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Urinary Myoinositol Index : A New and Better Marker for Postmeal Hyperglycemia

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Abstract : We investigated the usefulness of the urinary myoinositol index (UMI) for identifying postmeal hyperglycemia in type 2 diabetics undergoing a meal tolerance test. Fifty-eight patients (18 males, 40 females) were enrolled, fasted overnight and blood collected prior to and 1 and 2 hours following the test meal. Urine was collected 2 hours after the test meal. Plasma 1,5-anhydroglucitol (1,5-AG) was measured enzymatically, and UMI with an improved enzymatic cycling method. Simple and multiple regression analyses were employed to determine correlations between plasma glucose (PG) and three PG markers ; HbA_{1C} (Japan Diabetes Society), 1,5-AG and UMI. Study population characteristics were age 67.6±7.9 years, body mass index 24.9±3.8 kg / m^2 and waist circumference 90.2±10.4 cm. Mean concentrations for PG were 130±23 mg / dL (fasting), 179±46 mg / dL (1 h postmeal) and 150±49 mg / dL (2 h postmeal), HbA_{1C} (6.3±0.6%), 1,5-AG (11.9±5.7 μg / mL) and 2 h UMI (52.0±35.9 mg / gCr). Correlation coefficients were calculated between 1 h postmeal PG and HbA_{1C} (r＝0.558), 1,5-AG (r＝0.256), and 2 h UMI (r＝0.496), and 2 h postmeal PG HbA_{1C} (r＝0.605), 1,5-AG (r＝0.306), and 2 h UMI (r＝0.606). Two hour UMI and HbA_{1C} (Japan Diabetes Society) were significant determinants of 2 h postmeal PG. As HbA_{1C} reflects PG excursion during the previous 1-3 months, UMI may be a useful marker for monitoring and management of postmeal hyperglycemia in type 2 diabetics.

Key words : postmeal hyperglycemia, glucose excursion, urinary myoinositol index, UMI, polyol

Introduction

A growing body of evidence suggests that reducing postmeal (postprandial) plasma glucose (PG) excursions as well as achieving hemoglobin A_{1C} (HbA_{1C}) goals is important for the prevention of cardiovascular disease (CVD)[1-4)]. There are many markers used to monitor PG control and diabetes management, e.g. HbA_{1C}, glycoalbumin (GA), fructosamine, 1,5-anhydroglucitol (1,5-AG). Plasma 1,5-AG is a naturally occurring dietary polyol that has

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also been proposed as a marker for postmeal hyperglycemia[5,6].

Myoinositol is one form of inositol, a circular D-glucose isomer, which is stable in urine samples at room temperature[7,8]. Plasma myoinositol concentration is stable and remains within physiological concentration even in patients with abnormal glucose tolerance. However, urinary myoinositol concentration increases during hyperglycemia[9,10]. Thus, urinary myoinositol concentration may be a useful new indicator of PG excursions. The Urinary Myoinositol Index (UMI), calculated as the ratio of urinary myoinositol to creatinine, has also been reported to be a new and appropriate marker for abnormal glucose tolerance, and is now covered by health insurance in Japan.

However, the relationship between UMI and postmeal PG excursion has not been fully clarified. Therefore, we examined the relationship between postmeal hyperglycemia and HbA_{1C}, plasma 1,5-AG or UMI in patients with type 2 diabetes mellitus (T2DM) undergoing a meal tolerance test.

Material and Methods

Fifty-eight T2DM patients (18 males and 40 females) were enrolled consecutively in this study. Patients with renal disease (plasma creatinine ≥1.2 mg / dL ; 110 μmol / L or proteinuria ≥2+), hepatic disease, those using insulin or α-glucosidase inhibitors (α-GI), and those with HbA_{1C} above 8.0% were excluded from this study. All study protocols and procedures were approved by the Ethics Committee of Toho University Medical Center Omori Hospital. The study objectives and intended procedures were individually explained to all study participants, and all completed the study. Written informed consent was obtained from all participants. This was a prospective study, and its term was between October 2007 and June 2008.

Test meal A was fed to all participants and was composed of cream of chicken soup, 6 crackers, and pudding. This food contained 51.4% carbohydrate (57.6 g), 15.3% protein and 33.3% lipid and had a total energy content of 460 kcal.

A venous blood sample was collected after an overnight fast of at least 10 hours. Thereafter, patients passed urine, and the meal tolerance test was performed. All subjects consumed test meal A within 15 minutes along with 100 ml of water, and blood samples were collected 1 and 2 hours postprandially. Urine samples were collected 2 hours postprandially (Table 1).

Fasting and postmeal PG and HbA_{1C} were measured by AutoAnalyzer. Plasma 1,5-AG was measured using a fully enzymatic method[11], and urinary myoinositol by an improved enzymatic cycling method[12]. The UMI was calculated as the ratio of urinary myoinositol to creatinine. The HbA_{1C} concentrations were expressed using values standardized by the Japan Diabetes Society (JDS).

All values are expressed as mean ± SD. Simple and multiple regression analyses were employed to determine correlations between PG and PG markers (HbA_{1C}, 1,5-AG and 2

Table 1. Blood and urine sampling points

	fasting	postmeal	
	0-h	1-h	2-h
PG	⊗	⊗	⊗
HbA_{1C} (JDS)	⊗	–	–
1,5-AG	⊗	–	–
2-h UMI	–	–	⊗

HbA_{1C} (JDS) : hemoglobin A_{1C} (Japan Diabetes Society), 1,5-AG : 1,5-anhydroglucitol, 2-h UMI : 2-hour urinary myoinositol index.

Table 2. Baseline characteristics of study subjects

Gender (male / female)	18 / 40
Age (years)	67.6 ± 7.9
BW (kg)	64.5 ± 10.7
BMI (kg / m^2)	24.9 ± 3.8
Waist circumference (cm)	90.2 ± 10.4
Duration of diabetes (years)	7.5 ± 4.4
HbA_{1C} (JDS) (%)	6.3 ± 0.6
1,5-AG (μg / mL)	11.9 ± 5.7
2-h UMI (mg / gCr)	52.0 ± 35.9
Treatment for diabetes	
diet / exercise	9 (16%)
monotherapy	13 (22%)
combination therapy	36 (62%)

All values are expressed as mean ± SD or number. BW: body weight, BMI : body-mass index, HbA_{1C} (JDS) : hemoglobin A_{1C} (Japan Diabetes Society), 1,5-AG : 1,5-anhydroglucitol, 2-h UMI : 2-hour urinary myoinositol index.

h UMI). All statistical analysis was performed employing Excel 2007-Microsoft Office® software package. A significant difference was defined as $P < 0.05$.

Results

Patient characteristics were age (67.6 ± 7.9 years), body mass index (BMI) (24.9 ± 3.8 kg / m^2) and waist circumference (90.2 ± 10.4 cm). HbA_{1C} (JDS), 1,5-AG and 2 h UMI were 6.3 ± 0.6%, 11.9 ± 5.7 μg / mL and 52.0 ± 35.9 mg / gCr, respectively (Table 2). Plasma glucose concentrations were 129.9 ± 22.9 mg / dL (fasting), 178.5 ± 45.9 mg / dL (1 h) and 149.9 ± 48.7 mg / dL (2 h) (Fig. 1). Figure 2 shows the relationship between HbA_{1C} (JDS) and 0 h PG, 1 h PG or 2 h PG. The highest correlation coefficient was between HbA_{1C} (JDS) and 2 h PG ($r = 0.605$). The relationship between 1,5-AG and 0 h, 1 h or 2 h PG is shown in Fig. 3, with the highest correlation found between 1,5-AG and 2 h PG ($r = 0.306$). Figure 4 shows the relationship between 2 h UMI and 0 h, 1 h and 2 h PG. The highest correlation coefficient was for the relationship between 2 h UMI and 2 h PG ($r = 0.606$). For all markers, the highest correlation coefficient was for the relationship between PG and PG markers at 2 h postprandially. Of the three PG markers, 2 h UMI and HbA_{1C} (JDS) showed the highest correlation coefficients for the relationship with postmeal PG.

The relationship between area under the glucose curve [AUC (PG)] measured at the three time points (0, 1 and 2 h postmeal) and the three PG markers is summarized in Table 3. All three PG markers were associated with AUC (PG). To identify markers that are associated with 2 h PG, we performed a multiple regression analysis between 2 h PG and the three PG markers. An association was found between 2 h PG and HbA_{1C} (JDS)

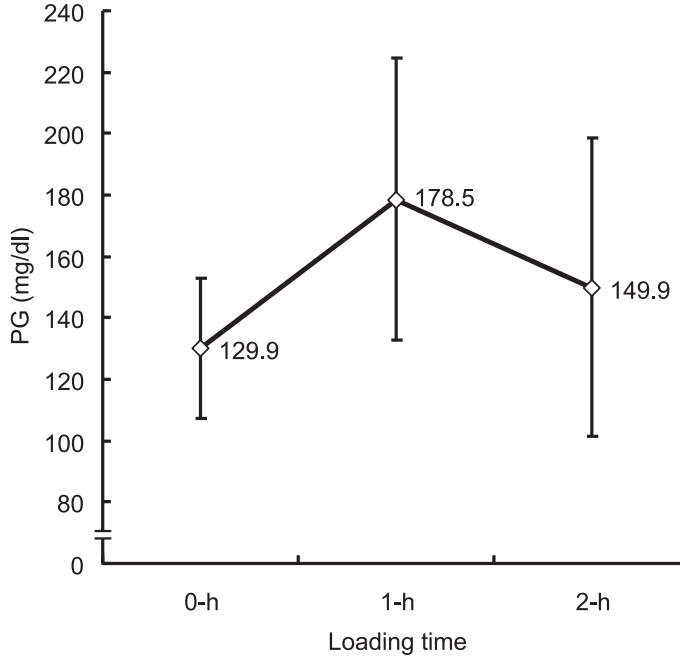

Fig. 1. Plasma glucose changes during test meal loading
Open diamonds indicate plasma glucose concentration. Vertical bars indicate mean ± SD.
PG : plasma glucose, 0-h : 0-hour, 1-h : 1-hour, 2-h : 2-hour.

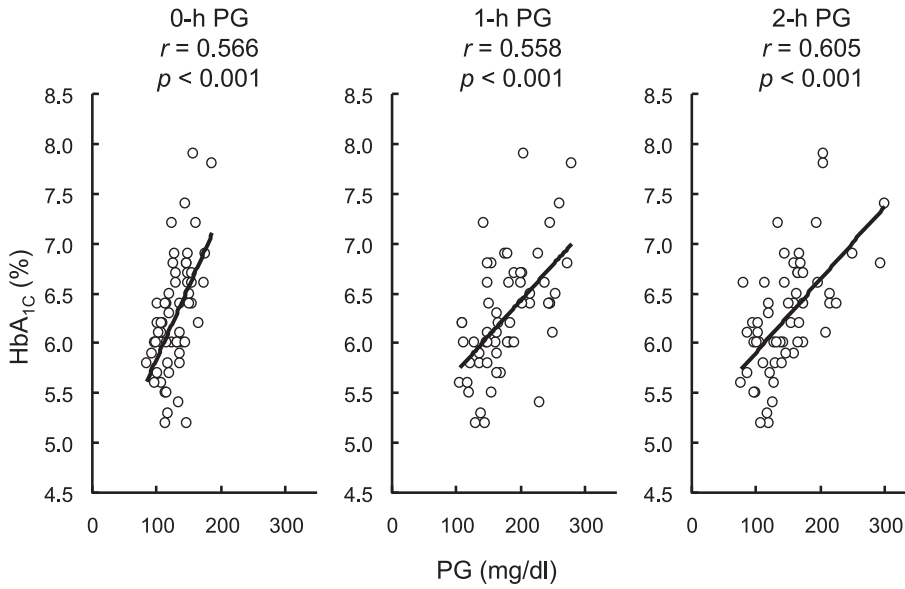

Fig. 2. Simple linear correlation between PG and HbA_{1C} (JDS)
Scatter diagram of association between PG and HbA_{1C} (JDS) with open circles indicating analyte values and showing an approximately linear relationship. Left, middle and right panels indicate the relationship between PG and HbA_{1C} at 0-hour, 1-hour, and 2-hour, respectively.
PG : plasma glucose, HbA_{1C} (JDS) : hemoglobin A_{1C} (Japan Diabetes Society), 0-h : 0-hour, 1-h : 1-hour, 2-h : 2-hour.

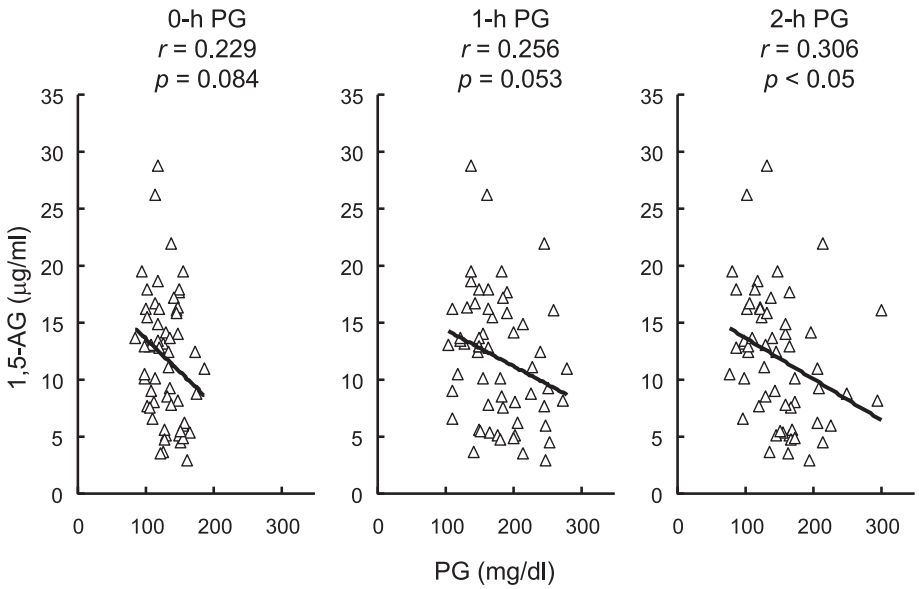

Fig. 3. Simple linear correlation between PG and 1,5-AG
Scatter diagram of association between PG and 1,5-AG with open triangles indicating analyte values and showing an approximately linear relationship. Left, middle and right panel indicate the relationship between PG and 1,5-AG, at 0-hour, 1-hour, and 2-hour, respectively.
PG : plasma glucose, 1,5-AG : 1,5-anhydroglucitol, 0-h : 0-hour, 1-h : 1-hour, 2-h : 2-hour.

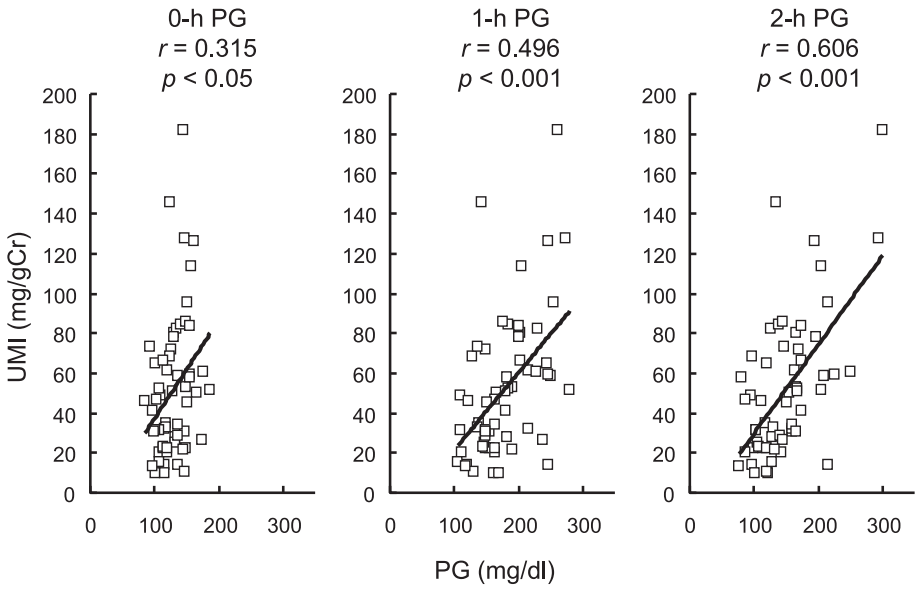

Fig. 4. Simple linear correlation between PG and 2-h UMI
Scatter diagram of association between PG and 2-h UMI with open squares indicating analyte values and showing an approximately linear relationship. Left, middle and right panel indicate the relationship between PG and 2-h UMI, at 0-hour, 1-hour, and 2-hour, respectively.
PG : plasma glucose, 2-h UMI : 2-hour urinary myoinositol index, 0-h : 0-hour, 1-h : 1-hour, 2-h : 2-hour.

Table 3. Correlation coefficients between AUC (PG) (0-2h) and HbA_{1C} (JDS), 1,5-AG or 2-h UMI

PG markers	*r*	*P* value
HbA_{1C} (JDS) (%)	0.6405	<0.00001
1,5-AG (μg / ml)	0.2985	0.02283
2-h UMI (mg / gCr)	0.5625	<0.00001

P values indicate the association between AUC (PG) (0-2h) and three PG markers.
AUC : area under the curve, PG : plasma glucose, *r* : correlation coefficient, HbA_{1C} (JDS) : hemoglobin A_{1C} (the Japan Diabetes Society), 1,5-AG : 1,5-anhydroglucitol, 2-h UMI : 2-hour urinary myoinositol index.

Table 4. Multiple regression analysis to determine factors that influence the value of 2-h PG

Factors	*P* value
HbA_{1C} (JDS) (%)	0.00853
1,5-AG (μg / ml)	0.88366
2-h UMI (mg / gCr)	0.00942

P values indicate the association between between 2-h PG and three PG markers.
PG : plasma glucose, HbA_{1C} (JDS) : hemoglobin A_{1C} (the Japan Diabetes Society), 1,5-AG : 1,5-anhydroglucitol, 2-h UMI : 2-hour urinary myoinositol index.

or 2 h UMI (Table 4).

Discussion

Patients with type 2 diabetes mellitus are at increased risk for atherosclerotic diseases such as coronary heart disease and stroke. Many long-term studies and meta-analyses have been published on the clinical significance of postmeal or post-glucose loaded hyperglycemia for macroangiopathy and mortality. The Funagata Study reported that the cumulative survival rates from CVD events associated with impaired glucose tolerance (IGT), were similar to those of diabetes, however that of impaired fasting glucose (IFG) was not significantly lower than that of normal fasting glucose[13]. Further, the Diabetes Epidemiology Collaborative analysis Of Diagnostic criteria in Europe Study (DECODE Study) reported that the 2 h postprandial blood glucose following the 75 g oral glucose tolerance test (75 g OGTT) was a better predictor of deaths from all causes and CVD than fasting blood glucose[14]. Moreover, The Diabetes Intervention Study (DIS), a prospective population-based multicentre trial of newly detected cases of non-insulin-dependent diabetes mellitus, used multivariate analysis to show that postmeal blood glucose is an independent risk factor for myocardial infarction[15]. Thus, postmeal hyperglycemia is now known to be an important risk factor for CVD, even in non-diabetic subjects and patients with IGT[3, 4, 13].

Therefore, identifying the best marker for postmeal blood glucose excursion has become important. Currently, there are many clinical markers used to monitor and manage PG and treat diabetes ; with urinary myoinositol concentration proposed as a useful marker of postmeal hyperglycemia.

Inositol is a naturally occurring nutrient that is usually classified as a carbocyclic polyol. Myoinositol, one form of inositol, is an extremely stable circular alcohol and is supplied daily from the diet and by renal biosynthesis[7,8]. Myoinositol in the plasma of healthy individuals is maintained at physiological concentration (approximately 25 μmol / L), but its

concentration in urine is reported to be higher in patients with diabetes or chronic renal failure[16-19]. Myoinositol is transported in renal tubules and excreted in urine, as is 1,5-AG. The reabsorption of myoinositol is competitively inhibited by the urinary glucosuria induced by hyperglycemia.

We used a highly sensitive method to measure urinary myoinositol. The measurable range for myoinositol using this method extends from 10 to 1,500 μmol / L. Urinary myoinositol / creatinine ratio was calculated following a meal load. A previous study which performed a 75 g OGTT and monitored UMI for the subsequent 3 hours in patients with normal glucose tolerance (NGT), IGT and diabetes, found peak values at 2 hours postprandially. Moreover, the UMI value was higher with greater degrees of glucose intolerance[20]. The mean UMI at 2 h following test meal loading for subjects with NGT was 18.0 ± 10.6 mg / gCr, and the estimated normal range was from 7.4 to 28.6 mg / gCr.

In this study, 1 h PG was greater than 2 h, but the three PG markers studied were more closely associated with 2 h than 1 h PG. Further, the correlation coefficient relating 2 h PG and 2 h UMI was greater than that between 2 h PG and 1,5-AG or HbA_{1C} (JDS). Multiple regression analysis revealed that 2 h UMI as well as HbA_{1C} (JDS) were significant determinants of 2 h PG.

Currently, HbA_{1C}, GA, 1,5-AG and PG are mainstream markers used to indicate glycemic control in diabetics. The benefit of HbA_{1C}, GA and 1,5-AG measured in blood and plasma samples is that they are unaffected by increases in PG following a single meal load. Of these PG markers, 1,5-AG has been proposed as a marker for postmeal hyperglycemia. It accurately reflects recent PG increases (within a few days,) and its daily recovery rate is constant (0.3 μg / mL / day) in and between those individuals with excellent glycemic control[21]. Dungan *et al* evaluated the relationship between postprandial hyperglycemia and glycemic markers using a continuous glucose monitoring system. They concluded that 1,5-AG reflected glycemic excursions, often in the postprandial state, more robustly than fructosamine or HbA_{1C}[22]. A similar study was carried out by Suwa *et al*[23]. They showed that GA, compared with HbA_{1C} and 1,5-AG, reflected not only short-term mean blood glucose concentration but also glycemic fluctuation. To our knowledge, there is no report definitively identifying the best marker for postmeal hyperglycemia. However, in the current study the correlation coefficient between postmeal PG and 1,5-AG was lower than that between postmeal PG and 2 h UMI. Thus, 2 h UMI may be a better marker for postmeal hyperglycemia than 1,5-AG.

In our study, HbA_{1C} was also a good marker for 1 h or 2 h postprandial PG as indicated by the correlation between AUC (PG) and HbA_{1C} (Table 3), and the results of the multiple regression analysis (Table 4). However, it neither accurately reflects transient elevations of PG occurring within a few days, nor responds quickly to changes in PG (including fasting and postmeal). Furthermore, anemic subjects often demonstrate lower HbA_{1C} values because of a rapid turnover of red blood cells. The values for 1,5-AG and 2 h UMI may,

therefore, be better for monitoring postmeal hyperglycemia than HbA_{1C}.

The advantages of UMI include non-invasive sample collection, and a rapid change in value in response to changes in PG. Thus, compared to HbA_{1C} and 1,5-AG, the measurement of 2 h UMI is easier because blood sampling is not necessary, however the test has several limitations. First, urine sampling at exactly 2 h postprandially was required ; second, similar to 1,5-AG, pseudo-positive reactions were found in patients with renal glycosuria[20] ; third, it is currently unknown what drugs may influence the UMI value.

Conclusion

UMI seems to be a better marker for monitoring postmeal hyperglycemia compared with plasma 1,5-AG or HbA_{1C}, and so it may help monitor and manage postmeal (postprandial) PG in diabetic subjects.

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