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Effects of Acute and Repeated Oral Doses of D-Tagatose on Plasma Uric Acid in Normal and Diabetic Humans

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D-Tagatose, a stereoisomer of D-fructose, is a naturally occurring ketohexose proposed for use as a low-calorie bulk sweetener. Ingested D-tagatose appears to be poorly absorbed. The absorbed portion is metabolized in the liver by a pathway similar to that of D-fructose. The main purpose of this study was to determine if acute or repeated oral doses of D-tagatose would cause elevations in plasma uric acid (as is seen with fructose) in normal humans and Type 2 diabetics. In addition, effects of subchronic D-tagatose ingestion on fasting plasma phosphorus, magnesium, lipids, and glucose homeostasis were studied. Eight normal subjects and eight subjects with Type 2 diabetes participated in this two-phase study. Each group was comprised of four males and four females. In the first phase, all subjects were given separate 75 g 3-h oral glucose and D-tagatose tolerance tests. Uric acid, phosphorus, and magnesium were determined in blood samples collected from each subject at 0, 30, 60, 120, and 180 min after dose. In the 8-week phase of the study, the normals were randomly placed into two groups which received 75 g of either D-tagatose or sucrose (25 g with each meal) daily for 8 weeks. The diabetics were randomized into two groups which received either 75 g D-tagatose or no supplements of sugar daily for 8 weeks. Uric acid, phosphorus, magnesium, lipids, glycosylated hemoglobin, glucose, and insulin were determined in fasting blood plasma of all subjects at baseline (time zero) and biweekly over the 8 weeks. The 8-week test did not demonstrate an increase in fasting plasma uric acid in response to the daily intake of D-tagatose. However, a transient increase of plasma uric acid levels was observed after single doses of 75 g of D-tagatose in the tolerance test. Plasma uric acid levels were found to rise and peak at 60 min after such dosing. No clinical relevance was attributed to this treatment-related effect because excursions of plasma uric acid levels above the normal range were small and were of short duration. Consistent with earlier observations on fructose, the increase of plasma uric acid was associated with a slight decrease of plasma phosphorus and a slight increase of magnesium. The daily ingestion of D-tagatose for 8 weeks had no effect on fasting plasma magnesium, phosphorus, cholesterol, triglycerides, glycosylated hemoglobin, glucose, and insulin levels. The ingestion of three 25-g doses per day for a period of 8 weeks resulted in varying amounts of flatulence in seven of the eight subjects, and some degree of diarrhea in six subjects. D-Tagatose holds promise as a sweetener with no adverse clinical effects observed in these studies.

Key Words: **D-tagatose; uric acid; humans.**

INTRODUCTION

D-Tagatose, a ketohexose proposed for use as a sugar substitute, has 92% of the sweetness level of sucrose (Levin *et al.*, 1995). It occurs naturally in various foods, such as sterilized and powdered cow's milk, hot cocoa, a variety of cheeses, yogurt, and other dairy products. D-Tagatose is a stereoisomer of D-fructose with an inversion at the fourth carbon atom, and is produced by a two-step process from lactose (Beadle *et al.*, 1991, 1992). In a rat study, ingested D-tagatose was reported to supply zero net metabolizable energy (Livesey and Brown, 1996).

Studies with pigs (Johansen and Jensen, 1997) have demonstrated that about 25% of ingested D-tagatose is absorbed. The absorbed D-tagatose is metabolized in the liver via a pathway similar to that of D-fructose. D-Tagatose is phosphorylated to D-tagatose 1-phosphate by fructokinase (Raushel and Cleland, 1973). Then D-tagatose 1-phosphate is cleaved by aldolase to D-glyceraldehyde and dihydroxyacetone phosphate (Martinez *et al.*, 1987). Infused D-fructose transiently decreases inorganic phosphate (P_i) in rat and human livers by formation of fructose 1-phosphate (Masson *et*

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TABLE 1
Prestudy Characteristics of Normal (NL) and Type 2 Diabetic (DM) Subjects[a]

Variable	NLs	DMs
N	8	8
Male/female	4/4	4/4
Age	43.6 ± 1.8[b,c]	53.8 ± 4.2
Weight (kg)	92.0 ± 8.0	83.0 ± 5.2

[a] There were no significant differences in gender distribution or weight between NLs and DMs.
[b] Data are means ± SE.
[c] Values are significantly different at $P < 0.05$.

al., 1994). Since D-tagatose 1-phosphate is cleaved at a slower rate than D-fructose 1-phosphate, P_i is complexed by D-tagatose for a longer time than by D-fructose (Vincent *et al.*, 1989). Thus, D-tagatose can also be expected to cause hepatic P_i reduction.

Since P_i is an inhibitor of adenosine deaminase, a rate-limiting enzyme in the degradation of AMP,[2] reduction of P_i in the liver may lead to increased degradation of purine nucleotides (Van den Berghe, 1986). Consequently, the release of uric acid from the liver may increase. Compounds given intravenously such as D-fructose (Kogut *et al.*, 1975; Narins *et al.*, 1974), sorbitol (Förster and Hoos, 1977), and xylitol (Förster and Hoos, 1977; Yamamoto *et al.*, 1995) produce a hyperuricemic effect in humans. Fructose (Buemann *et al.*, 1998b; Davies *et al.*, 1998; Förster and Ziege, 1971; Förster *et al.*, 1972; Macdonald *et al.*, 1978; Oberhaensli *et al.*, 1987; Reiser *et al.*, 1984; Stirpe *et al.*, 1970), sorbitol, and xylitol (Förster and Ziege, 1971; Förster *et al.*, 1972) given orally at approximately 1 g/kg body weight also produce hyperuricemia in humans.

The main purpose of this study was to determine if either acute or repeated oral ingestion of D-tagatose would cause elevations in plasma uric acid in normal and/or Type 2 diabetic humans.

MATERIALS AND METHODS

Subjects and Study Design

Eight normal human subjects and eight subjects with Type 2 diabetes mellitus (DM) were enrolled in the study, with each group consisting of four males and four females. The Type 2 diabetics ranged in age from 31 to 70, were diagnosed with diabetes at least 1 year prior to this study, and were under treatment by diet only, or by diet combined with sulfonylurea therapy. Prior to study initiation, there were no significant differences between diabetic and normals with respect to gender distribution and body weight (Table 1). Using the criteria of the World Health Organization (WHO, 1985), Type 2 DM was confirmed by the results of a 75-g oral glucose tolerance test (GTT). Except for diabetes, the subjects were healthy. No subject was admitted with poorly controlled or insulin-dependent diabetes, gastrointestinal disorders such as gastroparesis or diarrhea, glucocorticoid treatment, or treatment with other research drugs or medications which could affect gastrointestinal motility and/or absorption. Prior to study initiation, protocols were approved by the Institutional Review Board of the University of Maryland, and subjects were allowed into the study only after giving written informed consent.

Materials

D-Tagatose was synthesized by Biospherics Incorporated, Beltsville, Maryland. The purity was found to be 99% by HPLC analysis. The doses of D-tagatose and sucrose to be consumed by the subjects on each of the experimental days were weighed and packaged by the University of Maryland pharmacy prior to distribution.

Tolerance Testing

On the first day of the study, each subject was given a single-dose 75-g 3-h oral glucose tolerance test. Over the next 3 days, all subjects were adapted to D-tagatose by consuming 5, 10, and 25 g single doses of D-tagatose on three consecutive days. On the fifth day of the study, each subject was given a 75-g 3-h oral D-tagatose tolerance test. After a 12-h fast, all tolerance testing was conducted at 8:00 AM. Diabetic subjects being treated with sulfonylureas ($n = 2$) received their typical morning dose at 7:30 AM. Blood samples were collected from each subject at 0, 30, 60, 120, and 180 min after dose and analyzed for magnesium, phosphorus, and uric acid.

8-Week Study

The eight normals were randomly selected to receive, respectively, either 75 g (25 g with each meal) D-tagatose or 75 g sucrose daily for 8 weeks. The eight diabetics were given either 75 g D-tagatose or no sugar supplementation daily for 8 weeks. For all subjects, fasting analyses of plasma magnesium, phosphorus, uric acid, lipids [total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides], glycosylated hemoglobin, glucose, insulin, body weight, and blood pressure (BP) were determined at baseline (Week 0) and biweekly over the next 8 weeks. Fasting plasma copper was measured only in the eight normals at baseline and biweekly for 8 weeks. Liver enzymes (fasting plasma alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and total bilirubin) were measured in all subjects at Weeks 6 and 8 only.

[2] Abbreviations used: AMP, adenosine monophosphate; ANOVA, analysis of variance; ATP, adenosine triphosphate.

Laboratory Evaluations

A Vitros 950 Chemistry System (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY) using Vitros slides was used to analyze plasma magnesium, phosphorus, and uric acid in the Chemistry Laboratory at the University of Maryland Medical Center. The laboratory is accredited by the College of American Pathologists (Chicago, IL). A Vitros 700 chemistry system using Vitros slides was used to analyze plasma total cholesterol, HDL cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and total bilirubin. All reactions needed for a single quantitative measurement occur within the multilayered analytical element (coated on a polyester support) of a Vitros slide. Slides are provided in cartridges that are specific for one type of test. A slide is used once for a single test and then discarded.

Magnesium was determined by a colorimetric test in which magnesium (both free and protein-bound) reacts with the formazan dye derivative in the reagent layer of the slide. The high magnesium affinity of the dye dissociates magnesium from binding proteins. The resulting magnesium dye complex causes a shift in the dye absorption maximum from 540 to 630 nm. The amount of dye complex formed is proportional to the magnesium concentration in the sample and is measured by reflection density.

Plasma phosphorus was determined by a colorimetric test in which phosphorus in the sample forms a complex with ammonium molybdate in the reagent layer of the slide. The complex is reduced by *p*-methylaminophenol sulfate to yield a stable heteropolymolybdenum blue chromophore. The concentration of phosphorus in the sample is determined by measuring the heteropolymolybdenum blue complex by reflectance spectrophotometry at a wavelength of 680 nm.

Uric acid was measured by a colorimetric test in which uric acid from the sample migrates to the reagent layer of the slide, where it is oxidized in the presence of uricase to form allantoin and hydrogen peroxide. Hydrogen peroxide reacts with a leuco dye to produce a colored compound that is measured by reflectance spectrophotometry at a wavelength of 670 nm.

Plasma copper was analyzed at Mayo Medical Laboratories (Rochester, MN) by inductively coupled plasma emission spectroscopy using a Model ICP-2000 from Perkin–Elmer (Norwalk, CT).

Total cholesterol was determined by a colorimetric test in which Triton X-100 surfactant in the spreading layer of the slide helps dissociate cholesterol and cholesterol esters from lipoprotein complexes in the sample. Hydrolysis of the cholesterol esters to cholesterol is catalyzed by cholesterol ester hydrolase. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to produce a colored dye. The density of dye formed is proportional to the cholesterol concentration present in the sample and is measured by reflectance spectrophotometry at a wavelength of 540 nm.

HDL cholesterol was determined with a colorimetric method after first pretreating the sample by separating HDL by precipitation of LDL and very low density lipoproteins (VLDL), using dextran sulfate (MW 50,000) and magnesium chloride. The precipitated lipoproteins are removed by centrifugation. HDL cholesterol in the pretreated sample undergoes reactions to form hydrogen peroxide. Hydrogen peroxide reacts with a leuco dye yielding a blue dye complex. After measuring the amount of light from the dye after a fixed incubation period, the amount of HDL cholesterol present in the pretreated sample is calculated. The wavelength used was 670 nm.

Triglycerides were measured by a colorimetric test in which Triton X-100 surfactant in the spreading layer of the slide helps to dissociate the triglycerides from lipoprotein complexes in the sample. The triglyceride molecules are then hydrolyzed by lipase yielding glycerol and fatty acids. Glycerol diffuses to the reagent layer of the slide, where it is phosphorylated by glycerol kinase in the presence of ATP. In the presence of L-α-glycerol-phosphate oxidase, L-α-glycerophosphate is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The final reaction involves the oxidation of a leuco dye by hydrogen peroxide, catalyzed by peroxidase, to produce a dye. The density of the dye formed is proportional to the triglyceride concentration in the sample and is measured by reflectance spectrophotometry at a wavelength of 540 nm.

LDL cholesterol was calculated from the results of total cholesterol, HDL cholesterol and triglycerides.

The formulas are as follow:

$$\text{VLDL} = \text{Triglycerides}/5$$

$$\text{LDL} = \text{Total cholesterol} - \text{HDL cholesterol} - \text{VLDL}$$

Calculation of LDL cholesterol is inappropriate for samples with triglyceride concentrations above 400 mg/dL.

Alanine aminotransferase (ALT) was determined by a multiple-point rate test in which the spreading layer of the slide contains the ALT substrates L-alanine and sodium α-ketoglutarate. Alanine aminotransferase catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate to produce pyruvate and glutamate. Lactate dehydrogenase then catalyzes the conversion of pyruvate and NADH to lactate and NAD^+. The oxidation rate of NADH is monitored by reflectance spectrophotometry at a wavelength of 340 nm. The rate of

change in reflection density measured in a linear region is then converted to enzyme activity.

Alkaline phosphatase was measured by a multiple-point rate test in which the spreading layer of the slide contains the *p*-nitrophenyl phosphate substrate and other components needed for the reaction. The alkaline phosphatase in the sample catalyzes the hydrolysis of the *p*-nitrophenyl phosphate to *p*-nitrophenol at alkaline pH. The *p*-nitrophenol, which absorbs light at wavelengths in the region of 400 nm, diffuses into the underlying layer, and is monitored by reflectance spectrophotometry at a wavelength of 400 nm. The rate of change in reflection density is converted to enzyme activity.

Aspartate aminotransferase was determined by a multiple-point rate test in which the amino group of L-aspartate is transferred to α-ketoglutarate in the presence of pyridoxal 5-phosphate to yield glutamate and oxaloacetate. The oxaloacetate formed by the deamination of the L-aspartate is converted to pyruvate and carbon dioxide by oxaloacetate decarboxylase. Pryuvate is oxidized to acetyl phosphate and hydrogen peroxide by pyruvate oxidase. The final reaction step involves the peroxidase-catalyzed oxidation of leuco dye resulting in a colored dye. The rate of oxidation of the leuco dye is monitored by reflectance spectrophotometry at 37°C with a wavelength of 670 nm. The rate of change in reflectance density is measured over a linear region and then converted to enzyme activity in units per liter. The low wavelength cutoff filter on the slide support minimizes the blank rate effects of incident light during dye development.

Total bilirubin was determined by a colorimetric method in which the spreading layer of the slide provides a reflective background for measuring the diazo products of bilirubin and contains all reagents to determine total bilirubin. The method uses dyphylline to dissociate unconjugated bilirubin from albumin. Unconjugated bilirubin, conjugated bilirubin, and albumin-linked bilirubin then react with the diazonium salt 4-(*N*-carboxymethylsulfamyl) benzenediazonium hexafluorophosphate to produce azobilirubin chromophores that have similar molar absorptivities and absorbance maxima around 520 nm. The concentration of total bilirubin is determined by measuring the azobilirubin chromophores at two wavelengths through the transparent support. Reflectance measurements at 540 and 460 nm are taken after 5 min incubation at 37°C. The measurement at 460 nm corrects for spectral interferences.

Glycosylated hemoglobin (GlyHb) values were determined by the Helena Glyco-Tek Affinity Column Method (University of Maryland Pediatric Endocrine Laboratories) which has a normal range of 4.4 to 7.7% and a mean intraassay coefficient of variation of 2.95%. Plasma glucoses were analyzed by the glucose oxidase method. Insulin levels were determined by the Coates-A-Count RIA method (Diagnostics Products Corp., Los Angeles).

Statistical Analysis

Two types of analyses were conducted on the data, correlation analyses and ANOVA repeated measures analyses. Even though the number of subjects enrolled in the study was relatively small, the statistical hypothesis testing was designed to take advantage of the fact that subjects were followed over time. This allowed for an effectively larger n value and increased the power for the tests performed. Correlation analyses were performed to validate the tests by confirming the expected relations between the various clinical parameters measured in the study. Pearson *r* product–moment correlation coefficients (Snedecor and Cochran, 1974) were calculated. This correlation determines the extent to which values of two variables are "proportional" to each other. The value of the correlation does not depend on the specific measurement units used. Repeated measures ANOVA designs (Neter and Wasserman, 1977) were used to take advantage of the multiple measurements on each of the subjects. These tests have two major advantages: a smaller number of subjects need to be enrolled, and the effects of large between-subject variations are minimized. All statistical testing was conducted at the $P < 0.05$ alpha level. Levene's test (Milliken and Johnson, 1984) was used to test the homogeneity of variance assumptions for the ANOVA designs. While there were scattered instances of nonhomogenous variances, most values met the necessary assumptions for the ANOVA procedures. When results indicated, specific contrasts were run to compare study endpoints and maximal values to pretreatment time intervals. In the tolerance testing, the variable uric acid–area under the curve (AUC) was calculated using the trapezoid method for curve integration. Comparisons of the prestudy values for age and body weight (Table 1) between the diabetic and normal groups were performed using a two-tailed Student's *t* test. Statistics were calculated using the statistical software package Statistica, version 5.1 H, 1998 (StatSoft, Inc., Tulsa, OK).

RESULTS

Tolerance Testing

For both normal and diabetic subjects, plasma uric acid levels on average peaked at 60 min in the D-tagatose tolerance test (Tables 2 and 3), although these increases did not reach statistical significance ($P = 0.06$). Each normal subject's individual values are presented in Table 2, and each diabetic subject's individual values are presented in Table 3. Only the normal males in the D-tagatose tolerance test (TTT) had mean values increase above the upper limit of the normal

TABLE 2
Plasma Uric Acid Values[a] (mg/dL) of Normal Subjects during the Tolerance Tests

	Glucose tolerance test[b]						Tagatose tolerance test[c]				
	Test time (min)						Test time (min)				
Age	0	30	60	120	180	Age	0	30	60	120	180
						Males					
42	10.1	9.3	9.7	9.8	9.9	42	11.2	12.6	12.8	12.9	12.4
50	7.4	6.9	6.8	7.0	7.5	50	7.7	8.5	9.1	7.5	6.0
40	6.6	6.1	6.3	6.4	6.0	40	6.7	8.9	8.4	8.5	7.7
53	6.2	6.2	6.0	5.9	6.2	53	6.2	8.5	8.9	8.6	7.9
Mean:	7.6	7.1	7.2	7.3	7.4	Mean:	8.0	9.6	9.8	9.4	8.5
SD:	1.8	1.5	1.7	1.7	1.8	SD:	2.2	2.0	2.0	2.4	2.7
						Females					
40	3.6	3.8	3.9	3.7	3.2	40	2.3	2.8	3.1	3.0	4.9
43	3.3	3.4	3.4	3.9	3.4	43	3.6	5.4	5.9	5.3	5.2
38	5.2	5.0	5.0	5.0	5.0	38	4.9	6.3	7.4	6.6	6.2
43	4.5	4.5	4.3	4.4	4.3	43	4.4	6.3	6.3	6.1	5.6
Mean:	4.2	4.2	4.2	4.2	4.0	Mean:	3.8	5.2	5.7	5.2	5.5
SD:	0.9	0.7	0.7	0.6	0.8	SD:	1.1	1.6	1.8	1.6	0.6

[a] Normal range: males, 3.5–8.5 mg/dL; females, <34 years, 2.5–6.2 mg/dL, 35–44 years, 2.5–7.0, 45 and over, 2.5–7.5.
[b] Subjects were given a 75 g dose of glucose orally at time 0.
[c] Subjects were given a 75 g dose of D-tagatose orally at time 0.

TABLE 3
Plasma Uric Acid Values[a] (mg/dL) of Type 2 Diabetic Subjects during the Tolerance Tests

	Glucose tolerance test[b]						Tagatose tolerance test[c]				
	Test time (min)						Test time (min)				
Age	0	30	60	120	180	Age	0	30	60	120	180
						Males					
55	8.0	6.1	8.2	5.7	8.2	55	8.8	9.3	9.4	10.0	7.6
47	6.6	6.1	6.3	6.0	6.0	47	7.2	7.8	6.6	6.9	7.0
53	5.5	5.3	5.8	5.1	5.4	53	5.2	6.6	6.9	6.3	6.9
67	8.4	8.3	8.1	5.9	5.5	67	7.6	9.0	9.5	9.3	7.3
Mean:	7.1	6.4	7.1	5.7	6.3	Mean:	7.2	8.2	8.1	8.1	7.2
SD:	1.3	1.3	1.2	0.4	1.3	SD:	1.5	1.2	1.6	1.8	0.3
						Females					
31	5.2	5.2	5.4	4.9	4.5	31	5.3	5.6	7.1	7.1	7.0
55	3.5	3.3	3.2	3.2	3.3	55	3.1	2.9	2.8	2.7	2.6
52	7.9	7.7	8.1	6.6	6.9	52	8.5	10.2	11.4	9.7	9.2
39	4.1	3.8	3.8	4.0	4.2	39	3.7	4.3	4.8	5.3	5.4
Mean:	5.2	5.0	5.1	4.7	4.7	Mean:	5.2	5.8	6.5	6.2	6.0
SD:	1.9	2.0	2.2	1.5	1.5	SD:	2.4	3.2	3.7	3.0	2.8

[a] Normal range: males, 3.5–8.5 mg/dL; females, <34 years, 2.5–6.2 mg/dL, 35–44 years, 2.5–7.0, 45 and over, 2.5–7.5.
[b] Subjects were given a 75 g dose of glucose orally at time 0.
[c] Subjects were given a 75 g dose of D-tagatose orally at time 0.

range of 3.5–8.5 mg/dL for uric acid. There was a statistically significant ($P = 0.03$) treatment-related increase in values for uric acid–AUC when both normal and diabetic TTT were compared with normal and diabetic GTT. Uric acid AUC (mg/dL × min) was highest for the normal male subjects in the TTT (1666 vs 1310 for normal males in the GTT), followed by the diabetic males in the TTT (1421 vs 1149 for diabetic males in the GTT), the diabetic females in the TTT (1097 vs 880 for diabetic females in the GTT), and the normal females in the TTT (948 vs 748 for normal females in the GTT). However, there were no statistically significant differences for TTT uric acid-AUC compared to GTT uric acid-AUC for any of the above groups.

A greater decrease in plasma phosphorus was observed during the 30- and 60-min periods of the D-tagatose tolerance test compared to the glucose tolerance test (data not shown). However, no statistically significant effects were observed, and all mean values were within the normal range. A slight non-statistically significant increase in plasma magnesium was observed during the D-tagatose tolerance test (data not shown). All mean values were within the normal range for plasma magnesium.

8-Week Study

No significant changes occurred in fasting plasma uric acid levels within each treatment group over the course of the 8-week study. Statistically significant differences in plasma uric acid levels between groups were found. These significant differences in uric acid levels were seen only in the females ($P = 0.01$ vs 0.85 for males), and were more pronounced in the diabetic females (Table 4). These significant differences were attributed to the much higher baseline levels for uric acid in the female diabetic group receiving D-tagatose (mean of 10.3 mg/dL) versus the female diabetics who did not receive D-tagatose (mean of 3.8 mg/dL). Baseline uric acid levels were also higher in normal females receiving D-tagatose (mean of 5.5 mg/dL) versus normal females receiving sucrose (mean of 3.6 mg/dL). These higher baseline levels did not increase during the 8 weeks of D-tagatose administration. Based on these findings, the elevated plasma uric acid levels observed were judged to be unrelated to D-tagatose administration. Within each treatment group, no statistically significant differences were found comparing values at Week 8 to those at Week 0.

Statistically significant differences ($P < 0.03$) between the control and treated groups were observed in systolic BP. However, elevated levels for systolic BP were seen at Week 0 of the study, prior to administration of D-tagatose, when comparing both diabetics and normals who received D-tagatose to their respective controls (Table 5). Increases in systolic BP during the 8-week study were observed in the male and female diabetic groups given D-tagatose and in normal males given D-tagatose. A statistically significant increase in systolic BP ($P < 0.03$) at Week 8 was observed when comparing the diabetic controls to the diabetics given D-tagatose. Within each treatment group, no significant differences were found comparing values at Week 8 to those at Week 0. No treatment-related differences were observed with respect to diastolic BP (Table 5).

There were no statistically or clinically significant results relating to administration of D-tagatose in the diet for 8 weeks in the plasma liver enzymes, lipids, phosphorus, magnesium, copper, glucose, glycosylated hemoglobin, insulin (data not shown), and body weight (Table 5) in normals or diabetics.

Seven of the eight subjects given daily doses of 75 g D-tagatose in the 8-week study (both diabetics and

TABLE 4
Plasma Uric Acid Values (mg/dL) of Normal and Type 2 Diabetic Subjects during the 8-Week Study

Age	Week 0	Week 2	Week 4	Week 6	Week 8
			Normals: Sucrose[a]		
Males					
42	11.7	9.5	5.1	7.8	9.8
50	6.3	5.6	6.0	6.9	5.6
Females					
40	3.8	3.9	3.3	3.1	1.9
43	3.4	3.3	3.5	3.2	3.0
			Normals: Tagatose[b]		
Males					
40	6.4	5.5	9.3	6.9	6.8
53	7.1	7.1	6.9	7.3	7.4
Females					
38	6.8	6.0	6.0	6.5	5.7
43	4.2	4.9	4.8	4.7	5.0
			Diabetics: No sugar[c]		
Males					
55	8.9	9.2	8.7	6.4	6.5
47	7.0	6.8	5.9	7.0	6.2
Females					
31	4.8	4.6	4.9	4.3	4.9
55	2.8	2.7	2.5	3.1	3.1
			Diabetics: Tagatose[b]		
Males					
53	9.1	6.7	8.1	6.7	7.7
67	8.5	8.0	7.5	8.8	8.2
Females					
52	11.3	9.5	9.7	9.7	9.6
70	9.3	6.0	9.7	7.5	8.5

[a] Subjects consumed 75 g sucrose (25 g with each meal) daily for 8 weeks.
[b] Subjects consumed 75 g D-tagatose (25 g with each meal) daily for 8 weeks.
[c] Subjects were given no sugar supplements.

TABLE 5
Body Weight and Blood Pressure Values[a] of Normal and Type 2 Diabetic Subjects during the 8-Week Study

	Normals: Sucrose[b]		Normals: Tagatose[c]		Diabetics: No sugar[d]		Diabetics: Tagatose[c]	
Week	Females	Males	Females	Males	Females	Males	Females	Males
				Body weight (kg)				
0	66.6	107.1	95.0	99.0	78.6	94.8	82.0	76.6
2	66.4	106.6	94.2	98.8	78.4	95.0	80.6	77.0
4	67.0	106.4	94.6	99.9	78.0	95.8	81.5	77.8
6	67.2	106.0	95.7	99.2	78.2	96.4	81.2	77.8
8	67.5	105.2	96.1	99.6	78.1	97.6	82.7	78.0
				Systolic BP (mm Hg)[e,f]				
0	98	128	120	130	110	128	144	132
2	96	128	124	129	121	112	150	128
4	100	132	106	127	112	124	158	146
6	96	124	118	134	124	124	171	149
8	100	130	122	144	98	129	168	152
				Diastolic BP (mm Hg)[g]				
0	57	72	73	83	75	81	83	76
2	57	76	66	85	74	76	84	74
4	54	76	64	74	70	74	86	74
6	55	70	68	80	78	82	91	76
8	60	78	72	86	70	82	90	82

[a] Values are means of 2 subjects.
[b] Subjects consumed 75 g sucrose (25 g with each meal) daily for 8 weeks.
[c] Subjects consumed 75 g D-tagatose (25 g with each meal) daily for 8 weeks.
[d] Subjects were given no sugar supplements.
[e] BP, blood pressure.
[f] Normal values <140 mm Hg.
[g] Normal values <90 mm Hg.

normals) experienced flatulence, and six of the eight subjects had some degree of diarrhea with varying frequencies. In general, no improvement over the 8 weeks was reported for these gastrointestinal side effects.

DISCUSSION

Because D-tagatose has potential for use as a sweetener, it is important to investigate possible safety issues. As part of a detailed safety study program, effects of D-tagatose on the blood chemistry of normal and Type 2 diabetic humans were studied. Although some statistically significant differences were found among the parameters measured, the variations from normal values were not considered clinically significant. In the statistical analysis, data were aggregated and means were presented. However, this was supplemented by examining each subject's values in tracking changes in a small population with considerable variation. The latter approach is a clinician's preferred method of analyzing the results. We found that the conclusions from the statistical analyses were compatible and consistent with the clinical evaluation.

There was a consistent rise in plasma uric acid levels in all subjects (except one diabetic female) during the D-tagatose tolerance test which peaked at 30 or 60 min, and generally did not return to baseline by 180 min. This effect was similar in diabetics and normals. It is likely that enhanced AMP degradation following inorganic phosphorus depletion accounts for this effect (Vincent *et al.*, 1989). The renal excretion of uric acid does not appear to be impaired by D-tagatose ingestion (Buemann *et al.*, 1998a, b). One of the normal male subjects was hyperuricemic throughout both the glucose and D-tagatose tolerance tests. The 8-week study data demonstrated that he was insulin-resistant, with fasting insulin levels greater than 20 μU/mL (the upper limit of the normal range). Insulin resistance is associated with hyperuricemia (Davidson, 1995).

An acute effect of D-tagatose on uric acid metabolism was also seen in a study in which 8 male subjects received 400 mL water with 30 g D-tagatose, 30 g fructose, or pure water only (Buemann *et al.*, 1998b). Serum uric acid levels reached slightly higher levels after ingestion of D-tagatose than after fructose or water. The urinary excretion of uric acid was also slightly higher after D-tagatose intake. In another study by Buemann *et al.* (1998a), no effect on plasma uric acid

was observed 21 h after a 30-g oral dose of D-tagatose, compared to a 30-g oral dose of sucrose given to eight subjects (three and five females). Analyses of 24-h composite urine samples revealed a slight, but nonsignificant, increase in urate excretion following a 30-g oral dose of D-tagatose, compared to a 30-g oral dose of sucrose.

The increase of plasma uric acid levels seen in tolerance testing was not reflected in fasting plasma uric acid values during the 8-week study. There was no significant trend in any subject's course. In another study (Buemann *et al.*, 1998b), plasma uric acid levels returned to baseline 5 h after a 30-g oral dose of D-tagatose.

Similar to earlier observations on fructose (Buemann *et al.*, 1998b), the increase of plasma uric acid was associated with a slight decrease of plasma phosphorus and a slight increase of plasma magnesium. During the 30- and 60-min periods of 75-g D-tagatose tolerance tests, there was a statistically nonsignificant greater decrease in plasma phosphorus than with the glucose tolerance tests, and it appeared to be consistent, although some subjects' changes were much larger than others. This decrease in plasma phosphorus levels is consistent with changes associated with an increase in phosphoribosyl pyrophosphate (PRPP), which is strongly rate-limiting for uric acid production (Mayes, 1993), as described in hepatocytes exposed to D-tagatose by Vincent *et al.* (1989). There was also a slight rise in plasma magnesium during the D-tagatose tolerance test. D-Tagatose decreases the concentration of ATP in hepatocytes, which in turn results in increased plasma magnesium. ATP is a strong chelator of magnesium (Vincent *et al.*, 1989).

In the 8-week study, there was some suggestion of improvement with D-tagatose in fasting HDL and LDL cholesterol, but the results did not show statistical significance. Some subjects had a normal lipid profile and others had an elevated lipid profile at baseline, and neither changed significantly. Changes in plasma phosphorus and magnesium followed no significant or consistent pattern and could have been caused by variations in dietary intake or episodes of diarrhea, but were not attributed to D-tagatose administration.

The statistically significant increase in systolic BP for Week 8 when comparing the diabetic controls to the diabetics given D-tagatose is probably anomalous. There are no known physiological mechanisms for monosaccharides to cause increases in systolic BP in the absence of significant weight gain.

In conclusion, it was found that the ingestion of a single 75-g dose of D-tagatose increased plasma uric acid levels during a 3-h period in normal and Type 2 diabetic humans. However, this increase was not statistically significant in comparison to a control treatment with 75 g glucose, and it was not apparent after overnight fasting following ingestion of 3 × 25 g/day D-tagatose for 8 weeks. The results of the 8-week study do not suggest a cumulative effect on plasma uric acid. The increases in plasma uric acid levels during the 75-g tolerance testing were not prolonged or severe enough to achieve clinical significance. Acute uric acid nephropathy is rare when plasma uric acid levels are less than 15 to 20 mg/dL (Brady *et al.*, 1996). Fessel (1979) examined the clinical course of 113 patients with asymptomatic hyperuricemia, and 168 patients with gout, and concluded that azotemia attributable to hyperuricemia is generally mild, and probably of no clinical importance, until serum uric levels reach 13 mg/dL in males and 10 mg/dL in females for a sustained time period (several decades). Among 1356 men, age 60 to 69 years when serum uric acid was measured, follow-up for 10 years revealed no death from renal failure attributable to hyperuricemia (Fessel, 1979). In the Framingham study (Hall *et al.*, 1967), 12-year follow-up of 5127 subjects identified only 4 in whom preexisting hyperuricemia was possibly a contributing factor to the development of unspecified renal disease. In a study of matched pairs of 391 gouty patients and 391 control subjects, only 12 (3.1%) of the gouty patients had azotemia. Furthermore, hyperuricemia preceded azotemia, or the two were found simultaneously, in only 0.25 to 1.0% of these patients (Fessel, 1979). In the Fessel study, subjects with asymptomatic hyperuricemia had a small risk of urolithiasis (formation or presence of renal calculi), approximately one stone per 295 patients per year, compared with normouricemic control subjects (one stone per 852 patients per year), and patients with established gout (one stone per 114 patients per year). For the gouty patients, initial serum uric acid levels were not good predictors of future occurrence of calculi. Serum uric acid levels were similar in gouty subjects, regardless of whether or not they developed stones. Therefore, the transient elevations in plasma uric acid levels observed after consumption of acute doses of D-tagatose are not expected to pose an increased risk to people with asymptomatic hyperuricemia or gout. In our study, one diabetic female attained plasma uric acid levels above 10 mg/dL, and then only at 30 and 60 min (10.2 and 11.4 mg/dL, respectively) after the 75-g dose of D-tagatose. Furthermore, similar acute increases have been observed with D-fructose, a safe and widely consumed sugar (average per capita consumption in the United States is 71 g/day; Hallfrisch, 1990) in the United States since the late 1970's.

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