Review

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The Role of Nucleotides in the Immune and Gastrointestinal Systems: Potential Clinical Applications

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Abstract

Nucleotides are low molecular weight biological molecules key to biochemical processes. Sources include de novo synthesis, recovery via salvage mechanisms, and dietary intakes. Although endogenous production serves as the main nucleotide source, evidence suggests that exogenous sources are essential to immune competence, intestinal development, and recovery. Dietary nucleotides serve a marked role in rapidly proliferating cells where they are necessary for optimal function. Accordingly, dietary nucleotides are deemed conditionally essential in the presence of various physiological stresses, including growth and development, recovery from injury, infection, and certain disease states. Clinical studies that evaluated nutrition formulations of nucleotides in combination with other specific nutrient substances demonstrated improved clinical outcomes in patients characterized as critically ill, injured, immune suppressed, or with chronic gastrointestinal conditions. However, conclusions regarding specific benefits of nucleotides are limited. Scientific substantiation of nucleotide supplementation in infant formula has been reported to improve the maturation and development of the intestinal tract as well as immune function. All medical nutrition products except for one immune-modulating formulation are devoid of nucleotides. In an effort to build on this, the current review presents the data to support potential clinical applications for nucleotides in enteral nutrition that may contribute to improved outcomes in physiologically stressed patients. (*Nutr Clin Pract*. 2012;27:281-294)

Keywords

nucleotides; purines; pyrimidines; immunity; gastrointestinal; nutrition therapy

Nucleotides are biological molecules integral to almost all biological processes in the body, specifically as components of the nucleic acids—DNA and RNA. Given their importance in physiological structure and function, the body is capable of de novo production, as well as salvage mechanisms by which they are effectively recycled from products of cellular turnover. In addition, nucleotides are normal components of the human diet, and as such, mechanisms for their absorption and incorporation into body tissues exist. The relative contribution of each source to the larger nucleotide pool is not clear; however, under normal conditions, endogenous production and salvage are believed to sufficiently fulfill the needs of healthy individuals.[1] In contrast, conditions characterized by an increased demand for nucleic acid synthesis, including gut injury, periods of rapid growth, immunosuppression, or decreased protein intake, may deplete endogenous supplies. Thus, under such circumstances, nucleotides may be termed conditionally essential, and exogenous sources may confer distinct biological benefits to tissues that undergo rapid turnover, particularly those of the gastrointestinal (GI) and immune systems.[2] Animal studies support this role.[3,4] Representative animal studies are summarized in Table 1. There are indications for a protective effect against diarrhea and immune benefits associated with nucleotide supplementation in infant formulas.[5-10] Selective infant studies are shown in Table 2. In this article, the physiological actions and biological benefits of exogenously supplied nucleotides are reviewed, and potential applications for their inclusion in clinical nutrition products are discussed.

Background

Nucleotide Structure

Integral to cellular processes, nucleotides actively participate in energetic, metabolic, catalytic, regulatory, and structural functions.[1] Nucleotides are structured on heterocyclic nitrogenous bases, either pyrimidine or purine, which constitute the fundamental molecules of nucleotides and the nucleic acids, DNA and RNA. Pyrimidines consist of a 6-membered ring

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Table 1. Representative Animal Studies With Nucleotides

Study	Tissue or System	Outcome	Reference
Dietary adenine, uracil, or RNA and sepsis	Immune	Improved survival in uracil and RNA groups Increased phagocytic activity by all test groups	111
Dietary adenine, uracil, or RNA and candidiasis	Immune	Longer survival time in all test groups Reduced recovery of *Candida albicans* in kidney and spleen	112
Dietary nucleotides and dextran sulfate sodium and distal colitis	Large intestine	Nucleotide group had increased inflammation and delayed healing	64
Dietary nucleosides fed to old mice after food deprivation	Gastrointestinal	Nucleoside-supplemented group restored jejunum and ileum faster upon refeeding	50

Table 2. Representative Clinical Studies in Infants With Nucleotides

Study	Tissue or System	Outcome	Reference
Term infants breastfed and fed formula with and without nucleotides for 2 months	Immune	Natural killer cell percent cytotoxicity higher in breastfed and nucleotide-supplemented groups	6
Infants fed formula with and without nucleotides for 12 months; third group had breast milk for 2 months and then formula	Immune	Nucleotide group had increased antibody to *Haemophilus influenzae* and diphtheria vaccine No difference in immune response to tetanus and oral polio vaccine Breastfed group had greater antibody response to oral polio vaccine	8
Infants fed formula with and without nucleotides for 12 months; third group had breast milk for 2 months and then formula	Immune	Nucleotide group had improved humoral immune responses vs nucleotide-free group Nucleotide group had shifts in T cell populations indicative of improved immune cell maturation	9
Infants fed formula with and without nucleotides for 12 weeks	Gastrointestinal and immune	Nucleotide group had less diarrhea Nucleotide group had 13% higher incidence of upper respiratory tract infections	116
Term infants fed formula with and without nucleotides for 5 weeks	Circulation and gastrointestinal	Nucleotide group had increased superior mesenteric artery blood flow 90 minutes postprandial	137

system containing 2 nitrogen atoms. Purines adopt the ring structure of pyrimidines with the addition of a fused 5-membered imidazole ring. Major pyrimidines include cytosine, uracil, and thymine, whereas key purines include adenine and guanine (Figure 1). Hypoxanthine, xanthine, and uric acid are additional naturally occurring purines.[11]

Pyrimidines and purines undergo conversions between bases, nucleosides, and nucleotides. A nucleoside is formed when a pyrimidine or purine links to a pentose sugar, ribose or 2-deoxyribose, via a glycosidic bond. Subsequently, a nucleotide results when phosphoric acid is esterified to the pentose of a nucleoside (Figure 2). Mono-, di-, or triphosphates may form, and these forms are readily interconverted as they function in cellular energetics and metabolism.[11]

Sources of Nucleotides

Although endogenous synthesis constitutes the major nucleotide source, nucleotides can be obtained in the form of nucleoproteins (proteins linked to a nucleic acid) naturally present in all foods of animal and vegetable origin.[12] Concentrations of RNA and DNA in foods are dependent on cell density; thus, meat, fish, and seeds have higher nucleotide content than milk, eggs, and fruits.[13] Consequently, organ meats, fresh seafood,

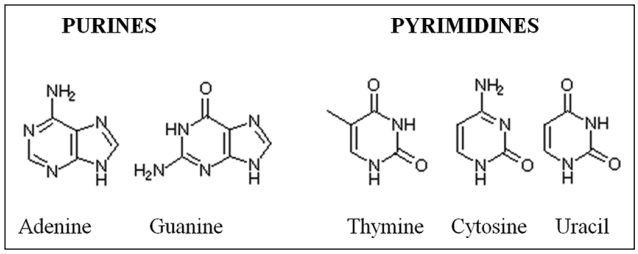

Figure 1. Structure of major bases.

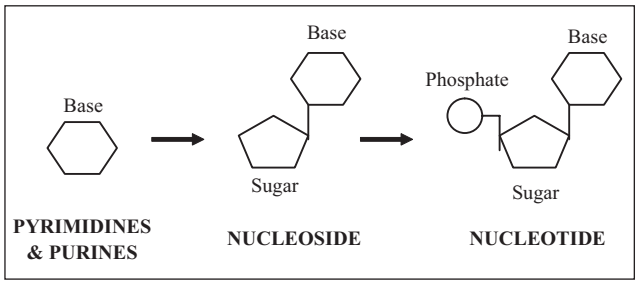

Figure 2. Basic structure of a nucleotide.

and dried legumes are rich food sources.[14,15] Total RNA content ranges from 50–400 mg/100 g for organ meats, 80–350 mg/100 g for seafood, and 140–490 mg/100 g for legumes.[13] Government databases such as the US Department of Agriculture (USDA) National Nutrient Database for Standard Reference, Release 23 do not list the purine, pyrimidine, or total nucleotide content of foods.[16] Thus, reliable data for exact quantities of nucleotides across a wide range of foods do not exist. The diet of healthy individuals normally contains 1–2 g of nucleotides per day.[17] One family of enteral nutrition (EN) formulas is supplemented with nucleotides in the range of 1.2–1.8 g/L.[18]

Soluble nucleotides contribute to the nonprotein nitrogen fraction of human milk. Their presence, although minor, has important physiological roles in immune function, lipid metabolism, and growth in the breastfed infant as well as those fed infant formulas supplemented with nucleotides.[19,20] Nucleotide contents isolated from human breast milk reveal ribonucleotide concentrations ranging from 69.4 mg/L in colostrum to 71.8 mg/L in mature milk.[20] These values are based on an enzymatic method capable of quantifying free nucleosides, free nucleotides, nucleotide adducts, and RNA in human milk to determine the total potentially available nucleosides (TPANs).[19] Generally, free nucleotide concentrations decrease with advancing lactation period or nursing time.[21]

Functions

Nucleotides serve unique physiological functions in the body. These are summarized in Table 3. Foremost, they serve as

Table 3. Summary of Major Metabolic Functions of Nucleotides

Precursors of Nucleic Acids
Energy metabolism
Activated intermediates
Physiological mediators
Allosteric regulation
Cellular agonists
Structural components of coenzymes

precursors of nucleic acids—monomeric units of DNA and RNA that play key roles in the storage and transfer of genetic information, cell division, and protein synthesis.[1,11,21-24] In addition, nucleotides and their derivatives serve diverse roles in energy metabolism, enzymatic regulation, and signal transduction and as structural components of coenzymes.[25]

At the cellular level, adenosine triphosphate (ATP) is the principal transducer of free energy. As the primary phosphoryl donor and acceptor, ATP and adenosine diphosphate (ADP) facilitate transfer of chemical energy from energy-yielding catabolic reactions to energy-dependent reactions of biosynthesis, specifically metabolic interconversions and oxidative phosphorylation.[1,11,21-24] Furthermore, nucleotides carry activated biosynthetic intermediates when linked to sugars or lipids. Uridine diphosphate (UDP)–glucose and UDP-galactose are involved in sugar interconversions and the biosynthesis of glycogen, glycoproteins, and proteoglycans; cytidine diphosphate (CDP)–diacylglycerol is an intermediate in lipid synthesis. S-adenosylmethionine is a major methyl group donor.[1,11,21-24] Nucleotides also serve as physiological mediators in metabolic regulation. The cyclic nucleotides cyclic adenosine monophosphate (cAMP) and guanosine monophosphate (cGMP) act as second messengers in physiological events of hormonal regulation. Adenosine, in its mono- and triphosphorylated forms; cytidine triphosphate (CTP); and guanosine triphosphate (GTP) are involved in the allosteric regulation of enzymes. Various enzymes are dependent on ATP for phosphorylation, and ADP plays a key role in the rate of oxidative phosphorylation. Guanosine diphosphate (GDP) and GTP are active in signal transduction cascades. Finally, adenine nucleotides are part of various coenzymes essential to vitamin biochemistry. These include nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), and coenzyme A.[1,11,21-24]

Nucleotides also play a role in the regulation of cellular energy and protein homeostasis to facilitate repair, recovery, and repletion of tissue function. The adenosine containing AMP-activated protein kinase (AMPK) is an established integrator of signals that control cellular and whole-body energy metabolism via regulation of various biochemical pathways.[25] AMPK activity is regulated by nutrients, hormones, adipokines, natural compounds, and small molecules to respond to increased AMP concentrations and/or an

increased intracellular AMP:ATP ratio.[25,26] This may occur under conditions of accelerated ATP consumption during muscle contraction or in response to elevation of cytosolic calcium concentrations in neurons, endothelial cells, and lymphocytes. Activation of the kinase results in the stimulation of various biochemical processes involved in the production of ATP, glucose utilization, and fatty acid oxidation, as well as repression of energy-consuming processes, including fatty acid and protein synthesis.[27-31]

Metabolism

Nucleotides can be obtained via de novo biosynthesis, salvage pathways, or exogenous dietary sources. Nucleotide concentrations are maintained within strict limits in the body, and their synthesis is a highly regulated metabolic process. Complex feedback mechanisms allow for adequate quantities and production according to physiological demands. Purines and pyrimidines can be synthesized de novo directly or indirectly from amino acids; however, synthesis and incorporation into nucleotides require great amounts of ATP and thus are metabolically costly.[1,32] Salvage pathways, in which nucleotides are formed from preformed bases and nucleosides, serve as a more energy-efficient process and as the primary pathway of synthesis in cells that cannot carry out de novo synthesis. For example, rapidly proliferating tissues of the immune and GI systems cannot meet nucleotide demands via de novo synthesis but rather preferentially use salvage pathways.[13] Furthermore, relative nucleotide contributions to the body nucleotide pool from de novo and salvage pathways exhibit tissue specificity and vary according to cell cycle phase.[23] However, overall contributions from these pathways are poorly understood. Exogenous dietary sources may become conditionally essential in the hypermetabolic state induced by cellular stress or rapid growth.[33,34]

Absorption

Nucleotides are primarily consumed in dietary protein and are converted to nucleic acids in the intestinal tract by proteolytic enzymes.[1] Phosphodiesterases then facilitate breakdown to nucleotides, which are hydrolyzed to nucleosides by alkaline phosphatase in the lumen.[19] Nucleosides are the preferred form for absorption by enterocytes[35-37]; however, they can be further degraded by nucleosidases to purines and pyrimidines (see Figure 3). Nucleosides and bases are translocated across the plasma membrane via facilitated diffusion and sodium-dependent transport in epithelial cells of the intestine and kidney.[38-40] Most absorption takes place in the upper regions of the small intestine, which is thought to preferentially use salvage pathways.[35,35-43] Once absorbed, nucleosides appear to be rapidly degraded in the cell; however, approximately

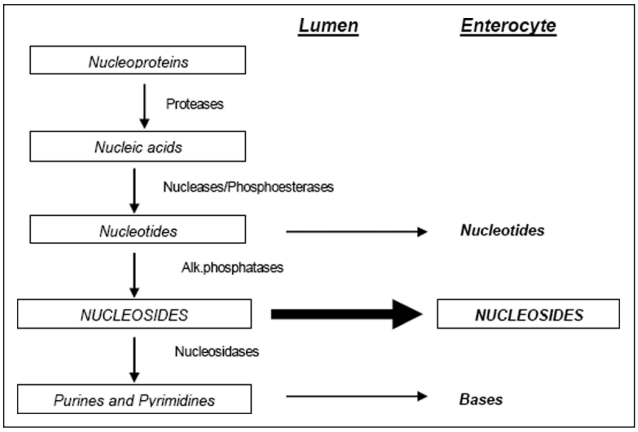

Figure 3. Digestion and absorption of dietary nucleotides.

5% may be incorporated into intracellular nucleotide pools throughout the body.[44] Dietary adenine and uracil appear to be incorporated into pools in significant levels compared with other nucleotides; however, these levels are both low and closely controlled.[22] The majority of absorbed nucleosides are extensively degraded and their end products excreted in the urine.[11]

Regulatory Aspects

The use of nucleotides as a component of infant formulas is strictly regulated around the world by various local regulatory authorities.[32] In the United States, nucleotides and nucleotide precursors are allowed at a maximum amount of 16 mg/100 kcal of formula.[45] The current European Union directive limits the use of nucleotides to 5 mg/100 kcal and a maximum of each individual nucleotide per 100 kcal as follows: cytidine monophosphate (CMP), 2.5 mg; uridine monophosphate (UMP), 1.75 mg; AMP, 1.5 mg; GMP, 0.5 mg; and inosine monophosphate (IMP), 1.0 mg.[46] Other countries have similar guidelines in place.[32]

Adult medical nutrition products that contain nucleotides use yeast extracts rich in RNA as the source of nucleotides. Yeast extracts are regulated as food ingredients that must comply with local requirements for purity and manufacturing.

Physiological Actions and Biological Benefits

Gastrointestinal

Growth and development. Exogenous sources of nucleotides provide benefit to enterocyte function during normal periods of growth and development characterized by rapid cell proliferation and high demand for DNA and RNA synthesis.

Multiple studies have shown that enterocytes have limited capability for de novo synthesis of all purines. Hence, this process is limited or absent in the small intestine except when the diet is totally devoid of purines and the intestine adapts to increase purine synthesis.[47-49] Salvage pathways are preferentially used in the intestinal mucosa whenever purines are part of the diet.[42,43] The capacity for pyrimidine synthesis is high; however, this process is inefficient and energy demanding.[1,50] In addition, intestinal nucleotide pools are low compared with other tissues, and dietary nucleotides are incorporated by the intestine in considerable amounts. Of the 2%–5% of dietary nucleotides incorporated into body pools and used for nucleic acid synthesis, 25%–50% are found in the GI tract.[44] Therefore, given the potential absence of the capability to synthesize purines and the inefficiency for synthesis of pyrimidines, exogenous sources of nucleotides may be necessary to meet the requirements of the GI tract.[51]

For instance, nucleoside supplementation (0.8% by weight) in weanling rats accelerated intestinal maturation via increased mucosal protein levels, DNA levels, villus height, and disaccharide activities compared with a control group on a nucleotide-free diet.[37] Similar findings were identified in adult rats in which dietary nucleotide restriction induced time-related decreases in the content and specific activity of established markers of intestinal maturation—alkaline phosphatase, leucine aminopeptidase, maltase, sucrase, and lactase—in the villus tip.[37]

Furthermore, physiological addition of a mixture containing equal amounts of 5 nucleotide monophosphates—AMP, CMP, IMP, GMP, and UMP—to a normal rat small intestinal epithelial cell line (IEC-6) revealed enhanced rates of proliferation and differentiation.[52] Data also suggest that AMP, in particular, may maintain homeostasis in the developing human small intestine by influencing proliferation and apoptosis.[53] In addition to being the main purine base used, adenine is better retained in fasted animals and as such is suggested to influence the balance between de novo and salvage pathways.[54]

Tests conducted with IEC-6 and Caco-2 cells showed that DNA, RNA, and mixtures of the nucleotides found in human milk stimulated growth in the normal IEC-6 cells. DNA-sodium salt from fish roe and RNA suppressed growth of vigorously proliferating Caco-2 cells grown under optimal conditions but stimulated growth when nutrients were limited in supply. The use of thymidine monophosphate (TMP) acted as a general growth inhibitor of both IEC-6 and Caco-2 cells.[55] However, TMP is not found in human milk or RNA and is not added to infant formulas. The authors speculated that the effect of these various sources of nucleotides had effects via Toll-like receptors, which go beyond the simple incorporation of these substrates into cells.

Recovery after injury. Intestinal cell turnover is increased in periods of GI repair after injury or malnutrition. Small bowel grafts of transplanted fetal rat intestine were used to examine ischemic reperfusion injury and long-term fasting managed by parenteral nutrition.[49] Nucleotide and nucleoside deprivation resulted in poor growth and development, abnormal nerve distribution, and degeneration of muscle layer structure in grafts as compared with grafts in rats fed a nucleotide-nucleoside mixture of inosine, cytidine, sodium 5′-guanylate, uridine, and thymidine. Nucleotides supported villous development in native and graft intestine. These findings suggested that nucleotides influence intestinal growth, protection, and maintenance in small bowel transplantation. In further support, rats maintained on standard PN supplemented with a nucleoside-nucleotide mixture after massive bowel resection demonstrated attenuated initial mucosal atrophy and improved intestinal cell turnover.[56]

Furthermore, dietary nucleotide intakes promoted accelerated recovery of intestinal mucosa in older rats after 5 days of food deprivation.[50] Those fed a nucleotide-supplemented diet exhibited greater activity in brush-border enzymes, markers of intestinal growth and differentiation. These findings were corroborated by similar studies in weanling rats in which nucleotide supplementation enhanced intestinal repair of injured mucosa after lactose-induced chronic diarrhea and malnutrition.[57,58] Additional studies of nucleotide-supplemented PN support a role for nucleotides in improved barrier function, intestinal turnover, and reduced incidence of bacterial translocation in protein-deficient mice.[59,60] Exogenous supplies of enteral or parenteral nucleotides improve gut function in experimental chronic diarrhea.[37,57]

Nucleotides also confer protective effects to the intestinal lumen. Physiological concentrations of nucleotides induce hyperemia in the intestine while decreasing hypoxanthine concentrations, intraluminal accumulations of leukocytes, protein leakage, and the production of nitrites during ischemia and reperfusion in a swine model.[61] Reductions in hypoxanthine and leukocytes are significant, as both have been proposed to result in the formation of free oxygen radicals during ischemia and reperfusion, whereas protein and nitrite levels are indicative of inflammation.

Indomethacin-induced enteritis, a condition that shares clinical, histological, and pathophysiological characteristics with Crohn's disease, exhibited significant acceleration in ulcer healing and significant decreases in total ulcer length and number in the small intestine after exogenous nucleotide supplementation.[62,63] However, one of these same labs found that when dextran sulfate sodium salt (DSS) was used to induce distal colitis in rats, the nucleotide-supplemented group showed more inflammation than the nucleotide-free group, which achieved faster healing of the colitis.[64] In another model of colonic damage that used trinitrobenzene sulfonic acid (TNBS) to induce colitis, the control group also had improved recovery as compared with the nucleoside-nucleotide-supplemented group. These authors also suggested

that an increase in local inflammation may be the cause of the impaired healing.[65] This shows that the experimental model selected is as important as the simulated clinical condition. These results in the DSS and TNBS models were unexpected because nucleotides are processed in the proximal small bowel and are not typically in the distal luminal milieu where tissue damage was induced.

In a rat model of short bowel syndrome, intravenous (IV) administration of OG-VI, a PN formula supplemented with a nucleoside-nucleotide mixture (inosine, 30 mmol/L; GMP, 30 mmol/L; cytidine, 30 mmol/L; uridine, 22.5 mmol/L; and thymidine, 7.4 mmol/L), increased jejunal villus height and cell proliferation.[56] Also, in a similar model of short bowel syndrome, dietary supplementation with orotate and uracil increased jejunal adaptive growth after massive small bowel resection.[66]

Microbiota influence. Nucleotides also modulate growth of intestinal microbiota.[67] Addition of nucleotides to cultured medium evidence increased growth of bifidobacteria, a beneficial bacterial strain in the gut that is protective against infection from enteropathogenic organisms.[68] A comparison of infant formulas with (31 mg/L) and without nucleotides showed that the nucleotide group had a lower *Bacteroides-Porphyromonas-Prevotella* group (BPP) to *Bifidobacterium* species ratio.[69] This showed that modulation of the microbiota was due to the added nucleotides. In addition, a study found a higher proportion of bifidobacteria than enterobacteria in the stools of infants fed human breast milk and formula supplemented with nucleotides (10.5 mg/L), with the inverse observed in infants fed commercial formulas without supplemental nucleotides.[70]

Microcirculation. Nucleotide-mediated increases in intestinal blood flow may enhance gut barrier function. Infants receiving nucleotide-supplemented formula demonstrated increased postprandial intestinal microcirculation as evidenced by increased superior mesenteric artery (SMA) blood flow velocity (BFV).[71,72] The SMA supplies blood flow to a significant portion of the small intestine; thus, changes in SMA BFV may influence bowel function.

The higher SMA BFV after nucleotide-supplemented feedings may be the result of dilation of the intestinal vasculature. Adenosine, an established vasodilator, is believed to play a large role in the regulation of postprandial and reactive hyperemia.[73-75] Episodes of inflammation or hypoxia result in large-scale release of 5′-AMP, which subsequently is converted to adenosine via surface-expressed ecto 5′-ectonucleotidase (CD73).[76,77] Adenosine then imparts immune-regulatory actions by binding to and activating G-protein-coupled cell surface receptors.[78,79] Notably, induced CD73 function has been shown to enhance 5′-AMP-mediated promotion of endothelial barrier function via increased intracellular cAMP levels and reduce intestinal permeability.[77,80] Furthermore, selective perfusion of the terminal ileum, the site of the majority of gut-associated lymphoid tissue (GALT), occurred in rats fed nucleotide-supplemented diets.[81,82]

Hepatic effects. Extracellular nucleotides modulate hepatic growth and repair.[33] Over the course of 5 weeks, hepatic cholesterol and lipid phosphorous were significantly higher, and liver weight and glycogen were lower in weanling mice on a nucleotide-free diet compared with those on nucleotide-containing diets.[83] Nucleoside-nucleotide mixtures added to amino acids and glucose and administered parenterally demonstrated favorable effects on nitrogen metabolism and liver function in rats with liver injury induced by D-galactosamine, a condition histologically comparable to viral hepatitis in humans.[84] Dietary nucleotides also demonstrated beneficial effects in rats with liver cirrhosis in which nucleotide supplementation showed extensive structural repair to cellular damage within 2 weeks.[85] In addition, supplementation of a nucleoside-nucleotide mixture to PN in rats after partial hepatectomy resulted in early restoration of nitrogen balance.[86] Furthermore, the liver demonstrates salvage of exogenous sources of adenosine or adenine to maintain the hepatocyte concentrations of ATP in cold ischemic rats; the restoration of ATP may readily facilitate hepatic recovery after insult.[87]

Immunity

Specific adaptive immunity. Experimentation in vitro and in animal models indicates that nucleotides become increasingly important in conditions characterized by immunological challenge. Rapidly proliferating cells of the immune system are not able to fulfill nucleotides' needs solely by de novo synthesis under conditions of cellular stress and subsequently rely heavily on the salvage pathways and dietary intake.[13] Early studies using diets free of preformed nucleotides confirmed their role in immune function; the absence of nucleotides produced significant reductions in host immune responses, including down-regulation of T cell function and antigen stimulation.[88,89] Nucleotide-free diets also produced prolonged cardiac allograft survival in rodents as well as synergistic immunosuppression, with cyclosporine A indicating that nucleotides influence T helper cell numbers and function.[90]

Nucleosides stimulate lymphocyte differentiation and proliferation, and thus, to some degree, stages of lymphocyte activation and function also influence nucleotide metabolism as de novo synthesis and salvage are increased in stimulated lymphocytes.[22] For instance, terminal deoxynucleotidyl transferase (TdT), a marker for undifferentiated T cells, was identified in undifferentiated bone marrow and thymocytes of rodents fed nucleotide-free diets.[89] Furthermore, splenic lymphocytes demonstrated a decreased proliferative response to mitogens, decreased interleukin-2 (IL-2) production, and lower levels of IL-2 receptors and Lyt-1 surface markers. IL-2 is a growth

Table 4. Representative Clinical Studies in Adults With Nucleotides

Study	Tissue or System	Outcome	Reference
Healthy adults supplemented with nucleotides and prolonged exercise	Immune	Smaller decrease in salivary IgA postexercise Smaller increase in cortisol postexercise	100
Healthy adults supplemented with nucleotides and maximal-intensity exercise	Immune	Smaller decrease in salivary IgA postexercise Smaller increase in cortisol postexercise	99
Healthy adults supplemented with nucleotides to analyze for uric acid levels in blood and urine	Blood and urine	2 g/d nucleotides maintained normal uric acid levels 4 g/d nucleotides caused slightly elevated plasma uric acid levels Protein deficiency reduced urinary uric acid excretion and increased plasma uric acid levels	135

factor for lymphocytes, whereas Lyt-1 is a marker of helper-inducer T cell immunity.[33,90]

Restriction of exogenous nucleotides is believed to influence the initial phase of antigen processing and lymphocyte proliferation via action on the T helper-inducer, as evidenced by increased levels of TdT in primary lymphoid organs.[3,88] This is also suggestive of suppression of uncommitted T lymphocyte response.[34] Nucleotide restriction may cause arrest of T lymphocytes in the G phase of the cell cycle, thus inhibiting transition of lymphocytes to the S phase to elicit necessary immunological signals.[22] Nucleotide restriction may also lower the cytolytic activity of natural killer (NK) cells and macrophage activity.[91] Therefore, dietary nucleotides may favor the balance of T cell differentiation to T helper-2 cells, primarily involved in B cell response.[92] In vitro findings in splenic rodent cells primed with T cell–dependent antigen displayed significant increases in the number of antibody-producing cells in yeast RNA-containing cultures.[93] RNA additions to normal strains demonstrated similar results and were nullified by T cell depletion; antibody did not increase in response to T cell–independent antigens or polyclonal B cell activation. The specific antibody response of yeast RNA was attributed to nucleotides. Immunoglobulin production also increased in vitro in adult human peripheral blood mononuclear cells in response to T cell–dependent antigen and stimuli. Specifically, this involved increased immunoglobulin M (IgM) and G (IgG) production. IgM production increased in the functionally immature umbilical cord mononuclear cells in response to T cell–dependent stimuli as well.[94]

Furthermore, antibody response to T cell–dependent antigen was suppressed in rodents maintained on nucleotide-free diets for prolonged periods. Immune function was rapidly restored with nucleotide supplementation.[84,95] In addition, significant increases in the numbers of antigen-specific immunoglobulin-secreting cells were observed in rodent splenic cells in the presence of nucleotides. Additions of AMP, GMP, or UMP have also resulted in increased IgG response in rodents; GMP was also shown to increase IgM response.[96]

Adult human studies evaluating the effects of dietary nucleotide single agents on immune function in patients have not been reported. Studies in preterm infants compared nucleotide-supplemented formulas, 11.7 mg/L (1.68 mg CMP, 1.98 mg AMP, 2.23 mg GMP, 5.13 mg UMP, and 0.68 mg IMP per liter), with formulas essentially free of acid-soluble nucleotides as determined by high-performance liquid chromatography (HPLC). The nucleotide-supplemented formula groups had increased circulating levels of IgM and IgA in the first 3 months of life as well as higher concentrations of specific IgG against α-casein and β-lactoglobulin in the first month of life.[97,98] Specific IgG levels to low-response antigens increased in term infants receiving dietary nucleotide formulas containing 73.1 mg/L (31.2 mg CMP, 17.7 mg UMP, 9.8 mg AMP, and 14.4 mg GMP per liter) as compared with nucleotide-free formula or fed breast milk for <6 months.[8]

Human studies assessing the effects of supplemental nucleotides as an isolated nutrient are limited to healthy individuals in the context of exercise and safety related to uric acid levels. These studies are listed in Table 4. The ergogenic effects of a nucleotide supplement (high levels of specific, purified, yeast-extracted dietary uridine, cytidine, thymine, adenosine, and guanosine nucleotide precursors and RNA) administered for 60 days on salivary IgA and cortisol response after short-term, high-intensity cycle exercise and prolonged endurance cycle exercise in trained males were investigated.[99,100] Postexercise salivary IgA levels were consistently significantly higher in the groups receiving a nucleotide supplement compared with placebo and control groups. Postexercise cortisol levels were significantly lower after nucleotide supplementation compared with placebo and control groups. Therefore, findings indicate that chronic nucleotide supplementation may counteract the hormonal response associated with physiological stress, resulting in an enhanced immune response.

Inflammation. Nucleotides have also demonstrated strong anti-inflammatory capabilities. For instance, extracellular adenosine has been shown to downregulate the potent inflammatory cytokine, tumor necrosis factor alpha (TNF-α) in the in vitro studies that involved macrophages or endothelium.[101,102] An in vivo animal model with endotoxin exposure demonstrated that adenosine receptor agonists almost completely inhibited TNF-α response.[103]

The potential for nucleotides to reverse the oxidative stress secondary to inflammation and immunosuppression also exists. Infusions of adenosine and ATP have demonstrated potent vasodilation in the treatment of hemorrhagic shock, tissue ischemia, and pulmonary hypertension.[104-106] Furthermore, infusions of nucleoside and nucleotide mixture OG-V (CMP, 72.2 mmol/L; UMP, 38.6 mmol/L; IMP, 120.4 mmol/L; and TMP, 9.5 mmol/L but lacking a source of guanosine) has produced reductions in markers of cardiac ischemia with restoration of myocardial contractibility, enhanced liver growth and increased rates of muscle and liver protein synthesis after partial hepatectomy, and reduced PN-associated mucosal atrophy in animal models.[107-110]

Resistance to infection. The absence of nucleotides decreases resistance to bacterial and fungal infections. Rodents receiving nucleotides demonstrated significant resistance to intravenous challenge of *Staphylococcus aureus* compared with those on nucleotide-free diets; also, a decreased ability to phagocytose *S aureus* was observed in the nucleotide-free group as compared with the groups receiving nucleotides as RNA (0.25% by weight of the diet) or uracil or adenine (0.06% by weight of the diet).[111] Moreover, decreased survival times were observed in rodents on a nucleotide-free diet after similar challenge with *Candida albicans;* additions of RNA or uracil, but not adenine, were shown to increase survival time.[112]

C57BL/6 mice were immunosuppressed by administration of dexamethasone and fed a diet with and without a 0.5% mixture of nucleotides and nucleosides before and after infection with *Cryptosporidium parvum*. The test group showed a lower level of *C parvum* oocytes and a greater cumulative survival rate.[113] These effects may be associated with a shift in T helper (Th) cell response to a Th1-dominated cellular immune response.[114,115] Nucleotide supplementation-induced Th1 cell response augments macrophage activation and delayed-type hypersensitivity reactions.

As part of a study in infants who received formula with (72 mg/L) and without nucleotides had diarrhea as the primary outcome, it was found that the nucleotide group had a small increase (13%) in upper respiratory tract infections. The investigators could not explain this because the nucleotide group had a decrease in diarrhea, an increase in IgA, and no difference in lower respiratory tract infections.[116]

Hematopoietic. De novo synthesis of nucleotides is also limited or absent in hematopoietic bone marrow cells.[117] Protein-deficient mice subjected to acute *S aureus* infection displayed increased proliferation in bone marrow cells and bone marrow DNA content after intraperitoneal (IP) administration of a nucleoside-nucleotide mixture. Similar IP supplementation resulted in increased bone marrow proliferation and peripheral blood neutrophil numbers in mice challenged with *S aureus*.[118] In contrast, mice on nucleotide-free diets demonstrated decreased in vivo hematopoietic growth factor production, with this tied to decreased immune response.[119] Therefore, exogenous nucleotide supplies may enhance the metabolism of rapidly dividing bone marrow stem cells or the production of materials necessary for their proliferation and differentiation.

Malnutrition. Energy and protein malnutrition is a recognized cause of cellular immune suppression and decreased resistance to infection.[120-122] Th cell–mediated immune response appears most vulnerable to nutrition deprivation and is associated with decreased IL-2 production and decreased normal T cell–mediated immune response.[121,123] Notably, the beneficial effects of nucleotides on the maintenance and improvement in immune response are well established in a number of in vitro and animal studies and are believed to exert significant influence on Th1 immune response.[3,115,124-126]

When examined under conditions of protein malnutrition and starvation in animal models, dietary nucleotides demonstrated a pronounced role in the restoration and maintenance of immune function.[127,128] Adequate protein alone did restore body mass in malnourished mice; however, cellular immune function was recovered only with additional RNA supplementation. RNA supplementation led specifically to recovery of Th function. Provision of calories and nitrogen balance alone did not restore lost immune function after conditions of deprivation, indicating that dietary nucleotides, in addition to energy or protein alone, are vital to the recovery and maintenance of the immune system after malnutrition. Interestingly, dietary regimens prior to starvation were also shown to influence post-starvation immune function, as RNA-supplemented mice were further conferred protection from the immune-suppressive effects of nutrition stress.

Potential Clinical Applications for Nucleotides

Immunonutrition constitutes the use of specific nutrients or combinations of nutrients as clinical interventions in critical care patients.[129] Cellular immune defense functions and the inflammatory response become potential targets for specific nutrition substances.[17] The primary goals of clinical nutrition interventions are to first provide the needed energy and nutrients essential for life and then reduce the initial inflammatory response and associated oxidative stress, prevent infection, and restore optimal immune function and inflammatory response to facilitate recovery from subsequent infection.[130] In

the framework of these events, nucleotides are considered one of several defined substances that potentially act to restore cellular defense function and alleviate inappropriate acquired immune responses, including systemic inflammation.[17]

Surgical stress or episodes of infection following injury and trauma demonstrate an increased demand for nucleotides to facilitate the synthetic capability of immune cells, for tissue repair, and to maintain organ function.[90,92,110] Clinically, the majority of life-threatening complications that occur in critical care can be attributed to failure to control infections associated with immune paralysis in postsurgical and trauma populations.[131] This creates a marked role for the immune-enhancing effects of nucleotide supplementation. As reviewed above, the absence of dietary nucleotides has been shown to adversely affect immune response as demonstrated by impaired mucosal integrity and function, impaired T cell function, weakened NK cell activity, suppressed lymphocyte proliferation, reduced IL-2 production, reduced phagocytosis, and decreased resistance to pathogens. Notably, many of these negative effects were prevented by nucleotide supplementation.

Although the above review of animal models shows promise for the clinical use of nucleotide supplementation, clinical studies in critical care patients have also produced encouraging results when tested as part of a blend of ingredients. A large meta-analysis addressed whether enteral nutrition with immune-enhancing feeds benefited critically ill patients after trauma, sepsis, or major surgery.[132] Fifteen randomized controlled trials comparing patients receiving standard EN with patients receiving a commercially available immune-enhancing feed with arginine with or without glutamine, nucleotides, and ω-3 fatty acids were identified by 2 independent reviewers. The meta-analysis demonstrated benefits of enteral immunonutrition in reducing episodes of infection, ventilator days, and length of hospital stay in critically ill patients. It is unclear which novel substrate was effective and/or if nutrient interactions were responsible for the improved outcomes.

Based on the data presented, it is anticipated that nucleotides can be important in the treatment of critical care populations facing large immunologic and metabolic challenges, although the specificity of these actions remains to be completely elucidated in future clinical trials. These studies would examine the benefit of nucleotides on GI structure and function, immune status, and lean body mass. These indicators of clinical status may then be directly linked to a decrease in infectious complications, lean body mass preservation, and decreased length of stay in the acute care setting.

Intestinal cells undergo rapid division and possess limited capacity for de novo nucleotide synthesis; therefore, it may be surmised that in instances of intestinal pathology, nucleotide synthesis may be further limited.[62,63] Accordingly, exogenous nucleotides may be conditionally essential dietary substrates in rapidly dividing GI tissues and have beneficial effects on the intestine in conditions of stress, including improved intestinal recovery, enhanced small bowel adaptive growth, and increased absorptive capacity in conditions of malabsorption.[53]

Furthermore, the concerted effects of exogenous nucleotide sources may restore or maintain cell energy. It has been reported that decreased glutamine can lead to an increase in the level of AMPK, which reduces protein synthesis.[25] It has been shown that supplemental glutamine stimulates de novo synthesis of nucleotides in the small bowel.[133] Although highly speculative, it is possible that the inverse may occur. Exogenous nucleotides suppress de novo nucleotide synthesis.[134] Thus, provision of exogenous nucleotides may conserve glutamine and potentially minimize the increase in intestinal AMPK levels. This possible indirect benefit of nucleotides on the maintenance of cell energy charge should be tested to confirm or refute this hypothesis. Regulation of the AMP:ATP ratios feeds back to inhibit AMPK and thus may preserve optimal protein turnover in skeletal and GI tissue to restore function and structure. Repletion and/or maintenance of skeletal muscle promote enhanced muscle function, mobility, repair, and healing. Moreover, adequate regulation of protein turnover in GI tissue improves digestion, absorption, and barrier function defense, as well as minimizes mucosal atrophy.

Clinical studies, primarily in infants, combined with review of the benefits of nucleotides outlined earlier, support the use of nucleotides alone or in combination with various other specific substrates in clinical EN. Inclusion of nucleotides in nutrition formulations is necessary for rapid restoration of essential body functions under hypermetabolic conditions.

Safety

Although dysfunctional nucleotide metabolism is rare in the general population, safety concerns do exist for individuals with altered nucleotide metabolism. Disturbances in the metabolic utilization and/or degradation of nucleotides may result in accumulation of intermediates associated with several diseases. Metabolic consequences arise most frequently in the presence of high nonphysiological levels of purines, resulting in elevated uric acid (hyperuricemia). Uric acid is a natural by-product of purine metabolism. Gout is characterized by hyperuricemia and monosodium urate monohydrate crystal deposition in the joints and uric acid calculi in the urinary tract. It is caused by lack of the enzyme uricase, which is necessary to oxidize uric acid to a soluble compound, or by overproduction of purine nucleotides via the de novo pathway.

The effect of different amounts of nucleic acid fed as yeast RNA has been studied in healthy men while protein as egg albumin was kept at 75 g/d. The results showed that 2 g/d RNA allowed for normal plasma and urine uric acid levels, whereas

4 g/d or more caused elevated levels in both plasma and urine.[135] This amount is similar to the current usage of yeast RNA in clinical nutrition products.[18]

The use of nucleotides in infant formulas has not been reported to cause an increase in GI intolerance. For adult medical nutrition products, elderly residents in a long-term care facility were fed enterally for 12 weeks either a standard formula without any added nucleotides or an immune-modulating formula that contained 1.3 g/L of nucleotides as yeast RNA. No differences related to feeding parameters or safety measures were observed.[136]

Conclusions

Extensive evidence indicates that, under conditions of physiological stress, dietary sources of nucleotides are required to support immune function, small intestinal development and function, hepatic function, and other processes of rapid cell growth. De novo nucleotide biosynthesis combined with efficient salvage pathways may be sufficient to meet the nucleotide demand in healthy individuals consuming a varied diet. However, requirements increase in various conditions, including rapid growth, tissue injury, infection, and disease states, as well as when biosynthetic capacity is impaired. Nucleotide demand is thereby increased to maintain optimal physiological function, and dietary nucleotides are considered conditionally essential. This review presents a number of potential clinical applications for nucleotides to improve outcomes in patients faced with cellular, metabolic, or nutrition stress; however, human studies examining the effects of dietary nucleotides as single agents are necessary to more fully understand the role nucleotides play in patients as well as demonstrate the expected clinical benefits.

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