ORIGINAL ARTICLE

Plasma mannose level, a putative indicator of glycogenolysis, and glucose tolerance in Japanese individuals

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ABSTRACT

Aims/Introduction: Mannose is a monosaccharide constituent of glycoproteins and glycolipids. Experiments in rats have shown previously that the plasma mannose level decreases after glucose load, but does not decrease in diabetic rats, and that hepatic glycogenolysis is a source of this plasma mannose; however, these results are not fully elucidated in humans. Plasma mannose levels before/after glucose loading in humans with various degrees of glucose intolerance were examined to analyze their association with clinical factors.

Materials and Methods: The 75-g oral glucose tolerance test was carried out in Japanese individuals not taking diabetes medications. Participants were classified into normal glucose tolerance, impaired glucose metabolism and diabetes mellitus groups. Insulinogenic index as an index of insulin secretion, and Matsuda Index as an index of insulin sensitivity were calculated. Mannose was assayed by the established method using high-performance liquid chromatography after labeling.

Results: After glucose load, the plasma mannose level decreased gradually in the normal glucose tolerance group, but did not decrease in the diabetes mellitus group. Plasma mannose changes during 120 min from baseline (M_{120}-M_0) were significantly different among the three groups (normal glucose tolerance: -16.7 ± 1.7; impaired glucose metabolism: -9.0 ± 1.9; diabetes mellitus: -1.4 ± 1.8 μmol/L [$n = 25$ in each group], $P < 0.0001$). Plasma glucose 120 min after glucose loading ($R^2 = 0.412$) or $\log_e$-insulinogenic index, $\log_e$-Matsuda Index and age ($R^2 = 0.230$) were determinants of M_{120}-M_0 in multiple regression analyses.

Conclusions: We clarified the relationship between plasma mannose level and glucose tolerance in humans. The present results are compatible with those using rats, in which mannose derived from glycogenolysis plays an important role in the alteration of mannose levels after glucose loading.

INTRODUCTION

Mannose is a monosaccharide constituent of glycoproteins and glycolipids. The mammalian serum mannose concentration ranges from approximately 50 to 100 μmol/L[1]. In humans, the mean concentrations of mannose in plasma determined by enzymatic methods were reported previously to be approximately 50[1], 20[2] and 40 μmol/L[3]. In a previous study[3] of patients with diabetes, the plasma concentration of mannose was found to be higher than that in participants with normal glucose tolerance, and plasma mannose and glucose concentrations were positively correlated in participants with various severities of glucose intolerance. However, the mechanism of alteration of plasma mannose levels after glucose load was not elucidated in that study.

Taguchi *et al.*[4] has shown using rats that hepatic glycogen is a source of plasma mannose. Oral administration of glucose increased the plasma glucose level, but decreased the plasma mannose level, showing that plasma glucose was not the direct source of plasma mannose. Interestingly, oral administration of glucose was found not to decrease plasma mannose levels in diabetic Goto-Kakizaki rats, in which the plasma insulin response to glucose is impaired[4]. The authors proposed that part of the plasma mannose is supplied by breakdown of glycogen in the liver by the route of glycogen → glucose 1-phosphate → fructose 6-phosphate → mannose 6-phosphate → mannose.

It is generally accepted that endogenous glucose production (EGP), which is the sum of gluconeogensis and glyogenolysis, is increased, and results in an elevated level of fasting plasma glucose in patients with diabetes. However, the relative and absolute contribution of glycogenolysis to increased EGP is controversial[5]. One of the reasons for this is the limited number of subjects available for such studies because of the complicated and expensive methods for using glucose isomer. A more convenient biomarker of glycogenolysis is necessary.

In the present study, to explore the possibility that the plasma mannose level is an indicator of glycogenolysis in humans, plasma mannose levels before and after glucose loading in participants with various degrees of glucose intolerance were examined and analyzed to clarify the association with clinical factors.

MATERIALS AND METHODS

Participants
Japanese outpatients who visited Fukuda Clinic, Kochi, Japan, for hypertension and/or lipid metabolism disorders during the period of April 2012 through April 2014, not diagnosed with glucose intolerance previously, and took no diabetes medications were recruited for the study. Participants were successively recruited until the number of each group reached 25. Informed consent was obtained from each patient. Mannose levels in plasma samples were determined at Meijo University. Analysis of data was carried out at Kochi Medical School. The study protocol was approved by local ethical review boards of Kochi Medical School, Meijo University and Fukuda Clinic.

Laboratory examination
The 75-g oral glucose tolerance test (OGTT) was carried out in the morning after an overnight fast. Samples were drawn just before, and 30, 60, 90 and 120 min after ingestion of glucose. Plasma glucose was measured by the glucose oxidase method. Plasma immunoreactive insulin (IRI) was measured using sandwich enzyme-linked immunosorbent assay (Access® Ultrasensitive Insulin; Beckman Coulter, Brea, California, USA). Mannose was assayed using an established method[4]. Briefly, after labeling with 4-aminobenzoyl ethyl ester, the concentration of mannose was determined using high-performance liquid chromatography (HPLC)[6]. The glucose contained in the samples was confirmed

not to affect the assay. To determine the time-course of mannose levels during OGTT, samples of five participants among 25 participants belonging to each group were randomly chosen, and mannose levels at 30, 60 and 90 min after glucose load were measured. Mannose levels before and at 120 min after glucose loading were measured in all participants. Glycated hemoglobin was measured by HPLC[7]. Using the 2006 World Health Organization criteria and Japan Diabetes Society criteria[8,9], participants were categorized as having normal glucose tolerance (NGT; fasting plasma glucose [FPG] <6.1 mmol/L and 2-h plasma glucose [2-h PG] <7.8 mmol/L), impaired glucose metabolism (IGM; either impaired fasting glucose [IFG]: FPG ≥6.1 and <7.0 mmol/L, and/or impaired glucose tolerance [IGT]: 2-h PG ≥7.8 and <11.1 mmol/L) or diabetes (FPG ≥7.0 mmol/L and/or 2-h PG ≥11.1 mmol/L). Anti-glutamic acid decarboxylase antibody was not measured in the participants.

Indices of insulin secretion and insulin sensitivity/resistance
In the 75-g OGTT, glucose (G), mannose (M) and IRI (I) levels in plasma were determined at fasting (G_0, M_0, I_0), 30 (G_{30}, M_{30}, I_{30}), 60 (G_{60}, M_{60}, I_{60}), 90 (G_{90}, M_{90}, I_{90}) and 120 min (G_{120}, M_{120}, I_{120}) after glucose loading, respectively. G_m and I_m were calculated by dividing the area under the curve of G and IRI during OGTT by 120 min, respectively. As insulin secretion indices, insulinogenic index (IGI) and homeostasis model assessment of β-cell function (HOMA-β) were calculated using the following formula[10,11]: IGI: $(I_{30} - I_0[\text{pmol/L}])/(G_{30} - G_0[\text{mmol/L}])$; HOMA-β: $I_0(\mu U/mL) \times 20 / (G_0[\text{mmol/L}] - 3.5)$. As an insulin resistance index, homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula[11]: $I_0(\mu U/mL) \times G_0(\text{mmol/L}) / 22.5$. As insulin sensitivity indices, the Quantitative Insulin Sensitivity Check Index (QUICKI) and Matsuda Index were calculated using the following formula[12,13]: QUICKI: $1 / (\log_{10} G_0[\text{mg/dL}] + \log_{10} I_0[\mu U/mL])$; Matsuda Index: $10,000 / (G_0[\text{mg/dL}] \times I_0[\mu U/mL] \times G_m[\text{mg/dL}] \times I_m[\mu U/mL])^{0.5}$. The oral disposition index (DI_O) was calculated using the following formula[14]: $IGI/I_0(\text{pmol/L})$.

Statistical analysis
Statistical analysis was carried out with the StatView 5.0 system (SAS Institute Inc., Cary, North Carolina, USA). Normally distributed continuous data are presented as mean ± standard error, and non-normally distributed continuous data are presented as median value, 25th percentile value and 75th percentile value. Differences among more than three groups were determined by analysis of variance (ANOVA) for normally distributed continuous data, and by Kruskal–Wallis tests for non-normally distributed continuous data. Scheffe's test was carried out as *post-hoc* analysis. The relationships between the parametric data and between non-parametric data were determined by Pearson's analysis and by Spearman's analysis, respectively. As dependent variables, M_0 and M_0-M_{120} were used. As

independent variables, normally-distributed $\log_e$-transformed HOMA-β, IGI, Matsuda Index and DI_O were used. In multiple regression analyses, to reduce the number of independent variables matching with the total number of participants, and to avoid confounding factors, factors with >0.7 coefficient in simple correlation among independent variables were omitted (Table S1). As $\log_e$-HOMA-β and QUICKI are strongly correlated with G_0 (multiple correlation coefficient 0.873), and $\log_e$-IGI and $\log_e$-MI are strongly correlated with G_{120} (multiple correlation coefficient 0.720), these variables were not included in the same analysis to avoid confounding results. Independent variables were selected by the forward stepwise selection method, according to the significance level for the addition of variables <0.10. P-values <0.05 were considered statistically significant.

RESULTS

Clinical and biochemical profiles

Clinical and biochemical profiles among the NGT, IGM and diabetes groups are shown in Table 1. Glycated hemoglobin, plasma glucose during OGTT and plasma IRI 120 min after glucose loading were significantly different among groups. Indices of insulin secretion, except HOMA-β and insulin resistance/sensitivity, were significantly different among groups.

Time-course of mannose levels during OGTT

Time-courses of mannose levels during OGTT were determined in five randomly-selected participants of each group. Plasma glucose was elevated after glucose loading in all groups, and were higher at 60, 90 and 120 min, and at 120 min after glucose loading in diabetes and IGM compared with those in NGT, respectively (Figure 1a). Plasma IRI was elevated after glucose loading in all groups, but did not differ among the three groups at any time-point during OGTT (Figure 1b). Plasma mannose levels gradually decreased after glucose loading in NGT and reached plateau after 90 min, but the decrease was blunted in IGM, and was not observed in diabetes. Plasma mannose levels in NGT compared with those in diabetes were lower at 60, 90 and 120 min after glucose loading (Figure 1c,d).

Plasma mannose levels before and after 120-min glucose loading

Fasting plasma mannose levels (M_0) and plasma mannose levels after 120-min glucose loading (M_{120}) were determined in all participants in the NGT, IGM and diabetes groups (n of each group = 25). M_0 did not differ among the three groups (Table 2). M_{120} in the NGT group was lower compared with those in the IGM and diabetes groups, and M_{120} in the IGM group was lower compared with that in the diabetes group.

Table 1 | Clinical and biochemical profiles of participants with normal glucose tolerance, impaired glucose metabolism and diabetes

	NGT	IGM	DM	P-value
n	25	25	25	
Age	60.0 ± 1.8	65.4 ± 2.6	65.6 ± 2.2	0.1364
Sex (male/female)	12/13	11/14	11/14	0.9476
BMI (kg/m^2)	24.0 ± 0.7	25.5 ± 0.7	25.3 ± 0.7	0.3117
WC (cm)	89.0 ± 1.9	90.2 ± 2.2	91.7 ± 2.0	0.6400
HbA1c (%)	5.70 ± 0.06	5.79 ± 0.09	6.28 ± 0.11*,**	<0.0001
G_0 (mmol/L)	5.35 ± 0.08	5.68 ± 0.14	6.48 ± 0.20*,**	<0.0001
G_{120}(mmol/L)	6.37 ± 0.22	9.17 ± 0.23*	13.53 ± 0.41*,**	<0.0001
G_m (mmol/L)	7.68 ± 0.23	9.91 ± 0.31*	12.60 ± 0.27*,**	<0.0001
I_0 (pmol/L)	35.5 ± 4.6	44.3 ± 4.6	49.9 ± 5.7	0.1291
I_{120} (pmol/L)	264 ± 43	538 ± 112*	494 ± 61*	0.0319
I_m (pmol/L)	293 ± 42	369 ± 50	306 ± 34	0.4005
IGI (pmol/mmol)	57.0 (36.3,96.5)	39.9 (23.1,65.6)	22.4* (13.2,41.7)	0.0005
HOMA-β	51.2 (41.0,86.6)	61.9 (41.9,91.4)	47.7 (37.7,80.2)	0.5153
HOMA-IR	1.21 (0.78,1.68)	1.53 (1.07,2.48)	1.97* (1.42,2.40)	0.0064
QUICKI	0.375 ± 0.006	0.357 ± 0.006	0.344 ± 0.006*	0.0025
Matsuda Index	6.55 (3.71,9.56)	4.36* (2.73,5.53)	3.61* (2.20,5.10)	0.0026
DI_O (mmol/L)	1.87 (1.30,2.60)	0.91 (0.72,1.58)	0.54* (0.40,0.81)	<0.0001

*$P < 0.05$ vs normal glucose tolerance (NGT); **$P < 0.05$ vs impaired glucose metabolism (IGM). G_0, G_{120} and G_m are plasma glucose at 0 (fasting) and 120 min after glucose loading, and mean plasma level in 75-g oral glucose tolerance test, respectively. I_0, I_{120} and I_m are plasma immunoreactive insulin at 0 (fasting) and 120 min after glucose loading and mean immunoreactive insulin in 75-g oral glucose tolerance test, respectively. G_m and I_m are calculated by dividing the area under the curve of plasma glucose and plasma immunoreactive insulin during oral glucose tolerance test by 120 min, respectively. BMI, body mass index; DI_O, oral disposition index; DM, diabetes; HbA1c, glycated hemoglobin; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; IGI, insulinogenic index; QUICKI, Quantitative Insulin Sensitivity Check Index; WC, waist circumference.
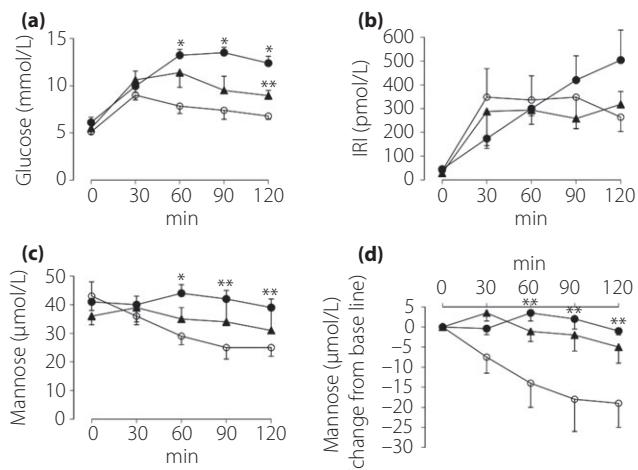
Figure 1 | Time-courses of (a) glucose, (b) immunoreactive insulin (IRI) and (c,d) mannose levels in plasma during the 75-g oral glucose tolerance test in participants with normal glucose tolerance (NGT; open circle, *n* = 5), impaired glucose metabolism (IGM; closed triangle, *n* = 5) and diabetes (DM; closed circle, *n* = 5). Samples of five participants among 25 participants belonging to each group were randomly chosen. *$P < 0.01$ vs NGT; **$P < 0.05$ vs NGT.

The decrease in mannose levels during 120 min after glucose loading from baseline in the NGT group was greater compared with those in the IGM and diabetes groups, and that in the IGM group was larger compared with that in the diabetes group.

Simple correlation between plasma mannose levels and clinical factors

M_0 was correlated with body mass index, fasting plasma glucose (G_0), fasting insulin (I_0) and QUICKI; M_0 was not significantly correlated with $\log_e$-HOMA-β (Table 3, Figure 2a,b). M_0 was significantly correlated in the diabetes group, tended to be correlated in the IGM group and was not correlated with G_0 (NGT: $R = -0.183$, $P = 0.380$; IGM: $R = 0.370$, $P = 0.068$; diabetes: $R = 0.371$, $P = 0.001$). The alteration of plasma mannose levels after glucose loading (M_{120}-M_0) was correlated with plasma glucose at 120 min after glucose loading (G_{120}), G_m, $\log_e$-IGI, QUICKI and $\log_e$-DI$_O$ (Table 3, Figure 2c,d).

Table 3 | Simple correlation between plasma mannose levels and clinical factors before and after glucose loading

Before loading Independent variables	Dependent variables M_0	
	R	P-value
Age	−0.213	0.0664
Sex	0.098	0.4016
BMI	0.253	0.0283
WC	0.205	0.0778
G_0	0.371	0.0010
$\log_e$-HOMA-β	0.022	0.8487
I_0	0.295	0.0102
QUICKI	−0.278	0.0156

After loading Independent variables	Dependent variables M_{120}–M_0	
	R	P-value
Age	0.147	0.2086
Sex	0.122	0.2957
BMI	0.043	0.7131
WC	0.018	0.8758
G_{120}	0.642	<0.0001
G_m	0.544	<0.0001
I_m	−0.127	0.2794
$\log_e$-IGI	−0.334	0.0034
$\log_e$-MI	−0.192	0.0991
QUICKI	−0.237	0.0410
$\log_e$-DI$_O$	−0.431	0.0001

Sex: male = 1, female = 0. G_0, G_{120} and G_m are plasma glucose at 0 (fasting) and 120 min after glucose loading, and mean PG in 75-g oral glucose tolerance test, respectively. I_0 and I_m are plasma immunoreactive insulin at 0 (fasting) and mean immunoreactive insulin in 75-g oral glucose tolerance test, respectively. BMI, body mass index; $\log_e$-DI$_O$, $\log_e$-transformed oral disposition index; $\log_e$-HOMA-β, $\log_e$-transformed homeostasis model assessment of β-cell function; $\log_e$-IGI, $\log_e$-transformed insulinogenic index; $\log_e$-MI, $\log_e$-transformed Matsuda Index; QUICKI, Quantitative Insulin Sensitivity Check Index; WC, waist circumference.

Multiple regression analyses for plasma mannose levels

G_0 and I_0 were determinants of M_0 in a model ($R^2 = 0.172$, $P = 0.001$) and QUICKI and $\log_e$-HOMA-β were determinants

Table 2 | Plasma mannose levels of participants with normal glucose tolerance, impaired glucose metabolism and diabetes

n	NGT 25	IGM 25	DM 25	P-value
M_0 (µmol/L)	40.8 ± 2.0	43.8 ± 2.2	45.0 ± 2.4	0.3977
M_{120} (µmol/L)	24.1 ± 1.4	34.7 ± 1.9*	43.6 ± 2.8*,**	<0.0001
M_{120}-M_0 (µmol/L)	−16.7 ± 1.7	−9.0 ± 1.9*	−1.4 ± 1.8*,**	<0.0001

*$P < 0.01$ vs normal glucose tolerance (NGT); **$P<0.01$ vs impaired glucose metabolism (IGM). M_0 and M_{120} are plasma mannose levels at 0 (fasting) and 120 min after glucose loading. M_{120}-M_0 is the alteration of mannose levels during 120-min glucose loading (M_{120} minus M_0). DM, diabetes.
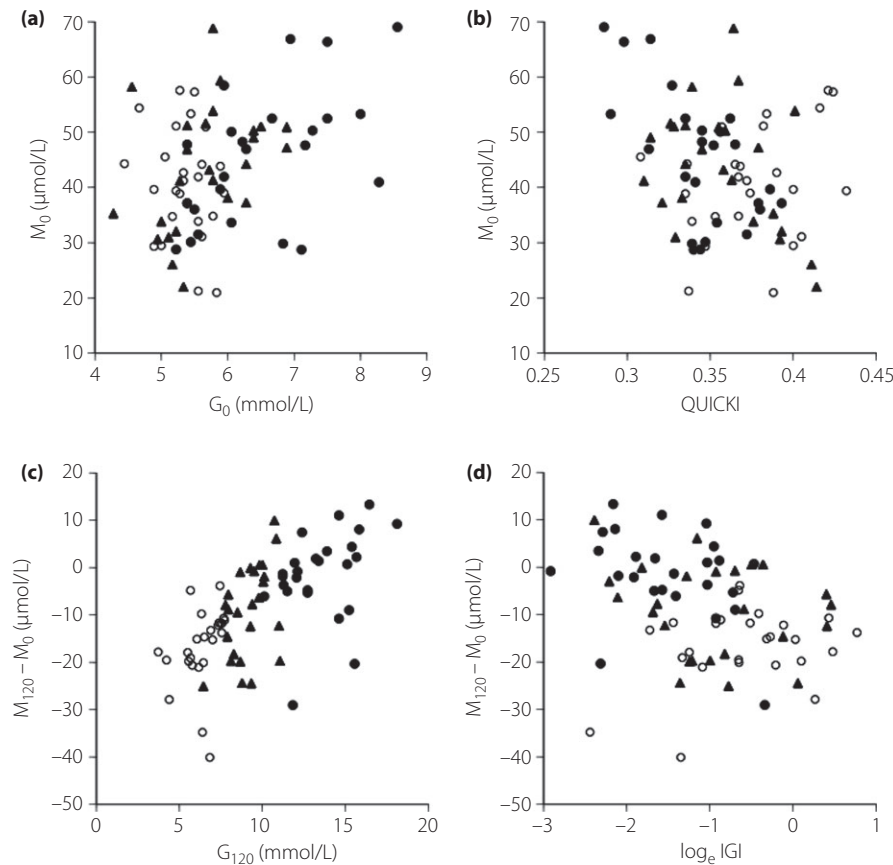
Figure 2 | Relationships between (a) glucose at 0 min (G_0) and mannose at 0 min (M_0), (b) Quantitative Insulin Sensitivity Check Index (QUICKI) and M_0, (c) glucose at 120 min (G_{120}) and plasma mannose changes during 120 min from baseline (M_{120}-M_0), and (d) $\log_e$ insulinogenic index (IGI) and M_{120}-M_0 in participants with normal glucose tolerance (open circle, $n = 25$), impaired glucose metabolism (closed triangle, $n = 25$) and diabetes (closed circle, $n = 25$).

of M_0 in another model ($R^2 = 0.118$, $P = 0.010$; Table 4a,b). G_{120} was a determinant of M_{120}-M_0 in a model ($R^2 = 0.412$, $P < 0.001$), and $\log_e$-IGI, $\log_e$-MI and age were determinants of M_{120}-M_0 in another model ($R^2 = 0.230$, $P < 0.001$; Table 4c,d).

DISCUSSION

We measured plasma mannose levels by HPLC after glucose loading, and confirmed that mannose levels decreased in participants with normal glucose tolerance, but did not decrease in patients with diabetes. These results are compatible with the results using normal and diabetic Goto-Kakizaki rats in which mannose derived from glycogenolysis was found to play an important role in the alteration of mannose levels after glucose loading.

To precisely analyze the correlation between plasma glucose and mannose levels in participants, mannose should be measured without affection of coexisting glucose in samples. Mannose, a C-2 epimer of glucose, is potentially metabolized by enzymes that metabolize glucose. In enzymatic methods, ~100-fold excess of glucose in plasma

samples potentially interferes with the assay in a glucose concentration-dependent manner. A strength of the present study was that measurement bias was excluded by using a method of HPLC not affected by coexisting glucose in plasma samples[6].

In dogs, portal infusion of catecholamine augments EGP by selectively stimulating glycogenolysis[15], showing a more important role of glycogenolysis over gluconeogenesis in the short-term regulation of glucose efflux from the liver. Intravenous administration of insulin decreased the plasma mannose level; in contrast, intravenous administration of epinephrine to fed rats increased plasma mannose. In addition, the increase in plasma mannose by epinephrine was canceled by fasting or by administration of glycogen phosphorylase inhibitor[4]. Furthermore, administration of lactate and alanine, which are gluconeogenic substrates to fasted rats, increased plasma glucose but did not increase plasma mannose[4]. These animal studies show the important roles of glycogenolysis in the short-term regulation of glucose efflux from the liver that are tightly linked to mannose efflux from the liver.

Table 4 | Multiple regression analyses for the determinants of mannose levels

(a)

Independent variables	Dependent variable: M_0	
	β	P
G_0	0.308	0.008
I_0	0.195	0.090
R^2	0.172	
P	0.001	

(b)

Independent variables	Dependent variable: M_0	
	β	P
QUICKI	−0.444	0.003
$\log_e$-HOMA-β	−0.260	0.074
R^2	0.118	
P	0.010	

(c)

Independent variables	Dependent variable: $M_{120} - M_0$	
	β	P
G_{120}	0.642	<0.001
R^2	0.412	
P	<0.001	

(d)

Independent variables	Dependent variable: $M_{120} - M_0$	
	β	P
$\log_e$-IGI	−0.396	<0.001
$\log_e$-MI	−0.348	0.003
Age	0.212	0.055
R^2	0.230	
P	<0.001	

Variables were selected by stepwise selection method following candidate variables: (a) age, sex, body mass index (BMI), glucose at 0 min (G_0) and insulin at 0 min (I_0); (b) age, sex, BMI, $\log_e$-transformed homeostasis model assessment of β-cell function ($\log_e$-HOMA-β) and Quantitative Insulin Sensitivity Check Index (QUICKI); (c) age, sex, BMI, glucose at 120 min (G_{120}) and plasma mean immunoreactive insulin in 75-g oral glucose tolerance test (I_m); (d) age, sex, BMI, $\log_e$-transformed insulinogenic index ($\log_e$-IGI) and $\log_e$-transformed Matsuda Index ($\log_e$-MI).

Fasting plasma mannose levels (M_0) were not significantly different among the NGT, IGM and diabetes groups, but the mannose levels after glucose loading differed. An implication of these results is that the FPG level overlaps largely among the three groups (Figure 2a); 68% of participants in the present study were classified to IGM and diabetes due to criteria for 2 h-PG, not because of the criteria for FPG in this study.

Therefore, the correlation between M_0 and fasting glucose (G_0) was analyzed in a combined population of the three groups. Simple and multiple regression analyses showed that M_0 was significantly correlated with G_0 (Tables 3 and 4).

In multiple regression analysis, QUICKI, an insulin sensitivity index, was an important determinant of fasting mannose level. In patients with type 2 diabetes, EGP, the sum of glycogenolysis and gluconeogenesis, is increased in the fasting state as a result of hepatic insulin resistance, which leads to fasting hyperglycemia[16]. The relative role of glycogenolysis and gluconeogenesis in increased EGP in type 2 diabetes is controversial. However, an increase in glycogenolysis might play some role in increased EGP, as metformin decreases EGP without affecting gluconeogenesis[17].

In multiple regression analysis, the glucose level at 120 min after glucose loading, which is an index of glucose tolerance, was an important determinant of the decrease in mannose level after glucose loading. In addition, the insulinogenic index and Matsuda Index, indices of insulin secretion and sensitivity, respectively, were important determinants of the decrease in mannose level after glucose loading. These results show the relationship between plasma mannose level and glucose tolerance. Acute suppression in EGP in response to insulin elevation by nutrient intake is much more dependent on glycogenolysis relative to gluconeogenesis[18–20]. Taken together, our present results are compatible with the possibility of mannose as an indicator of gycogenolysis in humans.

The present study had limitations. First, insulin sensitivity was not measured by hyperinsulinemic euglycemic clamp, and EGP also was not measured. Second, as this study was cross-sectional, causal association could not be evaluated. Third, glycogenolysis *in vivo* was not evaluated by methods using a glucose isomer. Fourth, hyperglucagonemia and reduced insulin/glucagon ratio after glucose loading were observed in patients with diabetes[21], which might contribute to the increase in glycogenolysis. However, glucagon levels were not evaluated in the present study.

In conclusion, we clarified the relationship between plasma mannose levels and glucose tolerance in humans. Further investigation regarding plasma mannose levels as an indicator of glycogenolysis in human is required.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Simple correlation coefficients between independent variables.