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. Insulinoenic 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 (M120-M0) were significantly different among the three groups (normal glucose tolerance: \(-16.7 \pm 1.7\); impaired glucose metabolism: \(-9.0 \pm 1.9\); diabetes mellitus: \(-1.4 \pm 1.8\) μmol/L [n = 25 in each group], \(P < 0.0001\)). Plasma glucose 120 min after glucose loading (\(R^2 = 0.412\)) or loge-insulinoenic index, loge-Matsuda Index and age (\(R^2 = 0.230\)) were determinants of M120-M0 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/L1. In humans, the mean concentrations of mannose in plasma determined by enzymatic methods were reported previously to be approximately 501, 202 and 40 μmol/L1. In a previous study3 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. 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. 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 gluconeogenesis and glycogenolysis, 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. 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 successfully recruited until the number of each group reached 25. Informed consent was obtained from each patient. Mannose levels in plasma samples were determined at Meij 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, Meij 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 \(^\text{R}\) Ultrasensitive Insulin; Beckman Coulter, Brea, California, USA). Mannose was assayed using an established method. Briefly, after labeling with 4-aminobenzoyl ethyl ester, the concentration of mannose was determined using high-performance liquid chromatography (HPLC). 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. Using the 2006 World Health Organization criteria and Japan Diabetes Society criteria, participants were categorized as having normal glucose tolerance (NGT; fasting plasma glucose [FPG] \(<6.1 \text{ mmol/L}\) and/or impaired glucose tolerance (IFG); 2-h PG \(\geq 7.8 \text{ mmol/L}\) or diabetes (FPG \(\geq 7.0 \text{ mmol/L}\) and/or 2-h PG \(\geq 11.1 \text{ mmol/L}\)). 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. Gm and Im were calculated by dividing the area under the curve of G and IRI during OGTT by 120 min, respectively. As insulin secretion indices, insulinoenic index (IGI) and homeostasis model assessment of \(\beta\)-cell function (HOMA-\(\beta\)) were calculated using the following formula:

\[
\text{IGI} = \frac{(I_{30} - I_0) \text{pmol/L}}{(G_{30} - G_0) \text{mmol/L}}
\]

\[
\text{HOMA-\(\beta\)} = \frac{I_0(\text{U/mL})}{(G_0(\text{mg/dL}) - 3.5)}
\]

As an insulin resistance index, homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula:

\[
\text{HOMA-IR} = \frac{(G_0(\text{mg/dL}) / 22.5) \times I_0(\text{U/mL})}{(G_0(\text{mg/dL}) / 22.5) \times I_0(\text{U/mL})}
\]

Indices of insulin sensitivity indices, the Quantitative Insulin Sensitivity Check Index (QUICKI) and Matsuda Index were calculated using the following formula:

\[
\text{QUICKI} = 1 / \left( \log_{10} (G_0(\text{mg/dL}) + 1) / \log_{10}(I_0(\text{U/mL})) \right)
\]

\[
\text{Matsuda Index} = 10,000 / (G_0(\text{mg/dL}) \times I_0(\text{U/mL}) \times G_{120}(\text{mg/dL}) \times I_{120}(\text{U/mL}))^{0.5}
\]

The oral disposition index \((D_{lc})\) was calculated using the following formula:

\[
D_{lc} = \frac{G_{120}(\text{mg/dL}) \times I_{120}(\text{U/mL})}{(G_0(\text{mg/dL}) \times I_0(\text{U/mL})}^{0.5}
\]

**Statistical analysis**

Statistical analysis was carried out with the StatView 5.0 system (SAS Institute Inc., Cary, North Carolina, USA). Normally distributed continuous data were presented as mean \(\pm\) standard error, and non-normally distributed continuous data were 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/m²)          | 24.0±0.7 | 25.5±0.7 | 25.3±0.7 | 0.3117  |
| WC (cm)              | 89.0±19 | 90.2±22 | 91.7±20 | 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_{O} (pmol/L)       | 35.5±4.6 | 44.3±4.6 | 499±57 | 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)      | 570 (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 (41.9,141.4) | 47.7 (37.8,80.2) | 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 (3.71,956) | 4.36* (2.73,553) | 3.61* (2.20,510) | 0.0026  |
| DI_{O} (mmol/L)      | 1.87 (1.30,2.60) | 0.91 (0.72,158) | 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_{O}, 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, insulinogetic index; QUICKI, Quantitative Insulin Sensitivity Check Index; WC, waist circumference.

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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 loge-HOMA-\( \beta \) (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 \( (G_{120}) \), \( G_m \), loge-IGI, QUICKI and loge-DIO (Table 3, Figure 2c,d).

**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 loge-HOMA-\( \beta \) were determinants of \( M_{120}-M_0 \). 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; loge-DIO, loge-transformed oral disposition index; loge-HOMA-\( \beta \), loge-transformed homeostasis model assessment of \( \beta \)-cell function; loge-IGI, loge-transformed insulinogenic index; loge-MI, loge-transformed Matsuda Index; QUICKI, Quantitative Insulin Sensitivity Check Index; WC, waist circumference.

**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) | 408 ± 2.0 | 438 ± 2.2 | 450 ± 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* | −14.2 ± 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.

**Table 3** | Simple correlation between plasma mannose levels and clinical factors before and after glucose 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 |
| loge-HOMA-\( \beta \) | 0.022 | 0.8487 |
| \( I_0 \) | 0.295 | 0.0102 |
| QUICKI | −0.278 | 0.0156 |

| 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 |
| loge-IGI | −0.334 | 0.0034 |
| loge-MI | −0.192 | 0.0991 |
| QUICKI | −0.237 | 0.0410 |
| loge-DIO | −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; loge-DIO, loge-transformed oral disposition index; loge-HOMA-\( \beta \), loge-transformed homeostasis model assessment of \( \beta \)-cell function; loge-IGI, loge-transformed insulinogenic index; loge-MI, loge-transformed Matsuda 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.
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 loge-IGI, loge-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.

In dogs, portal infusion of catecholamine augments EGP by selectively stimulating glycogenolysis, 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. Furthermore, administration of lactate and alanine, which are gluconeogenic substrates to fasted rats, increased plasma glucose but did not increase plasma mannose. 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.
Fasting plasma mannose levels (M0) 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 M0 and fasting glucose (G0) was analyzed in a combined population of the three groups. Simple and multiple regression analyses showed that M0 was significantly correlated with G0 (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. 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.

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. Taken together, our present results are compatible with the possibility of mannose as an indicator of glycogenolysis 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/glucacon ratio after glucose loading were observed in patients with diabetes, 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 humans is required.

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DISCLOSURE
The authors declare no conflicts of interest.

REFERENCES
1. Alton G, Hasilik M, Niehues R, et al. Direct utilization of mannose for mammalian glycoprotein biosynthesis. Glycobiology 1998; 8: 285–295.
2. Soyama K. Enzymatic determination of D-mannose in serum. Clin Chem 1984; 30: 293–294.

3. Sone H, Shimano H, Ebinuma H, et al. Physiological changes in circulating mannose levels in normal, glucose-intolerant, and diabetic subjects. Metabolism 2003; 52: 1019–1027.

4. Taguchi T, Yamashita E, Mizutani T, et al. Hepatic glycogen breakdown is implicated in the maintenance of plasma mannose concentration. Am J Physiol Endocrinol Metab 2005; 288: E534–E540.

5. Treadway JL, Mendys P, Hoover DJ. Glycogen phosphorylase inhibitors for treatment of type 2 diabetes mellitus. Expert Opin Investig Drugs 2001; 10: 439–454.

6. Miwa I, Taguchi T. A simple HPLC assay for plasma D-mannose. Clin Chim Acta 2013; 422: 42–43.

7. Committee on the Standardization of Diabetes Mellitus-Related Laboratory Testing of Japan Diabetes Society. International clinical harmonization of glycated hemoglobin in Japan: from Japan Diabetes Society to National Glycohemoglobin Standardization Program values. J Diabetes Investig 2012; 3: 39–40.

8. World Health Organization. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a World Health Organization/IDF Consultation. Geneva: World Health Organization, 2006.

9. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Investig 2010; 1: 212–228.

10. Seltzer HS, Allen EW, Herron AL Jr, et al. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetic mellitus. J Clin Invest 1967; 46: 323–335.

11. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and ß-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419.

12. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000; 85: 2402–2410.

13. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22: 1462–1470.

14. Utzschneider KM, Prigeon RL, Faulenbach MV, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care 2009; 32: 335–341.

15. Chu CA, Sindelar DK, Neal DW, et al. Direct effects of catecholamines on hepatic glucose production in conscious dog are due to glycogenolysis. Am J Physiol Endocrinol Metab 1996; 271: E127–E137.

16. DeFronzo RA. The triumvirate: ß-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988; 37: 667–687.

17. Consoli A, DeFronzo RA. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1996; 81: 4059–4067.

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

Table S1 | Simple correlation coefficients between independent variables.