Physiological Changes in Circulating Mannose Levels in Normal, Glucose-Intolerant, and Diabetic Subjects

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Mannose is an essential hexose that is required for glycoprotein synthesis. Although circulating mannose levels are known to be influenced by metabolic disorders, how physiological levels of mannose fluctuate in normal and diabetic subjects is largely unknown. We describe a new accurate and sensitive assay for determining circulating mannose levels, which we used to measure plasma mannose levels in 273 normal and diabetic (DM) subjects. Our results revealed a clear correlation (r = 0.754) between fasting plasma mannose (FPM) and fasting plasma glucose (FPG) levels. Our mannose assay showed sensitivity and specificity comparable to that seen for hemoglobin A_{1c} (HbA_{1c}) assay in subjects with impaired glucose tolerance (IGT) or DM whose FPG levels were normal. Mannose levels were found to increase less than glucose levels in response to an oral glucose tolerance test (OGTT). Furthermore, plasma mannose levels did not significantly change following a meal and more closely correlated with the coefficient of variation (CV) of daily glucose levels than did glucose itself. In conclusion, the close correlation between FPM and FPG levels taken together with the small fluctuations seen in plasma mannose in response to glucose suggests that the measurement of mannose using our assay could potentially play a supplementary role in the diagnosis and screening of patients with mild DM.

MANNOSE ($C_6H_{12}0_6$), a C2 epimer of D-glucose, is one of the carbohydrate components of the glycoproteins that are attached to the surface of cells. This sugar plays important physiological roles by participating in N-glycosylation reactions,[1] glycophospholipid anchor synthesis,[2] and the mannosylation of proteins such as mannose-binding lectin,[3,4] the latter of which plays a critical role in innate immune system function. There have been a few reports[2,5-7] describing the significance of mannose metabolism as an energy source under physiological and pathological conditions. Dietary mannose is absorbed by the digestive tract,[8] although mannose can also be synthesized from glucose via a cellular enzymatic pathway involving the phosphorylation of hexose intermediates.[7]

Circulating levels of monosaccharides are a reflection of systemic glucose/energy metabolism and characteristic changes in these levels are seen in patients with diabetes and other metabolic disorders.[9,10] Unbound, plasma mannose in humans has been measured using enzymatic,[11-14] gas-liquid chromatographic/mass spectrometric,[15,16] and capillary electrophoretic[17] techniques and preliminary data in a small number of subjects suggested that its levels were elevated in diabetic patients.[12,14,16,18] Pitkanen et al[19] reported that serum mannose levels in 20 diabetic patients correlated with their triglyceride, high-density lipoprotein (HDL)-cholesterol, and glucose levels, suggesting that mannose levels might be influenced by metabolic pathways other than those involving carbohydrates. However, the relationship between circulating levels of mannose and the pathophysiology of impaired glucose tolerance (IGT) and diabetes mellitus (DM) is largely unknown. We report the successful development of a new method for measuring circulating mannose levels that utilizes bacterial-derived aldohexose dehydrogenase (ALDH), which enabled us to determine plasma and serum mannose levels accurately and simply without interference from blood glucose. We used this new assay system to determine the circulating levels of mannose in normal, glucose-intolerant, and DM subjects and to evaluate its potential for clinical application.

MATERIALS AND METHODS

Study Subjects

Blood samples were collected from 273 consecutively recruited subjects (age, 25 to 75 years) who received diabetes care or screening at our clinic from January until March of 2001; of these, 140 (108 men, 32 women, 48.9 ± 11.5 years of age; age range, 27 to 73) underwent oral glucose (75 g) tolerance testing (OGTT). The OGTT was performed between 8 and 10 AM after an overnight fast of between 10 and 14 hours. Subjects were classified as having normal glucose tolerance (NGT), IGT, or DM using the World Health Organization (WHO) criteria.[20] Subjects who had abnormal hepatic or renal function were excluded from this study. Blood samples were taken from all other subjects after an overnight fast and, where relevant, before subjects took their first dose of insulin or hypoglycemic medication. Blood samples were immediately centrifuged at 4°C, and plasma was removed and frozen at −20°C until analyzed. This study was approved by the Institutional Review Board of the University of Tsukuba and written informed consent was obtained from all subjects.

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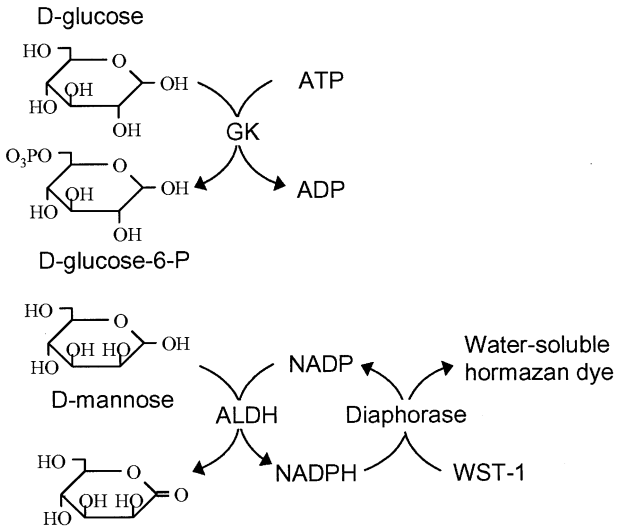

Fig 1. Reaction scheme of our newly developed assay for determining circulating mannose levels.

Determination of Plasma Mannose Concentrations

Mannose concentrations were determined using a colorimetric technique that utilized the water-soluble hormazan dye. The basis of the assay is the reduction of this dye by diaphorase and tetrazolium compounds (WST-1) and the subsequent quantification of NADPH generated by the enzymatic action of NADP-dependent ALDH. This method necessitated the prior elimination of glucose from the samples, since ALDH also catalyses glucose, 2-deoxy-glucose, and 2-amino-2-deoxy-mannose in addition to mannose.[21] To remove high concentrations of glucose from our samples, we converted the glucose into glucose-6-phosphate (G-6-P) using glucokinase (GK)-adenosine triphosphate (GK-ATP) (Fig 1); ALDH does not react with G-6-P, and GK from *Bacillus stearothermophilus* does not react with mannose.[22] Purified ALDH was prepared according to previously described methods,[21] with the exception that we used the IFO3276 strain of *Gluconobacter asaii* (Institute for Fermentation, Osaka, Japan) and added purification steps. ALDH activity was determined at 37°C by measuring the absorbance at 340 nm in the preparative buffer (50 mmol/L mannose, 1 mmol/L NADP, and 100 mmol/L Tris, pH 9.0). One unit of enzyme activity was defined as the amount of the enzyme that produced 1 μmol of NADPH per minute under the above assay conditions.

Reagents used in the mannose assay contained the following ingredients: reagent 1 contained 6.25 kU of GK (Unichika, Osaka, Japan), 6.25 kU of Diaphorase (Toyobo, Osaka, Japan), 10 mmol of ATP, 1.25 mmol of NADP, 0.75 mmol of WST-1 (Dojindo Laboratories, Tokyo, Japan), 3 mmol of $MgCl_2$, and 1.25 (wt/vol)% of Tween 20 per liter of Tris-HCl buffer (125 mmol/L, pH 8.5). Reagent 2 contained 2.3 kU of ALDH per liter of potassium phosphate buffer (20 mmol/L, pH 7.0). The concentration of plasma mannose was determined using a clinical automated analyzer (Hitachi 7170, Tokyo, Japan). The mannose assay was performed as follows: 6 μL of sample was incubated with 192 μL of reagent 1 for 5 minutes at 37°C, and the absorbance at 700 (sub)/450 (main) nm was measured. The reaction was then immediately initiated by the addition of 42 μL of reagent 2. After 5 minutes, the absorbance was measured again. The mannose concentration in our samples was determined by referring to a calibration curve generated with a calibrator, a 10-μg/mL concentration of mannose, and saline (0 μg/mL of mannose). Typical time courses for the reaction using the Hitachi 7170 Automated Analyzer are shown in Fig 2. A D-mannose calibrator and two serum samples were run as controls. About 5 minutes after the addition of reagent 2 (ALDH), the reaction reached a plateau.

The detection limit of the assay, defined as the minimum concentration of mannose that was detectably different than 0 at the 95% confidence level, was 0.5 μg/mL. The degree of imprecision of the assay was evaluated by analyzing 2 samples with low (3.4 μg/mL) and high (16.6 μg/mL) concentrations of mannose. The intra-assay coefficients of variation (CVs) (n = 10) were 3.2% and 0.8%, respectively, whereas the interassay CVs (n=5) were 4.0% and 1.2%, respectively. The linearity of the assay was evaluated by measuring mannose levels in pooled serum samples to which increasing concentrations of mannose (0 to 500 μg/mL) were added (1:9 by volume). The linear regression formula of absorbance (y) versus mannose concentration (x) was $y = 4.59x + 35.2$ mOD ($r = 0.999$). Analytical recovery of the assay was evaluated by measuring mannose levels in 5 serum samples to which a 100-μg/mL solution of mannose was added (1:9 by volume), and showed 92% to 98% recovery. To determine the degree of assay interference that was due to glucose, we measured mannose levels in pooled serum samples to which varying concentrations of a glucose solution (0 to 10 g/dL) were added (1:9 by volume). Similarly, different types of sugars such as fructose, galactose, maltose, fucose, sorbitol, mannitol, xylitol, and myo-inositol were individually added into the test blood samples to determine whether they interfered with the assay. Our results confirmed that the assay was capable of accurately measuring the concentration of mannose without interference from glucose and other sugars when they were present at concentrations less than 1,000 mg/dL and 5 mmol/L, respectively. Similarly, the degree to which other substances such as hemoglobin, ascorbic acid, and bilirubin interfered with the assay was also determined. Our results showed no interference from less than 500 mg/dL of hemoglobin, less than 50 mg/dL of ascorbic acid, and less than 20 mg/dL of bilirubin. Finally, we determined that blank reagents did not induce nonspecific reactions in this assay.

Other Biochemical Assays and Statistics

Plasma glucose levels were determined by the glucose oxidase technique (Determiner L/Glu II, Kyowa Medex, Tokyo, Japan; and Hitachi 7070 Automated Analyzer), which had intra assay CVs of less than 5%. Hemoglobin A_{1c} (HbA_{1c}) was measured by high-pressure liquid chromatography (Glycohemoglobin Analyzer HLC-723GHbV, TOSOH, Inc., Tokyo, Japan), which had intra-assay CVs of 0.95%,

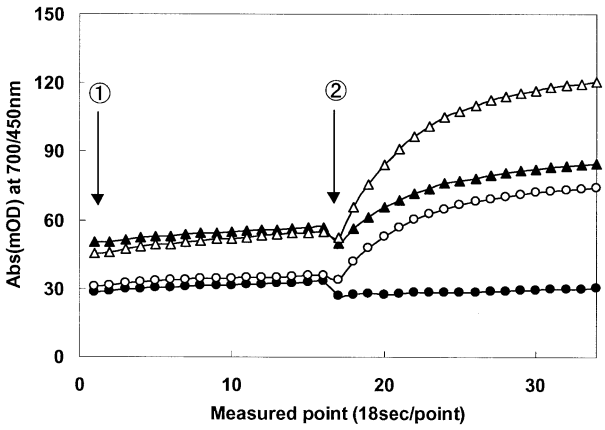

Fig 2. Typical time course of the mannose assay reaction measured with a Hitachi 7170 analyzer. (1) Mixing of reagent 1 and sample; (2) addition of reagent 2; ●, reagent blank; ○, D-mannose calibrator (10 μg/mL); ▲, serum 1 (9.1 μg/mL); △, serum 2 (18.6 μg/mL).

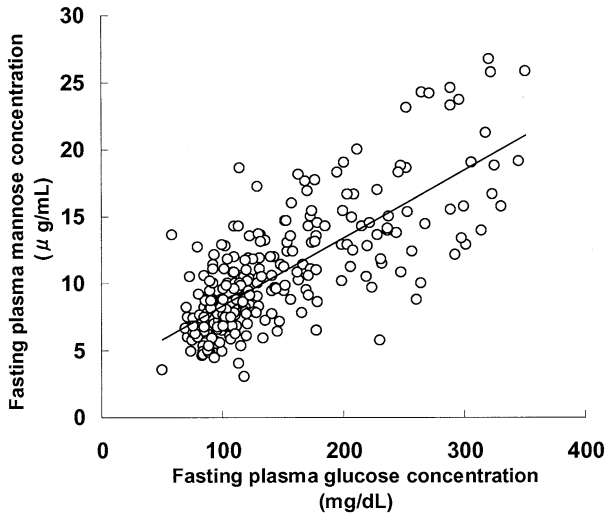

Fig 3. Relationship between FPM and FPG levels in all subjects (n = 273). Pearson's correlation coefficients, $r = 0.754$, $P < .0001$).

1.76%, and 2.63% at HbA_{1c} levels of 4.9%, 5.5%, and 9.9%, respectively. The reference range was less than 5.8%. Serum fructosamine levels were determined using a colorimetric assay (FRUC, Roche Diagnostics, Mannheim, Germany) with a reference range for subjects without diabetes of 205 to 285 μmol/L.

Data were expressed as the mean ±SD unless otherwise noted. The clinical characteristics of the patients in our 3 groups were compared using a factorial analysis of variance (ANOVA) followed by the Scheffé's test. Time-courses of the OGTTs and the subjects daily profiles were compared using a repeated-measures ANOVA; significant differences were then further evaluated by using either a paired *t* test (if the data followed a normal distribution as determined by the *F* test), or, if the data did not follow such a distribution, by the Wilcoxon signed-rank test. $P < .05$ was considered to be statistically significant. Pearson's correlation coefficients (*r*) were computed to identify significant correlations between measurements or indices. Statistical analyses were performed using Excel (Microsoft, Redmond, WA) and StatView (Abacus Concepts, Berkeley, CA) software.

RESULTS

Mannose Levels After an Overnight Fast

A clear positive correlation was found between fasting plasma mannose and glucose levels in all subjects, as shown in Fig 3. Among the subjects in whom OGTT was performed (Table 1), fasting plasma mannose (FPM), FPG, HbA_{1c}, and fructosamine levels were significantly higher in subjects with DM than in subjects with IGT or NGT ($P < .05$). Plasma mannose levels were found to be about 8% of plasma glucose levels, which is similar to previously reported results.[12,14,16,19] The mannose/glucose ratio did not change when basal glucose levels changed (Table 1).

The relationships between the FPM concentration and HbA_{1c}/fructosamine levels, and between the FPG concentration and HbA_{1c}/fructosamine levels are shown in Fig 4. Plasma mannose showed weaker correlations with HbA_{1c} and fructosamine, which are established indices of overall glycemic control, than did glucose. Data collected in subjects with NGT, IGT, or treated diabetes whose FPG levels were normal were used to calculate assay sensitivities and specificities for measurement of mannose and HbA_{1c} levels; cut-off values were measured in subjects whose FPG levels were less than 110 mg/dL (NGT, n = 50; IGT, n = 31; DM, n = 15) (Table 2). Sensitivity was calculated by dividing the number of true-positive subjects by the number of subjects with IGT and DM. Specificity was calculated by dividing the number of true-negative subjects by the number of subjects with NGT. Mannose sensitivity and specificity were found to be comparable to that seen for HbA_{1c}, and better than that seen for fructosamine. Unlike HbA_{1c}, mannose concentrations were determined in the same sample that was used for determining the glucose concentration, without any additional treatment of the samples.

Mannose Levels After an Oral Glucose Load

Circulating mannose levels were quantified and compared to glucose levels following the administration of an oral glucose load. Sequential changes in mannose levels during a 75-g OGTT are shown in Table 3, together with the accompanying changes in glucose concentrations; the degree of deviation of mannose and glucose levels from basal levels is illustrated in Fig 5. There were significant differences in mannose and glucose levels among our 3 groups as determined by a repeated-measures ANOVA ($P < .05$). All plasma glucose concentrations were significantly elevated at 30 and 60 minutes, but the values in subjects with NGT and IGT were significantly lower than those in the diabetic group, the latter of which remained elevated until 120 minutes after glucose intake. In contrast, plasma mannose levels showed only a slight and temporary elevation at 30 minutes, after which they returned to baseline. In subjects with NGT or IGT, mannose levels declined to below baseline levels at 120 minutes. Mannose levels at 60 and 120

Table 1. Clinical Characteristics of Subjects With NGT, IGT, and Type 2 DM

	NGT	IGT	DM
No. of subjects (M/F)	50 (41/9)	48 (37/11)	42 (30/12)
Age (yr)	46.5 ± 12.1 (27-72)	51.4 ± 7.4 (35-66)	54.6 ± 10.9 (31-73)*
Plasma glucose (mg/dL)	95 ± 11 (70-109)	104 ± 12 (76-123)*	116 ± 21 (71-157)*†
Plasma mannose (μg/mL)	7.4 ± 1.6 (4.9-11.9)	8.0 ± 1.9 (4.3-12.5)	9.3 ± 2.4 (5.8-15.1)*†
Mannose/glucose ratio ($\times 10^{-3}$)	7.9 ± 1.6 (5.0-12.3)	7.7 ± 1.7 (4.3-11.1)	8.2 ± 2.3 (4.4-15.9)
HbA_{1c}(%)	5.4 ± 0.6 (4.4-6.9)	5.6 ± 0.4 (4.5-6.5)	6.1 ± 0.6 (4.7-7.4)*†
Fructosamine (μmol/L)	267 ± 20 (215-308)	275 ± 24 (241-364)	290 ± 34 (183-364)*†

NOTE. Glucose and mannose levels were determined in the same plasma sample. Values are expressed as the mean ± SD.
Statistical differences between subjects were determined by factorial ANOVA followed by the Scheffé's test; *$P < .05$ *v* NGT, †$P < .05$ *v* IGT.

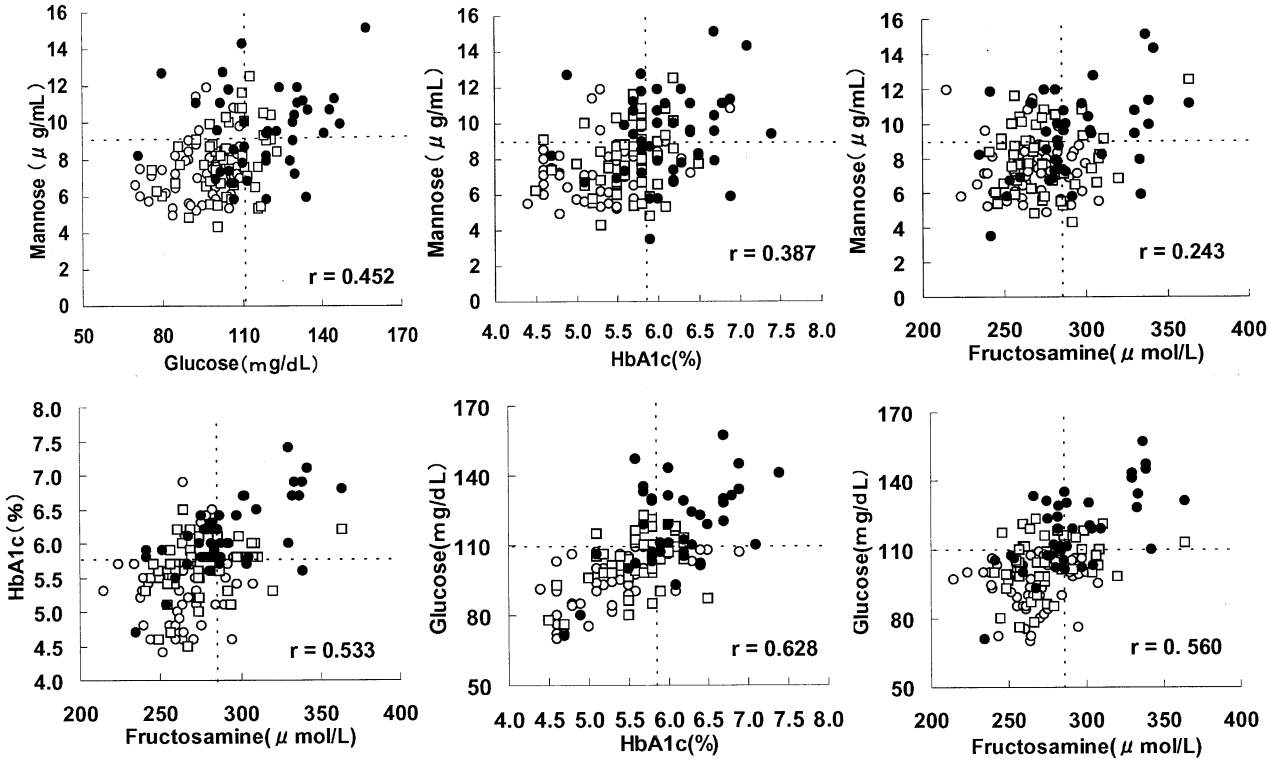

Fig 4. Relationships between the FPM concentration and HbA_{1c}/fructosamine levels, FPG concentration and HbA_{1c}/fructosamine levels, FPG and FPM, and between HbA_{1c} and fructosamine levels in subjects who underwent OGTTs (n = 140). Cut-off lines were added at 9.0 μg/mL, 110 mg/dL, 5.8%, and 285 μmol/L for mannose, glucose, HbA_{1c}, and fructosamine respectively.

minutes in patients with IGT and DM were significantly higher than those seen in individuals with NGT. In subjects with DM, mannose levels increased together with glucose, although the rise in mannose concentration was smaller than that seen with glucose. The increase in mannose concentration after oral glucose loading was only approximately 10% of baseline levels, while glucose levels doubled. The relationship between plasma mannose and glucose levels at each time point of the OGTT is shown in Fig 6. The Pearson's correlation coefficients between plasma mannose and glucose levels decreased 30 minutes after

Table 2. Sensitivity and Specificity of Mannose, HbA_{1c}, and Fructosamine Measurements Calculated by Determining Cut-Off Values in Subjects Whose FPG Levels Were Less Than 110 mg/dL

	Cut-off Values (μg/mL)								
	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
Mannose									
Sensitivity	0.83	0.78	0.61	0.52	0.43	0.35	0.33	0.22	0.17
Specificity	0.18	0.28	0.34	0.54	0.64	0.80	0.88	0.90	0.94
	Cut-Off Values (%)								
	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4
HbA_{1c}									
Sensitivity	0.91	0.89	0.83	0.72	0.52	0.46	0.20	0.13	0.70
Specificity	0.16	0.26	0.36	0.48	0.64	0.80	0.88	0.92	0.94
	Cut-Off Values (%)								
	220	230	240	250	260	270	280	290	300
Fructosamine									
Sensitivity	0.98	0.98	0.96	0.83	0.59	0.43	0.24	0.15	0.11
Specificity	0.20	0.40	0.10	0.18	0.28	0.54	0.74	0.82	0.96

NOTE. NGT, n = 50; IGT, n = 31; and DM, n = 15. Sensitivity = true-positive subjects (N) over cut-off values/IGT (N) + DM (N); specificity = true-negative subjects (N) below cut-off values/NGT (N).

Table 3. Plasma Mannose and Glucose Concentrations at Each Time Point During a 75-g OGTT in Subjects With NGT (n = 50), IGT (n = 48), and Type 2 DM (n = 42)

	Basal	30 min	60 min	120 min
Mannose (μg/mL)				
NGT	7.4 ± 1.6 (4.9-11.9)	7.8 ± 1.7 (5.2-13.3)*	7.1 ± 1.8 (4.1-12.3)*	5.0 ± 1.3 (2.5-10.1)*
IGT	8.0 ± 1.9 (4.3-12.5)	8.6 ± 1.9 (4.3-14.0)*	8.5 ± 1.9 (4.9-13.5)*	6.8 ± 1.6 (3.5-10.2)*
DM	9.3 ± 2.4 (5.8-15.1)	10.1 ± 2.3 (6.3-15.9)*	10.4 ± 2.5 (6.5-17.0)*	9.9 ± 2.9 (4.0-16.9)
Glucose (mg/dL)				
NGT	95 ± 11 (70-109)	160 ± 27 (102-217)*	161 ± 44 (58-271)*	110 ± 19 (54-138)*
IGT	104 ± 12 (76-123)	177 ± 25 (122-239)*	203 ± 39 (97-262)*	153 ± 27 (75-208)*
DM	116 ± 21 (71-157)	194 ± 37 (122-260)*	241 ± 43 (122-343)*	236 ± 55 (101-336)*

NOTE. Values are expressed as the mean ± SD.

Significant group vs. time course interactions were detected by a repeated-measure ANOVA ($P < .05$) and statistical differences between basal levels and levels at each time point were evaluated by an F-test followed by a paired *t* test (*$P < .05$).

the initiation of the test but then returned to levels that were above baseline values.

Daily Profile of Blood Mannose Levels and Its Relationship to Glucose

Based on our findings that plasma mannose levels closely correlated with plasma glucose levels but that their levels showed delayed equilibration after an oral glucose load, it was suggested that mannose might be useful as an index of glucose control or deviation over the very short term, ie, a 1-day period. To investigate this further, daily profiles of plasma mannose/glucose levels sampled before and after each meal (6 times per day) were determined in 80 consecutively recruited patients with DM who were admitted to our hospital. A CV of 6 daily glucose measurements represents a measure of the stability of daily plasma glucose excursions; the lower the CV, the more stable the daily glycemic control. Our results showed that there were no significant changes in plasma mannose levels before and after each meal, despite dynamic alterations in glycemic levels (Table 4). Because postprandial glucose levels varied widely at the same time that mannose levels fluctuated only somewhat, a close correlation between plasma mannose and glucose levels was only seen under fasted conditions (Fig 6). (The reason why the mannose levels of the subjects shown in Table 4 are much higher than those shown in Table 3 is that only diabetic patients that were admitted to our hospital were included in Table 4).

The CVs of glucose versus mannose were significant both before and after meals, whereas the CVs of glucose versus glucose were only significant after the meals (Table 4). The typical relationship between the CVs of 6 daily glucose measurements and mannose, and glucose levels measured before and after dinner are shown in Fig 7. In these subjects (n = 19), FPG/FPM levels were determined immediately after admission

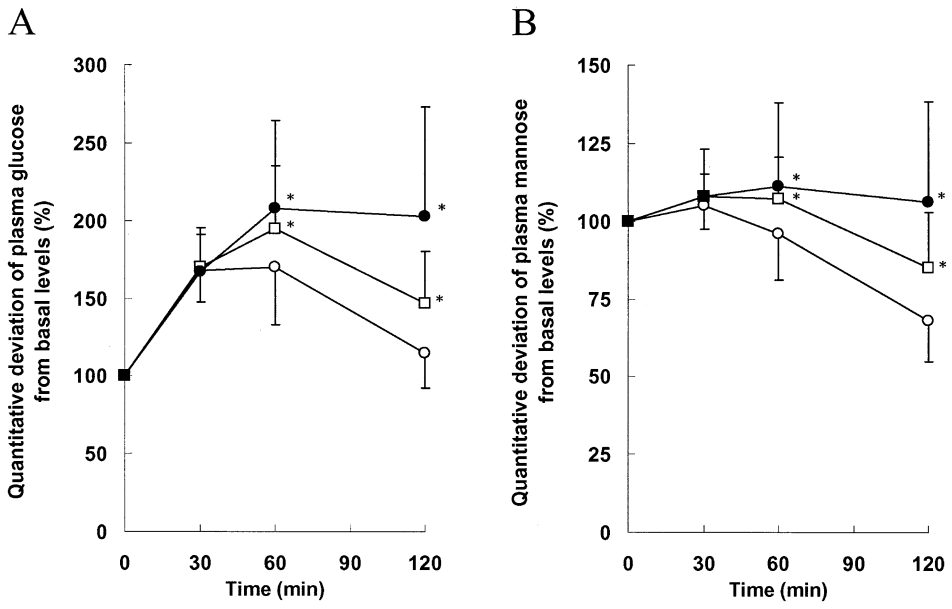

Fig 5. Quantitative deviation from basal levels, of (A) mannose and (B) plasma glucose during OGTTs in subjects with NGT (n = 50, ○), IGT (n = 48, □), and type 2 DM (n = 42, ●). The data were analyzed using repeated-measures ANOVA and Wilcoxon signed-rank test. *$P < .05$ *v* NGT).

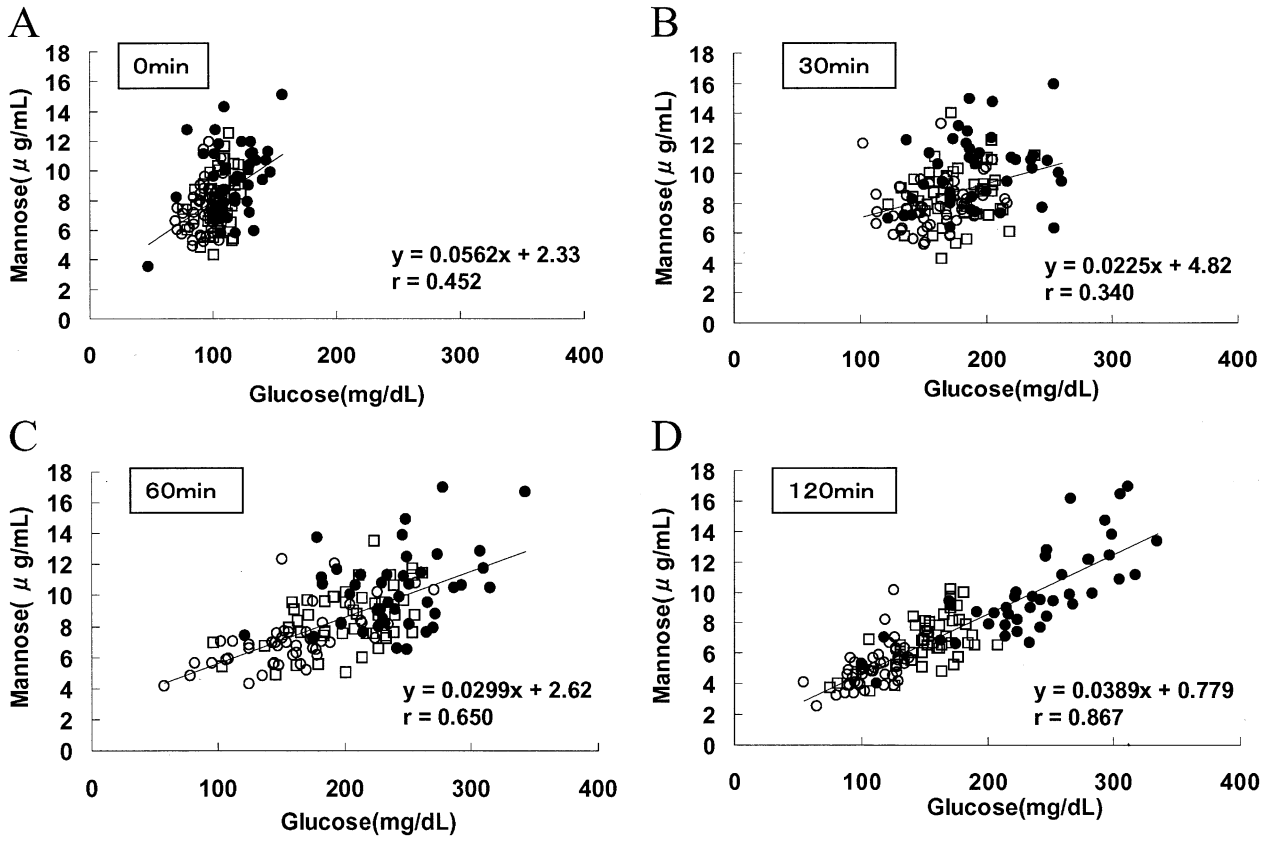

Fig 6. Relationship between plasma mannose and glucose concentrations at each time point measured during OGTT in subjects with NGT (n = 50, ○), IGT (n = 48, □), and type 2 DM (n = 42, ●).

and before discharge. Results (Fig 8 and Table 1) showed that the ratio between these 2 hexoses remained relatively unchanged regardless of the basal glucose levels.

DISCUSSION

The present study confirmed, in a large cohort of subjects, the earlier small-scale observations[12,14,16,19] that there is a positive correlation between FPM and FPG levels (Fig 3). Also confirmed were the previous findings[14,16,19] of an elevation in plasma mannose levels in patients with DM, which we extended by showing similarly elevated mannose levels in subjects with glucose intolerance (Table 1). To date, there have not been any reports in which circulating mannose levels were compared to other, established indices of glycemia, or in which dynamic measurements of mannose levels were made during periods of plasma glucose variation. Our new findings are important in determining the clinical usefulness of determining plasma mannose levels.

Table 4. Daily Profiles of Plasma Mannose/Glucose Levels Sampled Before and 2 Hours After Each Meal (6 times per day) and Pearson's Correlation Coefficients of the CV Between Six Glucose Measurements and Each Mannose or Glucose Level (n = 80)

	Before Breakfast	After Breakfast	Before Lunch	After Lunch	Before Dinner	After Dinner
Mannose (μg/mL)	17.6 ± 7.2	18.7 ± 7.5*†	17.4 ± 7.7	15.9 ± 7.4*†	15.7 ± 7.4†	16.5 ± 7.1*†
	(7.5-48.3)	(7.7-47.4)	(6.2-47.5)	(5.3-46.7)	(6.1-46.2)	(5.8-46.1)
Glucose (mg/dL)	147 ± 60	232 ± 71*†	182 ± 71†	207 ± 76*†	155 ± 67	220 ± 75*†
	(62-424)	(105-377)	(61-345)	(86-443)	(65-365)	(86-406)
Correlation coefficients (*r*)						
CV(Glu) *v* mannose	0.547‡	0.485‡	0.493‡	0.466‡	0.537‡	0.452‡
CV(Glu) *v* glucose	0.463‡	0.292‡	0.477‡	0.294‡	0.575‡	0.196

NOTE. Values are expressed as the mean ± SD.

Significant differences were detected using a repeated-measures ANOVA ($P < .05$) and statistical differences between values were evaluated by the Wilcoxon signed-ranks test (*$P < .05$ *v* before each meal; †$P < .05$ *v* before breakfast).

‡$P < .05$.

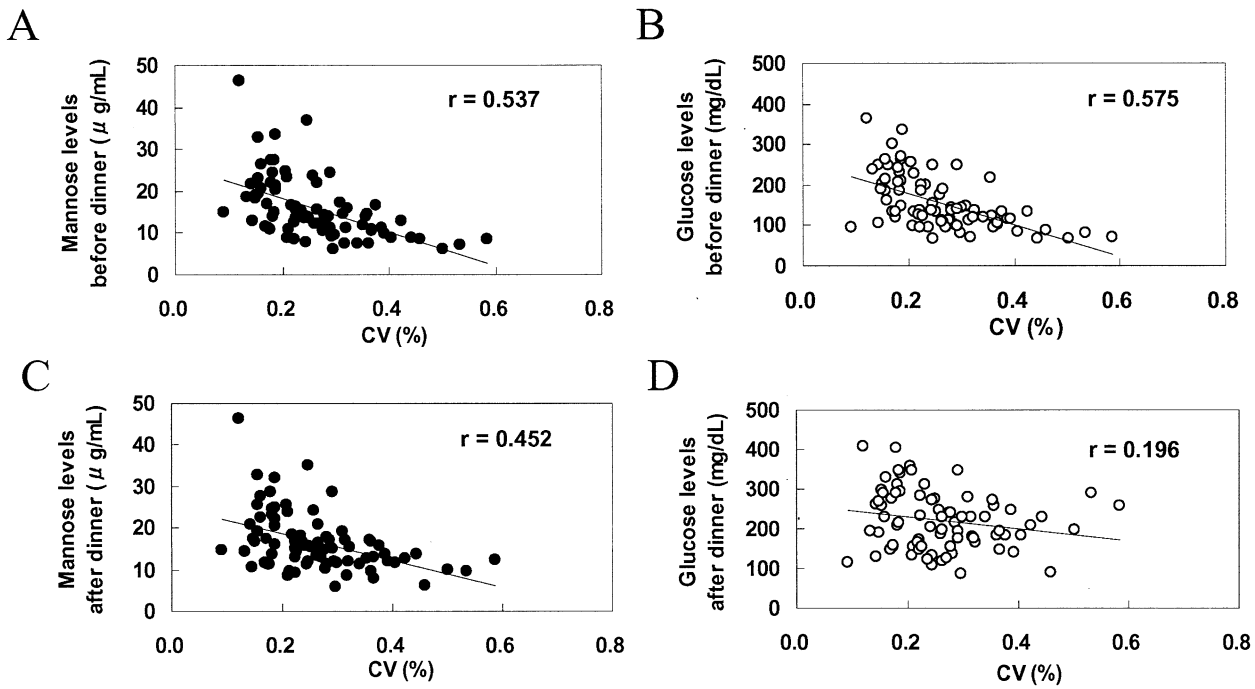

Fig 7. Typical relationship between the CV of 6 daily glucose measurements, and mannose (A and C) or glucose (B and D) levels measured before and after dinner.

Like HbA_{1c} and fructosamine, FPM levels were not significantly elevated in subjects with IGT compared to normal subjects, although levels were significantly elevated in subjects with DM (Table 1). Fasting mannose levels correlated only weakly with HbA_{1c} ($r = 0.387$), and fructosamine ($r = 0.243$) concentrations, while FPG levels correlated strongly with these compounds (HbA_{1c}, $r = 0.628$; fructosamine, $r = 0.560$), suggesting that fasting mannose levels were not a better indicator of overall glycemic control over a period of several weeks than was glucose (Fig 4). However, the lack of a significant correlation between fasting mannose and fructosamine levels despite the close correlation between mannose and glucose levels (Figs 3 and 4) suggests that mannose levels might be an indicator of shorter-term glycemic control, as discussed below. (The reason why the r value for the mannose v glucose comparison in Fig 4 [$r = 0.452$, $P < .05$) was lower than the value shown in Fig 3 [$r = 0.754$, $P < .05$) was because only subjects in whom an OGTT was performed were included in the data in Fig 4, while Fig 3 included data from a wider range of subjects.) The reason for the large overlap in the glucose and HbA_{1c} values seen in Table 1 was that in our hospital-based study, blood samples were collected from subjects with both definite and probable diabetes. Because of the overlap or possible bias of our sampling, we simply compared the sensitivity and specificity of our mannose determinations with those of HbA_{1c} for diagnosing diabetes. We are currently carrying out a population-based study to further clarify the above findings.

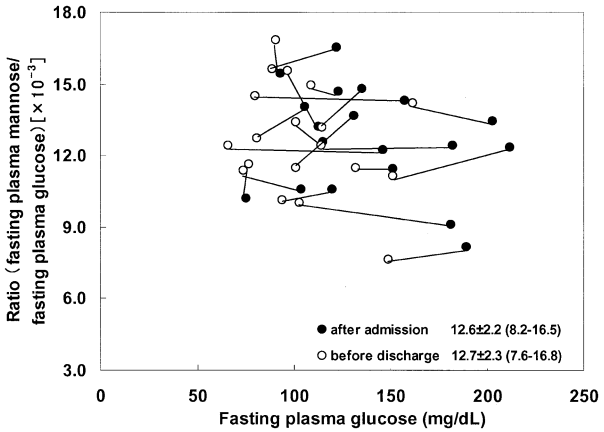

Fig 8. Relationship between FPG levels and the ratio of FPG levels to FPM levels in 19 diabetic patients on 2 different days (immediately after admission, ●; and before discharge, ○). The inset shows the mean ± SD and range of the ratios.

Mannose assay sensitivity and specificity were similar to that seen for the HbA_{1c} assay (Table 2). Though the measurement of circulating mannose levels alone is not sufficient to diagnose diabetes, in conjunction with the measurement of circulating glucose levels, it may be useful in diagnosing borderline diabetic patients whose FPG is still normal. In fact, this approach may be particularly attractive because measurement of FPG levels alone is generally insufficient in diagnosing borderline diabetes,[23] and such borderline patients are known to face an increased risk of cardiovascular disease.[24] Our mannose assay is as sensitive as the HbA_{1c} assay but is more convenient and requires a smaller volume of blood.

Patients with a deficiency in phosphomannose isomerase

(PMI), one of the key enzymes required for mannose formation from glucose develop a condition referred to as congenital disorder of glycosylation Ib,[25] which is marked by a significant decrease in antithrombin III levels due to the hypoglycosylation of glycoproteins. This condition can be successfully treated by oral mannose administration.[25,26] This finding, together with the results of other studies by Freeze et al[2,6,8] confirms that efficient absorption of mannose is possible through the intestine. However, the average diet does not contain enough mannose to even satisfy the body's need for physiological glycosylation. This fact provides a possible explanation for our inability to find changes in circulating levels of mannose in diabetic subjects following ingestion of a meal (Table 4). It is speculated that the main source of circulating mannose is not the diet but rather the liver or other tissues which synthesize it. Since mannose can be synthesized from glucose,[7] an increase in circulating mannose levels seen after glucose loading might be directly derived from ingested glucose. Our data showed that mannose levels increased only transiently after glucose loading (Table 3), possibly reflecting the complexity of the mannose synthetic pathway as well as its rapid clearance.[2,6]

Interestingly, our data also showed that mannose levels fell after glucose loading in normal and IGT subjects (Fig 5). The reason for this latter finding is unknown and is currently being investigated both in vivo and in vitro. Based on the limited available data concerning the mannose transport system and the in vivo accelerated clearance of mannose after insulin stimulation,[2,27-29] it is speculated that mannose transport into cells may be facilitated by the increased insulin induced after glucose loading. The correlation between glucose and mannose levels after an oral glucose load demonstrated a temporal decline after 30 minutes and a subsequent increase after 60 and 120 minutes (Fig 6), suggesting that changes in mannose levels were dependent on prior changes in circulating glucose levels. Taken together with the data showing that mannose levels changed far less than did glucose levels (Fig 5), it was speculated that this delay was due to the synthesis of mannose from the loaded glucose. It is possible that we would have had more proof for this had we continued our measurements past 2 hours. Alternatively, mannose synthesis and secretion could be temporally decreased acutely (30 minutes) but restored to normal after that time, as mentioned above.

The OGTT data in Fig 6 suggest that 2-hour mannose values are the most appropriate values to use in potentially diagnosing borderline diabetics. However, the OGTT is not the most convenient tool to use for diabetic screening purposes. On the other hand, by measuring FPM as well as FPG levels, both of which are more readily determined, we may be able to diagnose borderline diabetic patients whose FPG levels are still normal. The efficacy of using these measurements in this way needs to be further investigated.

It was surprising that circulating mannose levels more precisely correlated with daily glucose CV values than did glucose itself, both before and after a meal (Table 4 and Fig 7), although mannose correlated only poorly with fructosamine (Fig 4), the levels of which are used as an index of averaged glycemic control over the prior few weeks. In other words, single random sampling of mannose levels may be useful as an indicator of recent glycemic control that is influenced only minimally by meal ingestion (Table 4). The fact that mannose levels correlated with daily glucose levels is of potential clinical significance. Accordingly, mannose levels, determined by a simple blood test could be a useful gauge of the average daily glucose excursion.

Our data showed that the ratio between FPM and FPG levels was maintained regardless of basal glucose levels, and seemed to be unique to each individual (Table 1 and Fig 8). Preservation of the mannose/glucose ratio in a given individual has not been previously reported, but may be significant since this ratio was previously shown to correlate with body mass index and uric acid levels in a small number of patients.[19]

Although enzymatic methods of quantification are readily applied in clinical settings, previously reported uses of assays employing this methodology for the measurement of plasma and serum mannose levels that utilized hexokinase, mannose-6-phosphate isomerase, G-6-P isomerase, and G-6-P dehydrogenase[11-14] were quite complicated and not ideally suited for the processing of large numbers of samples. Furthermore, because only relatively low levels (~1% of glucose levels) of mannose are present in the blood, the sensitivity and/or specificity of the current assays need to be improved. The new enzymatic method described here relies on the use of bacterial-derived ALDH and demonstrated improved accuracy, precision, and linearity without interference from other blood constituents such as glucose. This methodology can be applied within a clinical automated analyzer. The effects of concomitant drug therapy, dietary alteration, exercise, aging, and urinary excretion on this assay system need to be investigated before this assay can be considered as a clinical tool.

In conclusion, the close correlation between FPM and FPG levels taken together with the small fluctuations seen in plasma mannose in response to glucose suggest that the measurement of mannose using our assay could potentially play a supplementary role in the diagnosis and screening of patients with mild diabetes.

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