Effect of Oral Purines on Serum and Urinary Uric Acid of Normal, Hyperuricemic and Gouty Humans

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ABSTRACT Adenine, guanine, hypoxanthine, xanthine, adenosine-5′-monophosphate (AMP), guanosine-5′-monophosphate (GMP), and inosine-5′-monophosphate (IMP) were given in single oral doses at 0.1 mmoles/kg body weight to normouricemic, hyperuricemic and gouty humans, and serum and urinary uric acid levels were monitored to evaluate the effect of dietary purines on serum and urinary uric acid. Oral hypoxanthine, AMP, GMP, IMP and adenine elevated serum uric acid levels while guanine and xanthine did not affect serum uric acid. Hypoxanthine, AMP, GMP and IMP produced a greater hyperuricemic effect on subjects with gout compared with hyperuricemic and normouricemic controls. Urinary uric acid levels were increased equally by all purines except for guanine, which did not alter urine uric acid levels. The effect of oral purines on urinary uric acid levels was the same for all groups of subjects. Although the purines are closely related compounds biochemically, they are metabolized differently and produce different alterations in uric acid metabolism when administered to normal, hyperuricemic and gouty humans. J. Nutr. *106:* 428–450, 1976.

INDEXING KEY WORDS food purines · gout · hyperuricemia · nucleic acids

Hyperuricemia appears to be associated with the development of gouty arthritis and may be due to increased endogenous production or reduced excretion of uric acid (1–3). Although hyperuricemia is exacerbated by diets high in fat (4), protein (5–12) or nucleic acids (1, 5, 6, 12–18), the purine nucleic acid intake has the greatest dietary influence on blood uric acid levels (19–21).

Many studies (12–18, 22, 23) have shown the role of diet and ribonucleic acid (RNA) in altering serum and urinary uric acid in humans. In these studies serum uric acid increased 0.56 to 0.90 mg/100 ml for each gram of dietary RNA while, urinary uric acid excretion increased 113 to 164 mg/24 hours for each gram of RNA consumed in the diet by normal healthy adults (controls). Hyperuricemic persons were fed levels of RNA ranging from 0 to 8 g/day/person added to a purine-free basic formula diet adequate and constant in all known essential nutrients (22). They showed a 50% greater increase in blood uric acid per unit of RNA consumed than did controls, while urinary uric acid output per gram of RNA consumed was similar for both groups.

Ribonucleic acid is a polynucleotide occurring mainly in the cytoplasm of cells and, only to a limited extent, in the nucleus. The constituents of the ribonucleic acids are purine (adenine, guanine) and pyrimidine (cytosine, uracil) bases, a sugar (D-ribose), and phosphoric acid. Only purines are metabolized to uric acid. Al-

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though considerable information is known concerning the effects of dietary RNA on blood and urinary acid levels in man, little is known concerning the effects of individual purines on these levels. This report describes the influence of oral doses of adenine, guanine, hypoxanthine, xanthine, adenine-5′-monophosphate (AMP), guanosine-5′-monophosphate (GMP) and inosine-5′-monophosphate (IMP) on uric acid metabolism of men who were normouricemic, hyperuricemic, and had a previous history of gout.

MATERIALS AND METHODS

Normal and hyperuricemic males over 21 years of age with no personal or family history of gout were selected from participants in a state-wide nutrition survey in Massachuetts who gave their consent for the present study. In this survey, a serum uric acid value of 8 mg/100 ml represented the mean plus two standard deviations of 605 males older than 21 years of age.[2] Thus, men with a fasting serum uric acid value of 8 mg/100 ml or greater were considered hyperuricemic individuals, while those with values less than 8 mg/100 ml (measured under identical conditions) were considered normal. While it is impossible to be sure that hyperuricemic subjects do not have latent gout, the thorough medical history excluded those who had manifested suggestive symptoms at any time in the past. Subjects with clinical gout were recruited through newspaper advertisement. Criteria for gouty subjects were repeated episodes of acute gouty arthritis confirmed by medical diagnosis, absence of renal or other complicating disease, and a demonstrated therapeutic response to colchicine therapy. Serum uric acid values were not a basis for their selection, but this group had a mean fasting serum uric acid value of 8.3 mg/100 ml.

Subjects were tested in the MIT Clinical Research Center and they followed their normal daily routine between tests. All the participants were allowed to eat free choice with the following exceptions: (a) glandular organ meats, dried legumes, lentils and meat extractives were not allowed; and (b) cheese and eggs were used as meat substitutes. The participants were fed the free choice low purine (24) diets for at least 5 days prior to each test and the subjects with gout discontinued all medication during this period. Published data on allopurinol excretion (16, 17) indicate that 1 week of abstinence from the drug is sufficient for its effects to wear off, and the high serum uric acid levels in our gouty subjects substantiates this.

Adenine, guanine, hypoxanthine, xanthine, AMP, GMP or IMP,[3] (0.1 mmoles/kg body weight) were suspended in fruit juice and given at breakfast. Blood was taken from the antecubital fossa before the test compounds were given and again 2, 4, 6, and 8 hours later, and serum was isolated by centrifugation.

Complete 24-hour urine collections in 0.1 N sodium hydroxide were obtained from the normal and gouty subjects during 2 days preceding and 1 day after ingestion of the test compounds. Spot urine samples were obtained from the hyperuricemic subjects immediately prior to, and 8 hours after the time when purines were given. Before the determinations of uric acid levels in the urine samples, lithium carbonate was added to each sample, which was then heated at 80° for 20 minutes and vigorously shaken to insure complete uric acid solubility. Uric acid (25) and creatinine (26) determinations were standardized against control serum and urine of known concentrations of these substances.

The fasting serum uric acid concentrations were subtracted from the highest subsequent value at 2, 4, 6 or 8 hours after ingestion of the test compounds to determine the hyperuricemic effect of each of the purines given. The effect of the purines on urinary uric acid was expressed as change in the ratio of uric acid to creatinine. Analysis of variance (27) was used to evaluate the data.

RESULTS

A description of the subjects tested and the hyperuricemic effects of individual purines are presented in table 1. All subjects were of similar age, but the hyper-

[2] Rand, W. M., Clifford, A. J., Young, V. R., Edozien, J. C. & Scrimshaw, N. S. (1971) Statistical description of serum uric acid values in a population. Federation Proc. *30*, 585 (Abstr.).

[3] The purines were purchased from the Sigma Chemical Company, St. Louis, Missouri.

TABLE 1

Effect of oral purines on serum uric acid concentrations of normal, hyperuricemic, and gouty subjects

	Subject group		
	Control	Hyperuricemic	Gouty
No. of subjects	6	11	8
Age (year)	45 ± 5[1]	45 ± 3	49 ± 5
Weight (kg)	87 ± 6	100 ± 2	87 ± 3
Fasting serum uric acid (mg/100 ml)	6.3 ± 0.5[a]	8.5 ± 0.2[a]	8.3 ± 0.7[a]
Changes[2] in serum uric acid due to:			
Adenine	+1.8 ± 0.3[1,b]	+1.6 ± 0.1[b]	+2.0 ± 0.2[b]
Guanine	−0.2 ± 0.1[a]	+0.1 ± 0.2[a]	+0.2 ± 0.3[a]
Hypoxanthine	+2.4 ± 0.2[b]	+2.8 ± 0.2[b]	+4.1 ± 0.4[z,b]
Xanthine	+0.7 ± 0.1[a]	—	+0.7 ± 0.1[a]
Adenosine-5′-monophosphate	+2.2 ± 0.3[b]	+1.9 ± 0.1[b]	+4.2 ± 0.4[z,b]
Guanosine-5′-monophosphate	+2.3 ± 0.6[b]	+2.2 ± 0.2[b]	+3.3 ± 0.2[b]
Inosine-5′-monophosphate	+1.5 ± 0.1[b]	+1.7 ± 0.2[b]	+3.2 ± 0.5[z,b]

[1] Values are means ± SE. [2] Changes in serum uric acid were determined by subtracting the fasting value from the highest subsequent value after purine intake and expressed as mg/100 ml. All purines except guanine significantly elevated serum uric acid levels above fasting values ($P < 0.05$). [a,b] Within columns, means not sharing a common superscript letter are significantly different ($P < 0.01$). [z] Within rows, means different from controls ($P < 0.01$).

uricemic group was heavier than the control or gouty groups.

When the purines were ranked with respect to their hyperuricemic effect per unit of purine fed, the following order was obtained: hypoxanthine = GMP = AMP = adenine = IMP > xanthine = guanine for control subjects; hypoxanthine = GMP = AMP = IMP = adenine > guanine for hyperuricemic subjects; and AMP = hypoxanthine = GMP = IMP = adenine > xanthine = guanine, for the gouty subjects. Hypoxanthine, AMP and IMP produced a greater hyperuricemia in the gouty subjects compared with the control and hyperuricemic groups. Control, hyperuricemic and gouty subjects responded equally to all remaining purines. Guanine did not alter serum uric acid, whereas GMP and adenine increased serum uric acid equally in all subjects. The magnitude of the change in serum uric acid produced by individual purines did not correlate with the fasting serum uric acid values prior to administering the test purines. This lack of correlation was also observed in a previous study

TABLE 2

Reproducibility of change in serum uric acid concentrations produced by orally administered adenosine-5′-monophosphate in normal and gouty subjects

Control				Gout			
		Serum uric acid				Serum uric acid	
Subject	Date	Fasting	Change[1]	Subject	Date	Fasting	Change[1]
		mg/100 ml	*mg/100 ml*			*mg/100 ml*	*mg/100 ml*
AC	6/22/70	4.98	2.78	RL	8/6/70	8.51	3.74
	8/1/71	6.06	3.32		8/3/71	7.00	4.15
FM	11/6/70	5.37	2.91	WS	8/5/70	9.80	4.30
	7/10/71	4.86	2.92		7/30/71	9.64	4.47

[1] Changes in serum uric acid were determined by subtracting the fasting value from the maximum value subsequent to AMP administration.

TABLE 3

Effect of oral purines on urine uric acid to creatinine ratio

	Subject group		
	Control[1]	Hyperuricemic[2]	Gouty[1]
No. of Subjects	4	4	4
	Urine uric acid/creatinine		
Before test purines	0.39 ± 0.03[3]	0.40 ± 0.04	0.34 ± 0.04
After administering			
Adenine	0.46* ± 0.02	0.53* ± 0.02	0.40 ± 0.02
Guanine	0.35 ± 0.02	0.50 ± 0.06	0.34 ± 0.03
Hypoxanthine	0.55* ± 0.07	0.58* ± 0.08	0.68* ± 0.09
Xanthine	0.40 ± 0.04	—	0.38 ± 0.03
Adenosine-5′-monophosphate	0.51* ± 0.04	0.54* ± 0.04	0.58* ± 0.06
Guanosine-5′-monophosphate	0.47* ± 0.01	0.64* ± 0.04	0.51* ± 0.01
Inosine-5′-monophosphate	0.47* ± 0.06	0.54* ± 0.06	0.57* ± 0.04

[1] Based on complete 24-hour urine collections. [2] Based on spot urine samples. [3] Values are means ±SE. * Greater than the ratio before loading ($P < 0.05$).

of 98 normal subjects in whom serum uric acid levels were measured initially and 4 hours after a test dose of either 0.2 or 0.4 mmoles of adenine/kg body weight.[4]

To determine whether or not the greater hyperuricemic effect of orally administered AMP in gouty subjects compared with normal subjects was reproducible, AMP was fed to two control and two gouty subjects approximately 1 year later. As shown in table 2, the greater hyperuricemic effect of AMP on gouty compared with normal subjects was characteristic of the individual subject and not markedly affected by time.

Oral purines increased the urinary uric acid to creatinine ratio equally in all groups of subjects except for guanine and xanthine which did not affect urinary uric acid in any group and adenine which did not affect urinary uric acid in the gouty group (table 3).

The data show that individual purines produced different hyperuricemic effects. They also indicate that gouty subjects had a greater response to hypoxanthine, AMP and IMP than did control or hyperuricemic subjects.

DISCUSSION

The use of new and unconventional proteins, particularly single cell protein sources, for human feeding has been limited by their high nucleic acid content. The published (15, 18, 22) relationships among diet and nucleic acid (RNA) and serum and urinary uric acid levels, summarized

[4] Riumallo, J. A. & Scrimshaw, N. S., unpublished data.

TABLE 4

Regression equations describing the relationship among dietary RNA intake and serum and uric acid levels in human adults

Type of subject	Regression equation of diet RNA g/day (X) on		Reference
	Serum uric acid mg/100 ml (Y)	Urinary uric acid mg/day (Y)	
Normal	Y = 4.84 + (0.65) (g RNA)[1]	Y = 367.8 + (147.2) (g RNA)[1]	Waslien et al. (15)
Normal	Y = 5.05 + (0.56) (g RNA)[2]	Y = 645.0 + (163.6) (g RNA)[1]	Edozien et al. (18)
Normal	Y = 3.25 + (0.90) (g RNA)[3]	Y = 377.0 + (113) (g RNA)[3]	Zöllner & Griebsch (22)
Hyperuricemic[4]	Y = 4.40 + (1.46) (g RNA)	Y = 286.0 + (120) (g RNA)	Zöllner & Griebsch (22)

[1] Represents g RNA consumed/person/day. [2] Calculated from the data of Edozien et al. (18). [3] Represents g RNA consumed/70 kg person/day. [4] Persons having a serum uric acid level in excess of 6.5 mg/100 ml.

in table 4, show a greater increase in serum uric acid per unit of RNA in hyperuricemic than in control subjects, while the response in urinary uric acid level per unit of dietary RNA was similar for both groups of subjects. Several reports (15, 16, 18) suggest that the maximum safe limit of RNA in the diet is 2 g/day. This intake of RNA would result in an increase in serum uric acid of 1.13 to 1.80 mg/100 ml, and an increase in urinary uric acid of 226 to 328 mg/24 hours for normal humans, while those with gout or hyperuricemia might be expected to show greater increases (50%) in serum uric acid (table 4). Because RNA is a polynucleotide of adenine and guanine, and hypoxanthine and xanthine are purine derivatives, the effects of each of these compounds on serum uric acid concentrations and on urinary uric acid excretion in humans were evaluated.

Inasmuch as age and body weight correlate with blood uric acid concentration (28, 29), it was important to select populations of subjects for this study who were comparable in these two variables. This was accomplished with the normouricemic and gouty subjects. The hyperuricemic subjects, however, were slightly heavier and thus received a slightly greater absolute amount of purines. With the criteria of a serum uric acid concentration of over 8 mg/100 ml to define our hyperuricemic subjects, we were confident of having selected truly hyperuricemic individuals.

In selecting the gouty subjects, we were especially careful to choose only those with very obvious and confirmed clinical gout. All the gouty subjects had experienced repeated attacks of typical, acute, gouty arthritis in the course of several years preceding this study. According to blood urea nitrogen, serum and urinary creatinine analyses, they had apparently normal renal function; however, the possibility of a diminished renal urate secretion per nephron (30) was not determined, and the observed differences between gouty and normal subjects in this study may, in part, reflect renal differences in the handling of uric acid. Support for a renal difference comes from the observation that urinary uric acid response was not different even though serum uric acid was much higher in gouty compared with the normal subjects. Some gout patients have been reported to have a higher than normal renal threshold for the excretion of uric acid (6, 7).

A differential response between hyperuricemic and normal subjects in serum uric acid per unit of RNA consumed has been reported by others (table 4). Although we do not understand the mechanism by which oral purines produced a greater hyperuricemia in gouty subjects than in normals, the differential response probably reflects a composite of the various biochemical deficiencies currently implicated in gout. The hyperuricemic effect of a single oral dose of hypoxanthine or AMP has been proposed for detecting, preclinically, a genetic tendency to gout.[5]

Although purine metabolism in man is not completely understood, recent studies have shown a relationship between dietary amino acid supply and rate of *de novo* purine biosynthesis and reutilization (31). Other studies (32, 33) have described adenine and guanine metabolism during endogenous purine degradation and reutilization. The reutilization pathways are not the same for adenosine and guanosine in that a kinase exists for adenosine [EC 2.7.1.20] (34–36), while with rare exceptions none are found for inosine or guanosine (37, 38). On the other hand, there is a single phosphorylase [EC 3.2.2.1] that converts guanosine and inosine to free bases (39), but none that act on adenosine.

From a consideration of these pathways, normal and gouty subjects might be expected to respond differently to dietary adenine and guanine compounds. Such a differential response may have predictive value in detecting pre-clinical gout, but for the moment must await a better understanding of purine metabolism in gout. When purines are given orally, absorbed adenine is converted to AMP, which would then be converted to adenosine, inosine, hypoxanthine and finally to IMP. Administered hypoxanthine could be converted to IMP by the action of hypoxanthine phosphoribosyltransferase [EC 2.4.2.8], or to xanthine and uric acid by the action of xanthine oxidase [EC 1.2.3.2]. It is well

[5] Clifford, A. J., Scrimshaw, N. S., Riumallo, J. A. & Young, V. R. (1971) Purine loading of hyperuricemic and gout diagnosed subjects. Federation Proc. *30*, 300 (Abstr.).

known that a deficiency of hypoxanthine phosphoribosyltransferase will lead to hyperuricemia and gout (40), and under these conditions hypoxanthine would be converted exclusively to xanthine and uric acid. Administered AMP and IMP would be converted to the corresponding nucleoside or free base prior to absorption, because nucleotides as such would not be absorbed (19, 41). It is conceivable that IMP, AMP, and hypoxanthine would yield a differential response between gout and normal humans in view of the fact that high adenine phosphoribosyltransferase activity and low hypoxanthine phosphoribosyltransferase activity often occurs in gout.[6] The fate of administered GMP and guanine would be different from AMP, IMP, or adenine because the pathway leading from IMP to GMP is not freely reversible in mammalian cells (32), thus GMP would be converted to guanosine or guanine and absorbed as such and then be readily converted to CO_2 by the action of guanase [EC 3.5.4.3], which is abundantly distributed in mammalian tissues.

A more complete understanding of the differential response to individual purines between gouty and normal subjects, and the different hyperuricemic effect of different purines, must await a further clarification of the role of purine reutilization and interconversion in relation to *de novo* purine biosynthesis and degradation. Nevertheless, the data presented here describe, for the first time, the effect of individual purines on purine metabolism in normal, hyperuricemic, and gouty subjects and show that purine metabolism can be altered by exogenous purines in the diet. Our data suggest that an evaluation of food purines in terms of individual purine components may enhance the understanding of purine metabolism and provide better guides for recommending safe maximum levels of purines in human diets.

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