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Review

The role of nucleotides in human nutrition

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Introduction

Nucleotides and their related metabolic products play key roles in many biological processes. Nucleotides can be synthesized endogenously and thus are not essential nutrients. Dietary nucleotides may, however, have beneficial effects upon the immune system, small intestinal growth and development, lipid metabolism, and hepatic function. The terms "semi-" or "conditionally" essential have been used to describe the role of dietary nucleotides in human nutrition. These nutrients may become essential when the endogenous supply is insufficient for normal function, even though their absence from the diet does not lead to a classic clinical deficiency syndrome. Conditions under which these nutrients may become essential include certain disease states, periods of limited nutrient intake or rapid growth, and the presence of regulatory or developmental factors that interfere with full expression of the endogenous synthetic capacity.[1] Under these conditions, dietary intake of the nutrient spares the organism the cost of de novo synthesis or salvage and may optimize tissue function.

Nucleotide biochemistry and metabolism

Nucleotide chemistry and nomenclature

Nucleotides (NT) consist of a nitrogenous base, a pentose sugar, and one or more phosphate groups. The nitrogenous base is either a purine or a pyrimidine whose atoms are primarily derived from amino acids (*Figure 1*). Pyrimidine bases are six-membered rings and include uracil (U), cytosine (C), and thymine (T). Purine bases have a second five-membered ring and include adenine (A), guanine (G), hypoxanthine, and xanthine (*Figure 2*). A purine or pyrimidine base linked to a pentose molecule constitutes a nucleoside (NS). A nucleotide (NT) is a phosphate ester of a NS, and may occur in the mono-, di-, or triphosphate forms. The pentose is either ribose or deoxyribose (*Figure 3*); the riboNTs and deoxyriboNTs serve as the monomeric units of RNA and DNA, respectively. RNA and DNA are linear polymers consisting of four different NTs linked together by 5′-3′ phosphodiester bonds. The immediate precursors for RNA synthesis are ATP, GTP, CTP, and UTP. The precursors for DNA synthesis are dATP, dGTP, dCTP, and dTTP. Purine and pyrimidine NT nomenclature is summarized in *Table 1*.

Metabolic functions. Mammalian, bacterial, and plant cells all contain a variety of nucleotides. Cellular NT may be found in millimolar concentrations and may have many metabolic functions including:

1. Energy metabolism: ATP is the main form of cellular chemical energy, and also serves as the phosphate donor for the generation of other NTs.[2–6]
2. Nucleic acid precursors: DNA and RNA are composed of monomeric units of NT.[2–6]
3. Physiological mediators: NT and their derivatives serve as mediators of many metabolic processes. For example, cAMP is a "second messenger," cGMP is a mediator of several cellular events, ADP is critical for normal platelet aggregation, and adenosine is a vasodilator.[2–6]
4. Components of coenzymes: Coenzymes such as NAD, FAD, and CoA are involved in many metabolic pathways.[2–6]
5. Activated intermediates: NTs serve as carriers of activated intermediates for many reactions. For example, UDP-glucose is an intermediate in glycogen and glyco-

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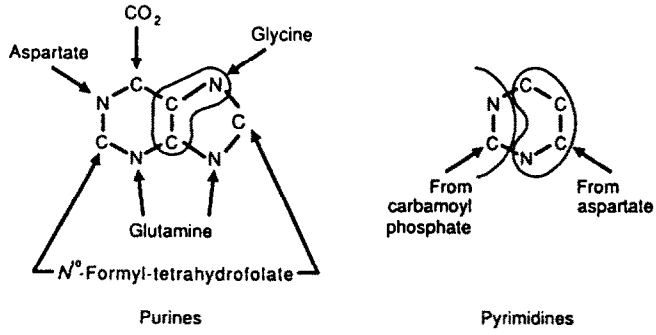

Figure 1 Biosynthetic origin of the atoms in purine and pyrimidine bases. Reprinted with permission from Ref. 2.

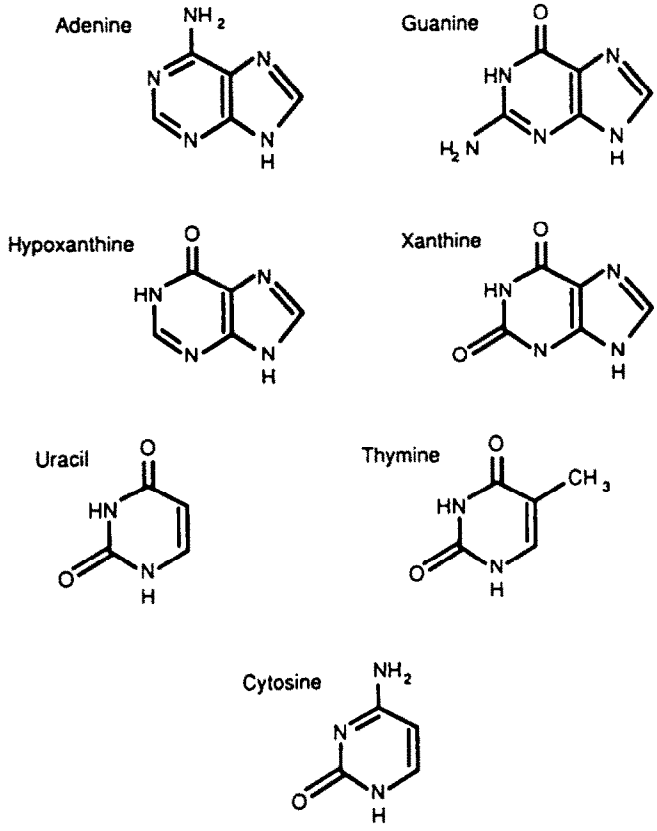

Figure 2 Structures of the major purine and pyrimidine bases. Reprinted with permission from Ref. 2.

protein synthesis; GDP-mannose, GDP-fucose, UDP-galactose, and CMP-sialic acid are intermediates in the synthesis of glycoproteins; CDP-choline and CDP-ethanolamine are involved in phospholipid metabolism; and S-adenosylmethionine serves as a methyl donor.[2–6]

6. Allosteric effectors: Intracellular concentrations of NT regulate the steps of many metabolic pathways.[2–6]
7. Cellular agonists: Extracellular NTs may function as potent agonists, triggering intracellular signal transduction cascades including the cAMP and inositol-calcium pathways. Evidence supports the existence of pyrimidine receptors in addition to the well-studied purine receptors.[7,8]

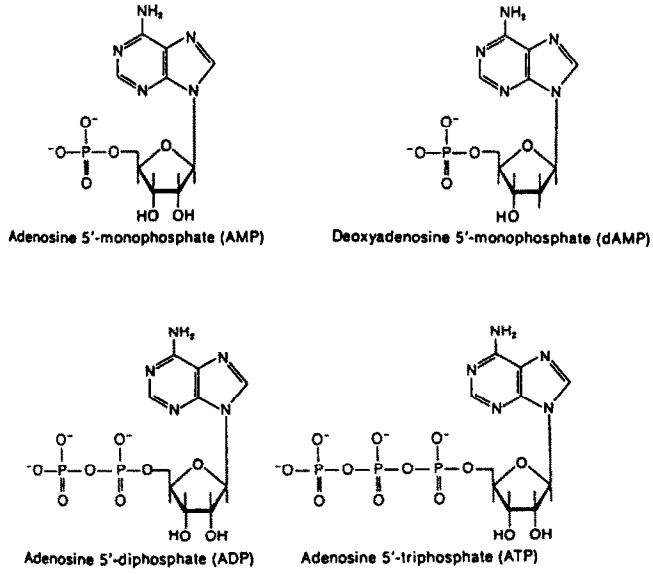

Figure 3 Adenine nucleotides.

Table 1 Nucleotide nomenclature

Base	Nucleoside	Ribo-nucleotide	Deoxyribo-nucleotide
Purines			
Adenine (A)	Adenosine	AMp	dAMP
Guanine (G)	Guanosine	GMP	dGMP
Hypoxanthine	Inosine	IMP	
Pyrimidines			
Cytosine (C)	Cytidine	CMP	dCMP
Uracil (U)	Uridine	UMP	
Thymine (T)	Thymidine		dTMP

Nucleotide metabolism

The 5′-nucleotide derivative is the principal form of cellular purines and pyrimidines. Cellular concentrations vary greatly, with ATP being present in the highest concentration. Free bases, NSs, and 2′- and 3′-NTs in the cell represent degradation products of endogenous NTs, exogenous NTs, or nucleic acids.

Cellular riboNT concentrations are in the millimolar range and remain relatively constant, while deoxyriboNTs are in the micromolar range and fluctuate greatly during the cell cycle. NT synthesis is a finely regulated metabolic pathway. Although the concentrations of individual components may vary, the total cellular NT concentration is fixed within narrow limits in normal cells.

Purines and pyrimidines can be synthesized de novo. However, de novo NT synthesis is a metabolically costly process that requires a great deal of energy in the form of ATP. An alternative mechanism for maintenance of cellular NT pools is the NT salvage pathway in which preformed purine and pyrimidine bases and NSs are converted to NTs. The salvage pathway conserves energy and permits cells incapable of de novo synthesis to maintain NT pools. For example, erythrocytes cannot carry out de novo NT synthe-

sis and thus depend upon salvage to replenish NT pools. Enzyme activities of the salvage pathways are higher than those of the de novo synthetic pathway.

Other enzymes important in NT metabolism include kinases. NTs are synthesized as the monophosphate, however, most reactions requiring NT require the di- or triphosphate form of NT. Kinases salvage NS to NT and convert the NS monophosphates to the di- and triphosphates.[2–6]

Purine metabolism

The purine ring is synthesized in mammalian cells from glycine, aspartate, glutamine, tetrahydrofolate derivatives, and CO_2 (*Figure 1*). All of the enzymes involved in purine NT synthesis and degradation are found in the cytosol of the cell.

The first step in purine NT synthesis is the formation of phosphoribosylpyrophosphate (PRPP) from ribose-5-phosphate and ATP (*Figure 4*). The next step, in which phosphoribosylamine is formed from PRPP and glutamine, is the committed step in the de novo purine synthetic pathway. In subsequent steps, additional N and C atoms are added, and in the final step, the ring is closed to form IMP. IMP serves as the common precursor for AMP and GMP and is not found in significant quantities in the cell under normal conditions. Altogether, six high-energy phosphate groups of ATP are utilized for the synthesis of IMP, in addition to one molecule of aspartate, one of glycine, and two of glutamine.[5]

Purine NT synthesis is regulated at several points, including the committed step of 5-phosphoribosylamine synthesis and the branch point of IMP to GMP and to AMP. An appropriate balance of purine NT is maintained through the activities of various enzymes which regulate conversions of GMP and AMP back to IMP.

There are two distinct enzymes involved in purine salvage: (1) hypoxanthine-guanine phosphoribosyltransferase, which catalyzes the conversion of guanine + PRPP to GMP and hypoxanthine + PRPP to IMP; and (2) adenine phosphoribosyltransferase, which catalyzes the conversion of adenine + PRPP to AMP. The generation of AMP and GMP through these salvage reactions shuts off the de novo synthetic pathway.

Purine NTs, NSs, and bases are degraded in humans to uric acid. Enzymes involved in purine degradation include: nucleases which show specificity for RNA or DNA and the 3′,5′-phosphodiester bonds; nucleotidases and acid and alkaline phosphatases which hydrolyze 3′- and 5′-NT; AMP deaminase and adenosine deaminase which are specific for adenine NT; and purine nucleoside phosphorylase which catalyzes the degradation of NS to base. In the final step of purine degradation, xanthine oxidase catalyzes the formation of uric acid from hypoxanthine and xanthine. Molecular oxygen is a substrate, and H_2O_2 is generated.[2–6]

Pyrimidine metabolism

The pyrimidine ring is synthesized de novo in mammalian cells from aspartate, glutamine, and CO_2. In the pyrimidine de novo synthetic pathway, the ring is formed first, followed by addition of the sugar phosphate. One of the enzymes involved in pyrimidine synthesis is located in the mitochondria, while the rest are in the cytosol.

The first step in pyrimidine ring synthesis is the formation of carbamoyl phosphate from glutamine and CO_2 (*Figure 5*); subsequent steps yield orotate, which reacts with PRPP, the ribose-5-phosphate donor, to form orotidine monophosphate. UMP is synthesized by the decarboxylation of orotidine monophosphate. The formation of cytidine NT proceeds from uridine NT but at the triphosphate

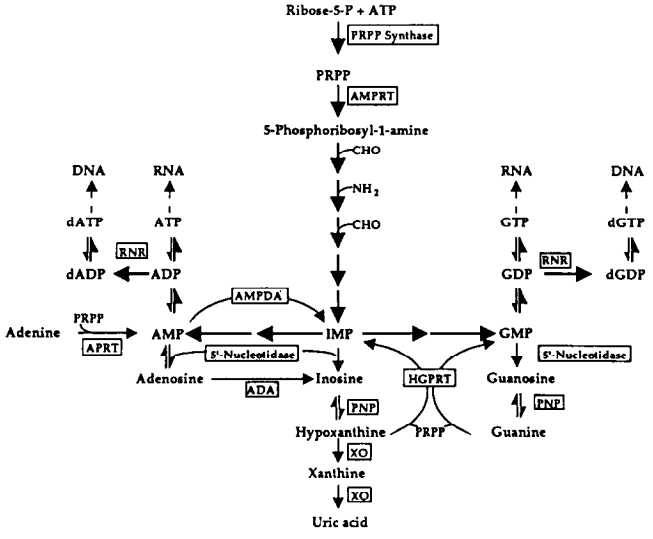

Figure 4 The pathways of purine metabolism. PRPP = 5-phosphoribosyl-1-pyrophosphate, AMPRT = amidophosphoribosyltransferase, APRT = adenine phosphoribosyltransferase, HGPRT = hypoxanthine-guanine-phosphoribosyltransferase, RNR = ribonucleotide reductase, AMPDA = adenylate deaminase, ADA = adenosine deaminase, PNP = purine nucleoside phosphorylase, and XO = xanthine oxidase. Reprinted with permission from Ref. 3.

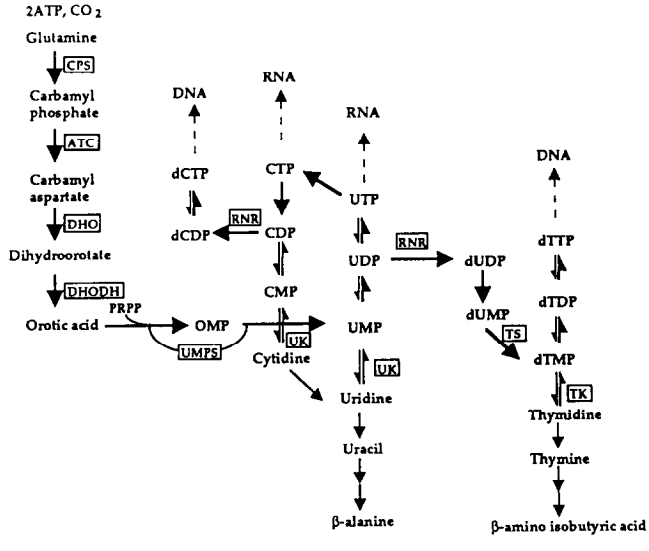

Figure 5 The pathways of pyrimidine metabolism. CPS = carbamylphosphate synthase, ATC = aspartate transcarbamylase, DHO = dihydroorotase, DHODH = dihydroorotate dehydrogenase, UMPS = UMP synthase, RNA = ribonucleotide reductase, TS = thymidylate synthase, UK = uridine kinase, TK = thymidine kinase. Reprinted with permission from Ref. 3.

level rather than at the monophosphate level. Four high-energy phosphate groups are utilized for the synthesis of UMP in addition to one molecule of aspartate and one of glutamine. In mammalian cells pyrimidine NT synthesis is regulated at the level of carbamoyl phosphate synthesis which is inhibited by UMP.[5]

Pyrimidines are salvaged to NT by the conversion of pyrimidine + PRPP to pyrimidine nucleoside monophosphate. This reaction is catalyzed by pyrimidine phosphoribosyltransferase.

The first step in pyrimidine degradation is the conversion to NS and then to the free base uracil or thymine. Cytidine is deaminated to uridine, which is dephosphorylated to uracil. The conversion of pyrimidine NT to NS is catalyzed by various nonspecific phosphatases.

Uracil and thymine are further degraded by analogous reactions to beta-alanine and beta-aminoisobutyric acid. Beta-aminoisobutyric acid originates exclusively from thymine degradation.[2–6]

Nucleotide metabolism and the cell cycle

The deoxyribonucleotide pool is extremely small in resting cells. During DNA replication, however, the DNA pool is increased to support nucleic acid synthesis. The periods of the cell cycle include mitosis (M), gap 1 (G_1), synthesis (S), and gap 2 (G_2); DNA replication occurs during the S phase. In most cells, the periods of M, S, and G_2 are constant, while G_1 varies widely depending upon the cell doubling time. RNA and protein synthesis occur continuously, although at varying rates during the cell cycle.

During the G_1 and early S phase, enzymes of purine and pyrimidine synthesis are elevated and DNA replication occurs. Many of the enzymes involved in NT interconversions are also elevated during the S phase. Rapidly growing tissues such as regenerating liver, embryonic tissue, intestinal mucosal cells and erythropoietic cells are geared toward DNA replication and RNA synthesis.[5]

Nucleotide metabolic disturbances

Several disturbances in NT metabolism result in accumulation of intermediates, which are associated with a variety of diseases. The Lesch–Nyhan syndrome is characterized by hyperuricemia and neurological problems. This disorder is associated with a deficiency of HGPRTase, which catalyzes the conversion of hypoxanthine and guanine to NT.

Immunodeficiency diseases may also result from defects in purine NT metabolism. Adenosine deaminase deficiency is associated with a severe combined immunodeficiency involving T-cell and usually B-cell dysfunction. This disorder is not associated with overproduction of purine NT, although deoxyadenosine triphosphate levels are elevated which may inhibit DNA synthesis. The elevated adenosine may also be toxic to cells due to the resultant increase in intracellular cAMP levels.

Purine nucleoside phosphorylase deficiency is associated with impaired T-cell function and normal B-cell function. The dGTP which accumulates in patients with this disorder may be toxic to the development of normal T cells.

Gout is characterized by elevated blood uric acid levels. In this disorder, a variety of metabolic abnormalities lead to the overproduction of purine NT via the de novo pathway. Sodium urate crystals deposit in the joints of the extremities and may also contribute to renal disease. Most clinical symptoms of gout are associated with the insolubility of uric acid.

Hereditary orotic aciduria is characterized by retarded growth, severe anemia, and the excretion of high levels of orotic acid. The biochemical basis of this relatively rare disease is the absence of the enzyme required to form UMP from orotate.[5,6]

Nucleotide antimetabolites

The de novo synthesis of purine and pyrimidine NT is critical to normal cell replication, maintenance, and function. Antimetabolites are structural analogs of purines and pyrimidines that act as specific inhibitors of enzymes involved in NT metabolism. These compounds may be synthesized or isolated as natural products. Purine antimetabolites include 6-mercaptopurine used in the treatment of acute leukemia, azathioprine for immunosuppression in organ transplant patients, acyclovir for the treatment of herpes virus infection, and allopurinol for the treatment of gout and hyperuricemia. Pyrimidine analogs used in the treatment of several forms of cancer include 5-fluorouracil and cytosine arabinoside.[5]

Nucleotide analogs as antiviral agents

Acyclovir (acycloguanosine) and 3′-azido-3′-deoxythymidine (AZT) are purine and pyrimidine analogs used in the treatment of herpes virus (HSV) and human immunodeficiency virus (HIV) infection, respectively. These compounds are metabolized by human cells to the phosphorylated compounds to yield the active drug. Acyclovir serves as a substrate for the HSV-specific DNA polymerase. It is incorporated into the viral DNA chain, resulting in chain termination. AZT blocks HIV replication by inhibiting HIV–DNA polymerase.[5]

Naturally occurring sources of nucleotides

Human milk

NTs are a component of the nonprotein nitrogen fraction of human milk. Nonprotein nitrogen accounts for approximately 25% of the total nitrogen in human milk[9] and includes compounds such as amino sugars and carnitine, which play specific roles in neonatal development. In contrast, nonprotein nitrogen accounts for only 2% of the total nitrogen in cow's milk and less than 20% in most cow's milk–based infant formulas.[9] Many of the nonprotein nitrogen components of human milk are present in significantly lower quantities in cow's milk and cow's milk–derived infant formulas.[10]

NTs are reported to account for 2 to 5% of human milk nonprotein nitrogen.[11] NT nitrogen may contribute to the more efficient protein utilization of the human milk-fed infant, who receives a relatively low protein intake compared with the formula-fed infant.[11]

Gil and Sanchez-Medina measured the NT content of the

milks of cows, goats, sheep,[12] and humans.[13] While the total NT content was lowest in human milk, relative quantities of cytosine and adenine derivatives were higher. A wide range of NT concentrations, from 0.4 to over 7 mg/dL, have been reported for human milk.[11,13–20] Purines, pyrimidines, NT derivatives, and cyclic NTs are reported, with values being highest at earlier stages of lactation.

Orotate, the major NT of cow's milk, is present in significant quantities in cow's milk–based infant formulas[11,18] but not in human milk. High levels of dietary orotic acid cause hepatic lipid accumulation,[21] however, this effect is unique to the rat.[22,23]

In addition to free NTs, NSs, and bases, human milk also contains nucleic acids, contributed primarily by cellular elements. The highest concentration of cells occurs in colostrum, with the principal leukocytes being neutrophils and macrophages.[24] Sanguansermsri et al.[25] reported DNA levels of 1 to 12 mg/dL, and RNA levels of 10 to 60 mg/dL in human milk. DNA and RNA levels in cow's milk were 1 to 4 mg/dL and 5 to 19 mg/dL, respectively. Digestion and absorption of nucleic acid NT occurs in the adult human,[26–30] however, the metabolic fate of nucleic acids ingested by the breast-fed infant is unknown.

Inosine and its metabolites enhance iron absorption in the rat by increasing the activity of intestinal xanthine oxidase.[31] Xanthine oxidase, present in human milk,[32,33] catalyzes the reduction of ferritin iron to ferrous iron and thus increases it's bioavailability.[34,35] Janas and Picciano[11] speculated that human milk inosine contributes to the enhanced iron absorption in the breast-fed infant.[36] However, other investigators do not report the presence of inosine in human milk.[13–19]

Human milk is generally considered to be the "gold standard" for infant feeding, and infant formulas are usually manufactured to be as similar to human milk as possible. Additional studies utilizing standardized methodology for collection and analysis are needed for accurate determination of human milk NT content. NT interaction with other human milk components that may affect NT bioavailability and biological action should also be considered in the design of NT supplemented infant formulas.

The role that human milk NTs play in the health of the breast-fed infant is not known, and the issue of NT supplementation of infant formulas remains controversial. However, infancy is characterized by rapid tissue growth and therefore increased nucleic acid synthesis. An exogenous source of NT supplied by formula or human milk may optimize tissue growth and differentiation by sparing the metabolic costs of de novo synthesis and salvage. NT supplemented formula may be particularly important for the infant born prematurely, since preterm birth is associated with limitations of many metabolic functions and limited opportunities for breast-feeding.

Infant formulas supplemented with NT at levels similar to those reported for human milk are currently being marketed in several countries, including the United States. Effects associated with the feeding of these formulas are reported.[18,37–49]

Food sources and effects on growth

The nucleotide content of foods has been of interest primarily as it relates to dietary purine effects upon gout. Foods that contain cellular elements supply dietary NT, primarily as nucleoproteins. Organ meats, seafood, and legumes are especially rich sources[17,50] (*Table 2*). Data regarding the pyrimidine content of food are scarce. However, since nucleoproteins contain equimolar ratios of purine and pyrimidine bases, contents in food are likely to be similar.[1]

NTs, particularly IMP and GMP, are used as flavor enhancers.[17,51,52] These NTs reportedly produce the sensation of "greater body and smoothness" in liquid products. In

Table 2 Purine and RNA content of selected foods. Adapted with permission from Ref. 50

	Adenine (mg/10 g)	Guanine (mg/100 g)	Hypoxanthine (mg/100 g)	Xanthine (mg/100 g)	Total purines (mg/100 g)	RNA (mg/100 g)	Protein (%)
Organ Meats							
Beef liver	62	74	61	0	197	268	20
Beef kidney	42	47	63	61	213	134	18
Beef heart	15	16	38	102	171	49	19
Beef brain	12	12	26	112	162	61	11
Pork liver	59	77	71	82	289	259	22
Chicken liver	72	78	71	22	243	402	20
Chicken heart	32	41	12	138	223	187	18
Fresh seafood							
Anchovies	8	185	6	212	411	341	20
Clams	14	24	12	86	136	85	17
Mackerel	11	26	5	152	194	203	23
Salmon	26	80	11	133	250	289	23
Sardines	6	118	6	215	345	343	23
Squid	18	15	24	78	135	100	15
Dried legumes							
Garbanza bean	17	14	18	7	56	356	21
Split peas	88	74	11	22	195	173	21
Lentils	54	51	15	42	162	140	28
Blackeye peas	104	82	20	16	222	306	22
Pinto bean	46	39	25	34	144	485	20

soups these characteristics are associated with products containing meat derivatives.[17] A pleasant "fifth" taste, umami, includes substances such as IMP and glutamic acid[53] and is also associated with protein-rich foods. Toxicology studies demonstrated that IMP and GMP fed to animals at 8% of the diet for several months did not produce adverse effects.[17]

Kobata[38] reported that infants consumed increased quantities of cow's milk when NTs were added and speculated that taste was improved. Other investigators, however, report no effect of feeding NT-supplemented formula upon weight gain.[19,37,39–49]

The role of NTs as flavor enhancers implies that the addition of NTs to foods may increase the level of intake. Therefore, intake and somatic growth should be carefully considered in the design and interpretation of dietary NT supplementation studies. Increased body weight in animals fed a NT-supplemented diet has been reported, however, observed biologic effects persisted when a pair-feeding model was utilized.[54]

Absorption and metabolism

Oral intake of NTs, NSs, and nucleic acids increases serum and urinary degradation products in animals,[50,55,56] and adult humans.[26–30] Dietary nucleic acids have the greatest influence upon serum uric acid levels,[26,27,29] and a maximum safe limit of RNA in the diet of 2 g/day has been suggested.[26,28,57] These observations provide presumptive evidence of NT absorption.

Nucleoproteins in foods are converted to nucleic acids in the intestinal tract by the action of proteolytic enzymes. The nucleic acids are degraded by pancreatic nucleases to a mixture of mono-, di-, tri-, and polyNT. Ribonuclease and deoxyribonuclease are specific for RNA and DNA, respectively. Intestinal polynucleotidases or phosphoesterases supplement the action of pancreatic nucleases in producing mononucleotides from nucleic acids. The liberated NTs are then hydrolyzed to NSs by alkaline phosphatase and nucleotidases, and may be further broken down by nucleosidases to produce purine and pyrimidine bases[5,6] (*Figure 6*). Investigations in animals suggest that NSs are the primary form absorbed,[1,56,58] and that over 90% of NSs and bases are absorbed into the enterocyte.[59,60]

Transport of NSs into the enterocyte occurs via both facilitated diffusion and specific Na^+-dependent carrier-mediated mechanisms.[61–65] The upper region of the small intestine has the greatest absorptive capacity.[64] Once absorbed, most of the NSs and bases are rapidly degraded within the enterocyte, and catabolic products are excreted in the urine and intestine.[56,59,66]

Catabolic enzymes for purines and pyrimidines predominate over anabolic enzymes in the small intestine,[67] which may also serve as an extrarenal route for the elimination of uric acid in humans.[68,69] The highest levels of purine catabolic enzymes are found in the upper alimentary tract.[70] Chinsky et al.[71] found that adenosine deaminase was one of the most abundant proteins of the epithelial lining of the alimentary mucosa in mice. Levels were low at birth and achieved very high levels within the first few weeks of life. Witte et al.[72] report that from the tongue to the ileum, diverse epithelial cell types lining the lumen of the mouse gastrointestinal tract strongly coexpress each of the five key purine catabolic enzymes, with dramatic increases in the expression of each enzyme occurring during postnatal maturation of the gastrointestinal tract. In light of these high levels of purine catabolism, the authors presume that exogenous purine nucleotides are probably not nutritionally significant.

Nucleic acids and their components are also released by the cellular turnover of the intestinal mucosa, yielding up to 30 mg of nucleic acid per day in the rat.[66] The metabolic fate of these endogenously released nucleic acids is not known.

Despite extensive catabolism, tracer studies in animals indicate that 2 to 5% of dietary NTs are incorporated into tissue pools, primarily within the small intestine, liver, and skeletal muscle.[59,73,74] Incorporation into tissues is reportedly increased at younger ages.[38] Gross et al.[75,76] also demonstrated significantly increased salvage and retention, and decreased catabolism, of orally administered bases and NSs in the fasted versus the fed state. Decreased catabolism may be due to a fasting-related decrease in xanthine oxidase activity.[75,77]

Extensive salvage of purines and pyrimidine NTs has been demonstrated in intestinal tissues,[78–80] however, the capacity for de novo synthesis remains unclear. Investigators have reported the presence of,[80,81] the absence of,[78,82] and limited[83] de novo NT synthesis within intestinal tissues.

Dietary NT may affect gene expression of intestinal enzymes. Feeding a purine- and pyrimidine-free diet to adult rats resulted in a highly significant decrease in total RNA and protein in the small intestine and colon, suggesting a mechanism by which dietary components differentially control the synthesis of specific proteins synthesized in the body.[84]

In summary, most orally administered nucleic acids, NTs, NSs, and bases are readily catabolized and excreted. However, tissue retention is increased during periods of rapid growth and limited food intake. The de novo synthetic capacity of mammalian gastrointestinal tissues remains unclear. Further studies are needed to characterize the metab-

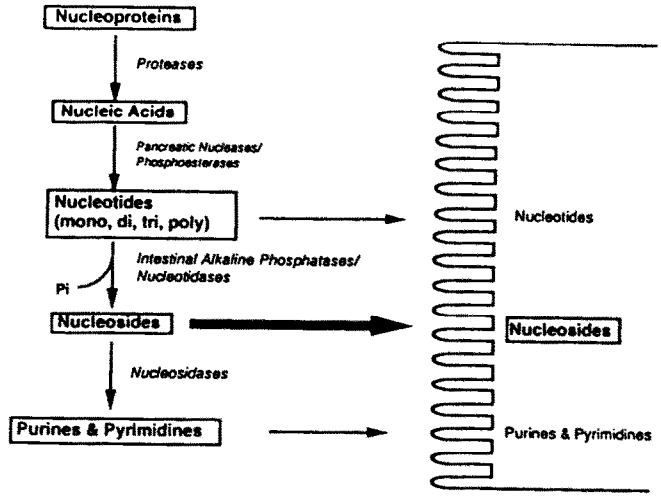

Figure 6 Digestion and absorption of nucleic acids and their related products. Reproduced with permission from Ref. 4.

olism of NT and NT derivatives in human gastrointestinal tissues and the impact of conditions such as immaturity, mucosal injury, and limited nutrient intake. The limited data regarding pyrimidine absorption and metabolism also suggest areas for additional study.

Gastrointestinal effects

Growth and differentiation

Dietary NT may play a role in the developing gastrointestinal tract. Uauy et al.[85] found increased mucosal protein, DNA, villus height, and disaccharidase activities in the intestine of weanling rats fed diet supplemented with 0.8% w/w dietary NT. Feeding 0.21% w/w dietary NT to weanling mice was associated with an increase in small bowel weight (as percent body weight) and weight/unit length; however, disaccharidase activities were not affected. Supplementation with AMP alone significantly increased jejunum wall thickness, protein, and villus cell number.[86,87] Other investigators report increased mucosal growth, maturity, and crypt cell proliferation in rats administered a NS/NT-supplemented TPN solution,[88,89] and intestinal hyperemia in newborn swine following intraluminal NT infusion.[90,91]

Response to injury

Dietary NT effects on diarrheal disease were recently studied in infants living in a relatively contaminated environment in urban Chile.[49] NT-supplemented infant formula was fed to 141 infants, and unsupplemented formula to 148 infants. Those who received supplemented formula experienced fewer episodes of diarrhea (109 versus 140), although clinical characteristics of the episodes and the pattern of enteropathogens isolated were not affected.

Beneficial effects of dietary NT upon intestinal injury have also been demonstrated in animals. The intestinal tissue content of DNA and the activities of lactase, maltase, and sucrase were increased following chronic diarrhea in rats whose diets were supplemented with NT. NT had no effect, however, upon intestinal dissacharidase activities in control rats.[92] Quan et al.[93] report that dietary NT supplementation decreased mortality and intestinal inflammation, and increased disaccharidase activities in rats following radiation-induced intestinal injury.

Intra-arterial infusion of adenosine interferes with leukocyte adherence and granulocyte extravasation in the intestinal mucosa during ischemia and reperfusion,[94] and attenuates the platelet-activating factor-induced adhesion of leukocytes and endothelial cells in postcapillary venules.[95] Luminal infusion of NT may also have effects upon intestinal tissues. Bustamante et al.[91] subjected isolated loops of piglet ileum to ischemia and reperfusion. Luminal infusion of a NT mixture was associated with reductions in leukocyte accumulation, protein leak, and nitrite production, and with intestinal hyperemia, particularly in younger animals. Effects were not changed significantly in the presence of an adenosine antagonist, although purine receptor specificity of the agent was not evident. Hypoxanthine was not increased in the intestinal mucosa in the presence of NT. The latter observation is significant, since xanthine oxidase catalyzes the degradation of adenine NT to hypoxanthine, xanthine, and uric acid during ischemia. Xanthine oxidase is a potential source of oxygen-free radicals and may play a role in the development of reperfusion damage to tissues.[96–98]

Intestinal flora

Bifidobacteria predominant in the stools of breast-fed infants, while gram-negative bacteria predominate in those of infants fed cow's milk–based formulas.[99,100] Bifidobacteria lower the pH of intestinal contents via hydrolytic action on various sugars. The lower pH may impede the proliferation and/or growth of pathogenic species such as Bacteroides and Clostridium. Bifidobacteria growth is enhanced in vitro when a selective medium with added nucleic acids is utilized.[101] In vivo as well as in vitro effects of NT upon bifidobacteria growth are suggested by a report of increased percentages of bifidobacteria and enterobacteria in the stools of infants fed NT-supplemented formula.[40]

Intestinal cell lines

In vitro studies demonstrate effects of exogenous NT upon the proliferation and differentiation of intestinal cell lines.[102,103] The uptake and transport of NS by a human colon carcinoma cell line, Caco-2, and a normal rat small intestine epithelial cell line, IEC-6, were characterized.[102] NT and NS were efficiently taken up by Caco-2 cells and were substantially metabolized during absorption by epithelial monolayers. The addition of NT enhanced the expression of the brush border enzymes sucrase, lactase, and alkaline phosphatase when the Caco-2 cell culture was stressed by glutamine deprivation. Enzyme activity was enhanced when NTs were added to the culture medium of IEC-6 cells, which require extracellular matrix (Matrigel) for brush border enzyme expression. These data suggest that exogenous NT may increase the growth and maturation of normal enterocytes as well as reduce their dependence upon exogenous glutamine.[103]

Due to rapid turnover, tissues of the gastrointestinal tract require increased levels of NT as precursors for nucleic acid synthesis. An exogenous source of NT may optimize tissue function particularly during periods of accelerated growth and during recovery from mucosal injury when the endogenous supply may limit nucleic acid synthesis. It is not known, however, if dietary NT effects are due to direct incorporation of NT into gastrointestinal tissue nucleic acid and/or to other biological mediator effects of NT. Additional studies utilizing whole animal models and adult and fetal intestinal organ culture will help to define the role of dietary NT in this metabolically active tissue.

Hepatic effects

The liver plays a major role in meeting the body's NT requirements through active synthesis and release of NT for use by other tissues.[104,105] The hepatic supply of NT is maintained through de novo NT synthesis and salvage in addition to sodium-dependent and independent transport of NS into the liver.[106,107] Extracellular NT and NS modulate

hepatocyte growth[108] and regeneration.[109] Following hepatic injury NT synthesis and salvage are activated, and regeneration of new tissue is accomplished by accelerated synthesis of RNA and DNA.[110]

Ogoshi et al. report that a parenterally administered NT/NS mixture improves hepatic function and promotes earlier restoration of nitrogen balance following liver injury[111] or partial hepatectomy[112] in rats. When the NT/NS mixture was infused 72 hr after partial hepatectomy in rabbits, the mitochondrial phosphorylation rate and DNA concentration in remnant liver was increased.[113] Earlier infusion, however, was associated with a decrease in the mitochondrial phosphorylative activity.[114] NT infusion is also reported to enhance hepatocyte respiration and improve survival following hypovolemia.[115]

Novak et al.[54] report that weanling mice fed NT-free diet had increased hepatic cholesterol, lipid phosphorous, and serum bilirubin, and decreased liver weight (as percent of body weight) and glycogen when compared with animals fed 0.21% w/w NT. Animals fed diet supplemented with AMP alone represented a greater contrast to animals fed NT-free diet than did animals fed a NT mixture, which may relate to the increased hepatic incorporation of dietary adenine versus other purines,[59,73,74,116] or to adenosine's role in increasing hepatic blood flow.[117]

These studies suggest that exogenously administered NT may affect hepatic composition and function. Dietary NT may be especially important in meeting NT needs when the liver's capacity to supply preformed NT is diminished due to disease or injury. Further studies are needed to determine if the beneficial effects of parenterally administered NT/NS upon hepatic function can be accomplished with oral administration.

Immunologic effects

Cellular immunity

Investigators have demonstrated a role of dietary NT in maintenance of the cellular immune response. While the mechanism is unclear, data suggest that exogenous NTs supplied by the diet contribute to the pool of NT available to stimulated leukocytes, which rapidly turnover and thus have increased NT requirements. Activation of lymphocytes causes a rapid increase in the synthesis of NTs, which are required first for the increase in energy metabolism and later as precursors for nucleic acid synthesis.[118] Induction of lymphocyte proliferation is accompanied by a dramatic increase in intracellular NT pools,[119] and the expression of large numbers of transmembrane NS transporters.[120]

Cohen et al.[121] demonstrated that de novo purine biosynthetic activity is present in S-phase thymic lymphocytes. G_1 phase lymphocytes, however, may have only salvage pathways to maintain their purine nucleotide pools. Perignon et al.[122] found a limited capacity of lymphocytes to salvage pyrimidines, while Marijnen and associates[123] suggest that the NT salvage pathway may not be capable of providing sufficient purine NT for proliferating lymphocytes. These studies suggest that proliferating lymphocytes require an exogenous supply of NT for optimum function.

Feeding NT-supplemented diet to mice has been associated with increases in the following immune parameters: (1) graft versus host disease mortality,[124] (2) rejection of allogeneic grafts,[125,126] (3) delayed cutaneous hypersensitivity,[126,127] (4) alloantigen-induced lymphoproliferation,[116] (5) reversal of malnutrition and starvation-induced immunosuppression,[128,129] (6) natural killer cell activity and interleukin-2 production,[130] (7) resistance to challenge with Staphylococcus aureus[131,132] and Candida albicans,[133] (8) macrophage phagocytic capacity,[131] and (9) spleen cell production of interleukin-2[134] and expression of interleukin-2 receptors and lyt-1 surface markers.[135]

In most of these studies, the addition of RNA or uracil restored immune function, which may relate to the limited capacity of rapidly proliferating lymphocytes to salvage pyrimidines.[122] It has further been suggested that the rapid turnover of plasma uridine indicates a role of this NS in the metabolism of pyrimidines by various tissues.[106]

In contrast to these studies, oral RNA[136] or intraperitoneally administered individual nucleosides[137] had no effect against methicillin-resistant *Staphylococcus aureus* infection in mice. Intraperitoneal administration of a NT/NS mixture, however, resulted in increased survival and lower recoveries of viable organisms from the kidney and spleen.[137]

A study of human infants demonstrated increased natural killer cell activity and interleukin-2 production in infants fed NT-supplemented formula or breast milk compared with infants fed nonsupplemented formula.[46] Hematologic profiles, incidence of documented infections, and rate of growth did not differ between infants fed NT-supplemented or nonsupplemented formula. Although the sample size was small, results suggest that NT in human milk may contribute to the previously reported enhanced cellular immunity of the breast-fed infant.[24]

Van Buren et al.[134] propose that dietary NTs exert effects upon immune responsiveness by acting upon the T helper/inducer population with the predominant effect upon the initial phase of antigen processing and lymphocyte proliferation. The presumed mechanism is suppression of uncommitted T lymphocyte responses, as demonstrated by higher levels of a specific intracellular marker for undifferentiated lymphocytes in primary lymphoid organs in mice fed a NT-free diet.[138] A regulatory role of dietary NT in immunohematopoiesis has also been proposed.[139] Rudolph et al.[140] suggest that dietary NT effects upon immunity were not previously observed since they are only evident under conditions of stress such as immune challenge.

Humoral immunity

Jyonouchi et al.[131,142] report that murine spleen cells primed with T cell–dependent antigen displayed a significant increase in the number of antibody-producing cells when RNA was added to the culture. No increase was noted in the absence of T cells.[142] In contrast, antibody production in response to T cell–independent antigens, and nonspecific polyclonal B cell activation were not increased by the addition of RNA.[142,143] RNA also increased IgM and IgG production in response to T cell–dependent stimuli in mononuclear cells from human peripheral[144] and umbilical cord blood.[145] Treatment with ribonuclease, but not deoxy-

ribonuclease, nullified RNA effects.[141] The actions of RNA were reduced by chemical degradation but not by the removal of small oligonucleotides.[146] The investigators conclude that yeast RNA affects specific antibody responses to T-cell dependent antigens and that these effects are largely attributable to polynucleotides.

Additional in vitro studies suggest the following effects of polyNT[145]: (1) influence antibody production via effects on T-helper cells at the initial stages of antigen presentation; (2) modulate the humoral immune response by interaction with cell surface molecules of T cells or other linage cells; (3) suppress nonspecific activation of T cells in the presence of antigen stimulus; and (4) increase specific antibody response mediated primarily through resting T cells.

Dietary NT may also produce in vivo effects upon humoral immunity. Specific antibody responses to T cell-dependent antigen were significantly decreased in mice fed NT-free diet.[146] Responses to T cell-independent antigens and to a nonspecific polyclonal B cell activator, however, were not affected. The feeding of NT-free diet to mice was also associated with lower numbers of immunoglobulin M and G secreting cells in the spleen, and with T cells less capable of inducing T-dependent antibody production in vitro.[147]

Nucleotides and immunity

These studies demonstrate significant in vivo and in vitro effects of dietary nucleotides upon both cellular and humoral immunity. Dietary NT enhancement of immunity may be particularly important for individuals at increased risk of acquiring infections. Infants, particularly those born prematurely, and individuals with disease-related immunosuppression are included in this category.

The mechanism of dietary NT effects upon immunity are unknown. Most dietary NTs are readily metabolized and excreted, however, a significant proportion of retained NTs are found in gastrointestinal tissues. Gut-associated lymphoid tissue can initiate and regulate T-cell development and may act as a thymus analog.[148] Dietary NT effects upon peripheral immunity may be mediated in part via effects upon this important, but poorly understood, immune tissue.

Use of nucleotides as a nutritional supplement

Recognition of the relationship between malnutrition and immunosuppression has led to the design of feeding formulas that might enhance immunocompetence. Enteral formula with added NT, fish oil, and arginine (Impact, Sandoz Pharmaceuticals, East Hanover, NJ USA) was studied. Twenty septic or critically ill adult patients were randomized to receive either Impact or an isocaloric high-nitrogen enteral formula (Osmolite, Ross Laboratories, Columbus, OH USA) for an average of 9 days.[149] Lymphoprolilferative responses to mitogen and antigen were significantly higher in the Impact-fed group, and the duration of hospital stay was shorter, although these differences were not statistically significant. In a second study, Osmolite and Impact were studied in 85 postoperative gastrointestinal cancer patients.[150] Caloric intake was equivalent in both groups, while nitrogen intake and balance were lower in the Osmolite-fed group. Infectious wound complications and the length of hospital stay were lower, and in vitro lymphocyte mitogenesis was higher in the Impact-fed group.

Additional studies in animals have demonstrated increased survival and enhanced natural killer cell activity following challenge with Listeria monocytogenes in mice fed Impact versus Osmolite.[151] Kulkarni et al.[135] also report an additive effect of RNA, fish oil, and arginine in enhancing lymph node response to injected allogeneic spleen cells.

Nutritional adequacy of the diet significantly enhances wound healing and improves recovery rates for hospitalized patients. NT may be a dietary component that contributes to these beneficial effects. Further studies are needed to determine if routine incorporation of NT into enteral feedings for hospitalized patients is justified.

Effects on lipids

Long chain polunsaturated fatty acids

Feeding a NT-supplemented formula has been associated with increases in long chain polyunsaturated fatty acids in erythrocytes of term[42,152] and preterm[45] infants, and in the plasma of term infants[39,41] and in rats.[153–155] An increase in plasma arachidonic acid in rats was associated with increased thromboxane B_2 levels.[153] In most studies absolute levels, and not percentages, of very long chain fatty acids were increased with NT supplementation. These data suggest that dietary NTs play a role in the conversion of 18C essential fatty acids to 20–22C very long chain polyunsaturated fatty acids. Other investigators, however, found no effect of dietary NTs upon long chain polyunsaturated fatty acids in the livers[54] or erythrocytes of mice (unpublished observations) or in erythrocytes of infants[156] (unpublished observations).

Serum lipoproteins

Sanchez-Poza et al.[41] report that term infants fed NT-supplemented formula or human milk had lower VLDL and higher HDL levels at 1 month of age compared with infants fed unsupplemented formula. More recently the investigators report that NT supplementation of formula fed to preterm infants resulted in increased levels of several plasma lipoproteins; effects in term infants were less significant. The authors speculate that dietary NTs enhance lipoprotein synthesis particularly in the intestine.[18] In contrast Villarroel et al.[43] found no effect of NT-supplemented formula upon serum lipoprotein levels in infants.

Alterations in tissue levels of very long chain fatty acids and serum levels of lipoproteins may have significant health effects. Additional studies are needed to further characterize dietary NT effects upon lipid metabolism.

Effects of individual nucleotides

Data regarding the effects of feeding individual NTs are limited. Orally administered purines have been the most extensively studied due to their effects on gout. Oral hypoxanthine, AMP, GMP, IMP, and adenine, but not guanine and xanthine, elevate serum uric acid levels.[30] The metab-

olism of dietary adenine is different from that of other purines in that a greater portion is absorbed and incorporated into tissues,[73,74,116] particularly during the fasted state.[59] Adenine may be absorbed with minimum alteration and is the most extensively reutilized purine, in contrast to other purines that are extensively degraded to uric acid in the gut.[59] Further, up to 20% of orally administered adenine may be recovered unmetabolized in the portal vasculature.[60] Excessive intake of adenine reduces growth rates in animals,[50,157,158] however, these effects are seen only when adenine is fed in the free form and not as the NS or NT.[158]

Adenosine administered intravenously has significant effects upon vascular, cardiac, and neuronal tissues,[159] and is approved for use in the treatment of paroxysmal supraventricular tachycardia. Many of adenosine's biologic effects are due to its role as a potent vasodilator.[160] Adenosine serves as an intrinsic dilator through which portal blood flow regulates hepatic arterial blood flow,[117] and also stimulates hepatic glucose production.[163] Intra-arterial infusion of adenosine to the small intestine increases blood flow to the intestinal wall[160–164] and mucosal layer.[165,166] Adenosine further regulates postprandial[163,167] and reactive hyperemia,[164,168] and when applied to the serosa increases blood flow[169] and arrests inflammatory changes associated with reperfusion-induced injury.[170]

Intraluminal administration of adenine NT affects the contractility of smooth muscle in rat duodenum in an age-dependent manner.[171] Kolassa et al.[58] report that adenosine uptake by intestinal epithelium is faster than that of other purines and suggest that adenosine is the most important source for maintenance of purine NT in intestinal epithelium.[79]

In addition to its vasodilatory effects, extracellular adenosine and its metabolites serve as intercellular signals that stimulate cell division and morphogenesis, regulate cellular response to injury,[172] and regulate blood vessel growth.[173] It has been postulated that adenosine is a hormone or a hormone second messenger.[174]

The effect of oral administration of adenosine upon these activities is unknown. Small intestinal and hepatic effects in animals fed diets supplemented with AMP alone, however, may be related to one or more of these phenomenon.[54,87]

Inosine pranobex[175] and methyl inosine monophosphate[176] are synthetic compounds derived from inosine which appear to have immunomodulating activity. Oral administration of these compounds augments proliferative responses to T-cell mitogens,[176] increases plaque forming and delayed-hypersensitivity responses,[177] and exerts antiviral and antitumour activities[175] in both mice and humans. In multicenter trials involving HIV-positive patients without AIDS, daily administration of a 3 to 4 g dose of isoprinosine was associated with a significant decrease in the development of new infections.[178–181] Hadden et al.[182] postulate that the similarity of these compounds to the hypothetical structure of transfer factor provides the basis for these activities.

Fewer studies report the biologic role of extracellular pyrimidines. In many studies of NT supplementation in mice, orally administered uracil significantly enhanced immunity while adenine did not.[127–129,131–135] These effects may relate to the limited capacity of lymphocytes to salvage pyrimidines and/or to the potentially greater need of dividing lymphoblasts for pyrimidine NT.[122] In fed rats, the uridine concentration in hepatic venous blood is higher than that in portal or arterial blood. Gasser et al.[106] suggest that the rapid turnover of plasma uridine indicates a role of this circulating NS in pyrimidine metabolism by various tissues.

Naturally occurring sources of NT in the adult diet probably provide a balanced supply of preformed NT, while human milk is reported to contain relatively higher levels of pyrimidine versus purine derivatives. The specific biologic effects of feeding individual NT and their related metabolic products require further investigation.

Summary

Dietary NT are reported to have significant effects upon lymphoid, intestinal and hepatic tissues, and lipid metabolism (*Table 3*). The mechanism remains unknown, and the nutritional role of NT remains controversial. However, maintenance of the endogenous NT supply via de novo synthesis and salvage is metabolically costly. Preformed NT supplied by the diet may contribute to tissue NT pools and thus optimize the metabolic function of rapidly dividing tissues such as those of the gastrointestinal and immune systems.

An exogenous source of NT may be particularly important for individuals whose dietary intake of NT is low and/or whose tissue needs are increased, for example, rapidly growing infants fed most cow's milk–based formulas and individuals with disease related immunosuppression, intestinal, or liver injury. Under these conditions, dietary NTs may play a role as conditionally essential nutrients.

In addition to serving as nucleic acid precursors, NTs and their related metabolic products are potent inter- and intracellular biological mediators. Certain effects of dietary NT may relate to one or more of these important functions.

Area for future study include:

1. The absorption and metabolism of nucleic acids, NTs, NSs, bases, and related metabolic products in humans, and the effects of age and disease upon these activities.
2. Dietary NT effects upon gut-associated lymphoid tissues.

Table 3 Reported effects of dietary nucleotides in humans and in animals

	Human	Animal
Promotion of small intestinal growth		+
Increased small intestinal dissacharidase activity		+/−
Intestinal hyperemia		+
Protection against diarrheal disease	+	+
Effects upon stool flora	+	
Enhanced cellular immunity	+	+/−
Enhanced humoral immunity		+
Effects upon hepatic composition		+
Increased blood levels of long chain polyunsaturated fatty acids	+/−	+/−
Effects upon serum lipoproteins	+/−	

3. The content of nucleic acid, NTs, NSs, bases, and their related metabolic products in human milk.
4. The relative contribution of nucleic acid, NTs, NSs, and free bases to observed biologic effects, and the effects of individually administered purine and pyrimidine compounds.

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