relative proportions? Are they safe? Are they stable in the formula? Should they be given on a free basis or as a more complex molecule? In an attempt to answer these questions the present review summarizes the biological effects of nucleotides as nutrients and provides an update on their known side effects.

Nucleotides in human milk

Human milk contains approximately 25% of its nitrogen as non-protein nitrogen, including substances such as urea, free amino acids, nucleic acids, creatine and creatinine, polyamines, nucleotides and carnitine. Nucleotides represent 2–5% of the non-protein nitrogen in human milk [1,2]. In the past, free nucleotide content of the acellular part of breast milk was measured. Recently however, it has been recognized that human milk digested by the infant can generate substantially more available nucleotides or nucleosides. Their main source is the live cells with DNA and RNA contained in human milk. The lack of a method capable of quantifying all available nucleotide sources prompted Leach et al. [3] to develop a novel assay for converting all sources of ribonucleotides to nucleosides, called TPAN (total potential available nucleoside). They found a geographical as well as stage-of-lactation variability in human milk nucleotides. The overall range of TPAN in human milk is 82–402 μmol/L and the overall mean 189 μmol/L.

Applying a different method, Thorell et al. [2] reported a mean of 163 μmol/L nucleotide equivalents. It seems that different methods confirm, independently, higher concentration of nucleotides in human milk than previously reported.

Biological effects of dietary nucleotides

Nucleotides have numerous biological effects, most of which have been demonstrated in experimental animal models. These biological effects have been assessed only rarely in human infants [1,4].

Intestinal growth and differentiation

Feeding nucleotides to weanling rats induced intestinal anabolic effects expressed by increased mucosal protein, DNA, villous height, and brush-order enzyme activities [5]. Trophic effects on the gastrointestinal tract were demonstrated also in mice and in a swine experimental model.

Intestinal repair

Rats with experimentally induced chronic diarrhea made a better recovery after nucleotide supplementation [6–8].

TPAN = total potential available nucleotide

The benefits of nucleotide-supplemented formula were demonstrated in a study on Chilean infants, with the supplemented group experiencing fewer episodes of diarrhea [9]. Another study showed a reduction in incidence of diarrhea in babies fed nucleotide-supplemented formula [10]. One possible anti-infective mode of action of nucleotide may be that dietary nucleotide can inhibit endotoxin-induced bacterial translocation in protein-malnourished mice [11].

Somatic growth

Weanling rats showed significant weight gain when fed a low protein diet with supplemented nucleotide [12]. Studies in human infants have not demonstrated any benefits of nucleotide in normal full-term or preterm infants. However, several studies have shown positive growth effects in the small for gestational age group. Improved growth in weight, length and head circumference was demonstrated in nucleotide-supplemented SGA babies [13]. This may explain the observation that breast-fed SGA babies show faster catch-up growth than those fed standard formulas.

Iron absorption

Studies on the rat everted gut have found that the nucleotides inosine, hypoxanthine and uric acid all significantly increased iron absorption [14]. It is possible that the larger amount of nucleotide in breast milk is a factor that improves iron bioavailability.

Intestinal flora

The effects of nucleotide-supplemented formula on intestinal flora are controversial. Gil et al. [15] showed that babies fed nucleotide-supplemented formula had intermediate intestinal flora as compared to breast-fed and standard formula-fed babies. The nucleotides induced an increase in the fecal *Bifidobacterium* level and a decrease in the number of lactobacilli and enterobacteria. The above observation was not substantiated by Balmer et al. [16] who found more *Escherichia coli* and less *Bifidobacteria* in nucleotide-supplemented infants.

Lipid metabolism

Feeding full-term infants with nucleotide-supplemented formula increases long chain polyunsaturated fatty acids in their red cells [17,18]. In preterm infants nucleotide supplement may improve lipid tolerance by enhancing plasma lecithin cholesterol acyltransferase activity [19].

Immune function

Nucleotides affect the humoral as well as the cellular immune system [20]. Pickering and co-workers [21] examined the influence of nucleotides on the immune system of normal term infants by measuring the antibody response to routine pediatric vaccination. The responses to both *Haemophilus influenza*e type B conjugate vaccine and to diphtheria toxoid were significantly increased at the age of 7 months in the nucleotide-supplemented group. Thus, oral nucleotides enhanced the infant immune system as indicated by increased vaccine response to some T dependent protein antigens. In the same study the authors showed that infants who were breast-fed for more than 6 months had significantly higher response to oral polio virus vaccine, as measured by an increased antibody titer. In another study [22], higher plasma immunoglobulin M and A concentrations were found in preterm infants fed nucleotide-supplemented formula.

Natural killer cell cytotoxicity was significantly higher in breast-fed and nucleotide-supplemented infants. In addition, phytohemagglutinin-stimulated interleukin 2 production was also increased in the nucleotide-supplemented group [23]. The study by Pelton et al. [24] found no effect of nucleotides on the number of natural killer cells.

Side effects of nucleotides

To date no side effects have been shown in clinical trials. However, since nucleotides are a relatively new supplement to infant formula, further long-term human studies are needed to assess its safety, tolerability and potential side effects. For example, dietary adenine has been shown to be nephrotoxic in animals and to adversely affect growth when fed at high levels [25]. Thus, it is important that stability of added adenosine or AMP be checked at all stages of manufacturing. Furthermore, heat treatment of powdered formula degrades pyrimidine nucleotide to orate. The latter interferes with apolipoprotein B synthesis that results in fatty infiltration of the rat liver [26]. Recently, extracellular ATP was found to induce apoptosis, inhibit growth of a colorectal originated cell line [27] and increase oxidative stress, resulting in hepatocyte injury [28]. Despite the fact that extracellular concentration *in vivo* does not reach the concentration used in the above *in vitro* studies, the *in vivo* potential apoptotic effect should be evaluated in the growing baby.

When discussing nucleotide-supplemented nutrition one must mention the existence of several nucleotide metabolic inherited diseases, such as Lesch-Nyhan syndrome, adenosine deaminase deficiency, purine nucleoside phosphorylase deficiency, gout, and hereditary orotic aciduria.

Finally, a study in mice used as a nasal allergy model [29] has shown that nucleotides increased the severity of allergic response compared to controls. The relevance to humans is still uncertain.

Conclusion

Nucleotide-enriched formula confers beneficial biological effects on the early stage of human life. While this statement is supported by animal studies and *in vitro* evaluations, data are limited. Thus, the quality and quantity of nucleotides and the factors affecting their bioavailability in infant nutrition remain fertile areas for research.

SGA = small for gestational age

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