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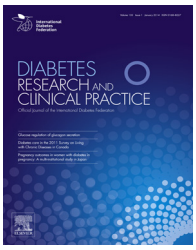

The utility of urinary myo-inositol as a marker of glucose intolerance

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ABSTRACT

Objective: The most common screening tests for glucose intolerance are fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c). Because it reflects the current status of hyperglycemia, urinary myo-inositol (UMI) may be useful. We evaluated UMI as a screening tool for glucose intolerance.

Design and methods: A cross-sectional, community-based population study of 1057 Japanese residents. 173 with an FPG level between 5.5 and 6.9 mmol/L and an HbA1c under 6.5% had an oral glucose tolerance test. We measured UMI level before (fasting UMI) and 2 h after (2 h-UMI) glucose ingestion. Δ-UMI was defined as the difference between fasting UMI and 2 h-UMI.

Results: Δ-UMI, 2 h-UMI and HbA1c levels significantly increased as glucose intolerance worsened. Δ-UMI level was significantly positively correlated with 2 h-UMI level ($r = 0.896$, $p < 0.001$). Using cutoff levels from receiver operating characteristic (ROC) analyses, the sensitivity of Δ-UMI (82.1%) and 2 h-UMI (79.3%) were higher than that of HbA1c (48.3%). The area under the ROC curve values for Δ-UMI (0.903) and 2 h-UMI (0.891) were higher than that for HbA1c (0.785).

Conclusions: 2 h-UMI is useful as a non-invasive screening of glucose intolerance.

1. Introduction

Type 2 diabetes mellitus (DM) is increasing rapidly and has become a worldwide health problem. The International Diabetes Federation estimates that 522 million people worldwide will have DM by 2030 [1] and the World Health Organization (WHO) that DM will become the seventh leading cause of death in the world by 2030 [2]. According to large scale epidemiological studies, even at the early stage of glucose intolerance, large glucose fluctuations are likely to cause macrovascular diseases, including cardiovascular and cerebrovascular diseases [3,4]. Because of its various complications, DM has a significant economic impact on patients, families, healthcare systems, and countries. Prevention and early diagnosis and intervention are effective for reducing the burden of DM [5,6].

A fasting plasma glucose (FPG) sample and a subsequent 75 g oral glucose tolerance test (OGTT) [7] are commonly used for the diagnosis of DM. The American Diabetes Association and WHO have proposed the measurement of glycated hemoglobin (HbA1c) as a diagnostic tool [8]. The advantages

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of HbA1c are that it does not require special preparation and that it can be measured at any time of day, but it requires a whole blood sample. It is often underestimated in women and can be inaccurate in persons with anemia or hemoglobinopathies [9,10].

Myo-inositol is a form of inositol, a circular D-glucose isomer, and it is an extremely stable circular alcohol. It is known that urinary myo-inositol (UMI) excretion is increased in persons with hyperglycemia [11–14]. Detection is possible without blood collection at the early stage of glucose intolerance, it is non-invasive to examinees, and it is possible to test many examinees simultaneously [15,16]. Recently, an easy to use, highly sensitive enzymatic cycling method for measuring UMI has been developed and approved for clinical use in Japan [17].

The aim of this large, cross-sectional, Japanese community-based population study was to evaluate the utility of UMI as a screening and diagnostic tool for the early stage of glucose intolerance and DM as part of a follow-up medical examination.

2. Subjects and methods

2.1. Study population and design

The study began in 2007 as a survey of the incidence of macrovascular events associated with lifestyle-related diseases among the general population as a part of the Kyushu and Okinawa Population Study (KOPS) [18,19]. This substudy evaluated the residents of two suburban areas: Iki city, an isolated island in southwestern Japan with about 28 400 residents, and Hoshino village, a rural, mountainous village with about 3300 residents. The lifestyle in these two areas is similar to that of other parts of Japan. The participants were notified, by local newspaper and public announcements, of a free annual health examination given by our department.

2.2. Study 1

A total of 1057 residents (348 men, 709 women, and age range 24–79 years) participated in the health examinations in 2010 and 2011. All underwent a medical evaluation and were interviewed about their personal and family medical histories and lifestyle-related habits. After the exclusion of 11 participants because of insufficient data, the data of 1046 were available for analysis (Study 1). Common laboratory tests were included in the examination, as outlined below.

2.3. Study 2

As shown in Fig. 1, from among the 1046 with complete data, 232 eligible participants were selected for OGTT based on an FPG level between 5.5 and 6.9 mmol/L, an HbA1c level under 6.5%, and no history of DM, viral hepatitis, thyroid disease, anemia, or chronic renal disease. Chronic renal disease was defined as an estimated glomerular filtration rate (eGFR) under 60 mL/min/1.73 m^2 or the presence of macro-albuminuria. An eGFR was calculated using the following formula proposed by the Japanese Society of Nephrology: $194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} \times 0.739$ (if women) [20]. Macro-albuminuria was considered positive when the dipstick test (Ames dipstick, Bayer Medical, Tokyo, Japan) for spot urine was positive, corresponding to a urinary protein level of over 300 mg/L.

Of the 232 eligible participants, 176 received an OGTT at a later date (about 3 months later). OGTT was performed following the standard protocol after at least 8 h of overnight fasting. Urine samples for UMI were collected just before and 2 h after glucose ingestion. The participants were categorized as newly diagnosed DM, impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and normal glucose tolerance (NGT) according to the WHO criteria [21]. The plasma glucose and serum insulin levels of each blood sample were measured,

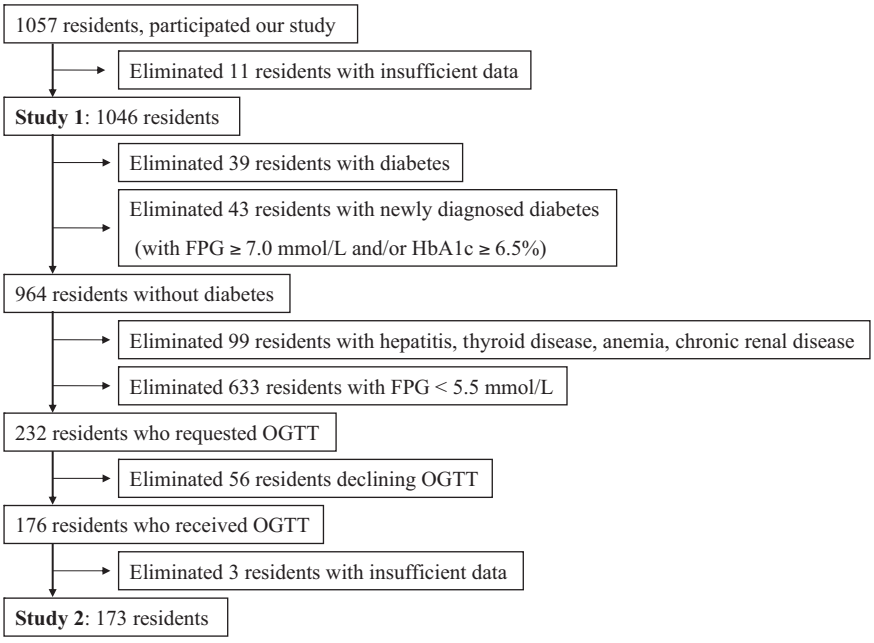

Fig. 1 – Cross-sectional study design. FPG, fasting plasma glucose; OGTT, oral glucose tolerance test.

as were UMI and creatinine in each urine sample. The difference between the UMI level after 2 h (2 h-UMI) and that just before glucose ingestion (fasting UMI) was calculated for each participant (Δ-UMI). The HbA1c level of each fasting blood sample was also measured.

Of the 176 participants who received OGTT, 3 were excluded because of insufficient data, leaving the data of 173 available for analysis in Study 2. To ensure the validity of the data, all doctors who participated in the study were staff members of the General Internal Medicine Department of Kyushu University Hospital who had been trained with regard to the study protocol and the medical procedures. This study was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. The study was approved by the Kyushu University Hospital Ethics Committee, and written informed consent was obtained from each resident prior to the examination.

2.3.1. *Anthropometry measurement*

Anthropometric measurements were done with the participant wearing indoor clothing and without shoes. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The waist circumference was measured midway between the lowest rib and the iliac crest, in a standing position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured on the right arm, in the sitting position, with an automated sphygmomanometer (HEM-780, Omron Healthcare, Kyoto, Japan) after a 5-min rest.

2.3.2. *Urinary myo-inositol assay*

The UMI level was measured with a Lucica MI kit (Asahi Kasei Pharma, Tokyo, Japan). This assay uses an enzymatic cycling method that incorporates myo-inositol dehydrogenase, which is highly specific for myo-inositol [17]. The measurement range of this assay is linear between 10 and 2000 μmol/L. UMI can be accurately measured because there is little influence from coexisting glucose in the urine samples in this assay and it is measured by a general-purpose biochemical auto-analyzer. The within-run and between-run coefficient of variation values were 0.5–1.1% and 0.4–1.3%, respectively.

To correct for the influence of the amount of urine on the measurement of UMI, urinary creatinine was measured using an enzyme method and UMI was calculated as urinary myo-inositol/creatinine (Cr) ratio (mg/g Cr).

2.4. *Laboratory measurements*

All blood samples were collected after an 8-h overnight fast. Aliquots of whole blood and fresh serum and plasma samples from each participant were immediately separated and sent at 4 °C to a clinical laboratory testing company (SRL, Fukuoka, Japan).

Plasma glucose concentration was measured using a hexokinase-glucose-6-phosphate dehydrogenase method (Quick Auto Neo GLU-HK, Sinotest, Tokyo, Japan). Serum insulin concentration was measured by a chemiluminescent enzyme immunoassay (Lumipulse Presto Insulin, Fujirebio, Tokyo, Japan).

The HbA1c level (Japanese Diabetes Society (JDS)) (%) was measured from fresh whole blood samples using an immunoassay method (RAPIDIA Auto HbA1c; Fujirebio) based on latex agglutination that has been shown to have acceptable precision and good correlation with the high performance liquid chromatography assay [22,23]. The values were adjusted with standard reference materials (JCCRM 411) certified by the Reference Material Institute for Clinical Chemistry Standards. The level for HbA1c (JDS) was converted to the US National Glycohemoglobin Standardization Program (NGSP) equivalent level (%) calculated by the formula; HbA1c (NGSP) = HbA1c (JDS) × 1.02 + 0.25 [24]. The HbA1c levels are expressed as NGSP level (%) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units (mmol/mol) [25].

2.5. *Statistical analyses*

All statistical analyses were performed using JMP® ver. 9 (SAS Institute Inc., Cary, NC, USA). Data are reported as mean ± standard deviation, median [first quartile, third quartile], or percentage within each category. Spearman correlation coefficient analysis was used to analyze the characteristics of the Study 1 population. The student's *t* test and the Mann–Whitney *U*-test were used to compare between-group differences, and the Tukey–Kramer honesty significant difference test, the Steel test, and the Steel–Dwass test were used for multiple group comparisons. The cutoff values were calculated by receiver operating characteristic (ROC) analysis using the Youden Index. Comparison of the diagnostic ability of each test was performed using the area under the ROC curve (AUC). Statistical tests for comparison of AUCs were conducted by the non-parametric approach [26]. A *p*-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. *Study 1*

Table 1 shows the characteristics of the Study 1 participants (1046 sets of complete data). Of these, 39 participants (3.7% of total) had a history of DM, 43 (4.1% of total) were newly diagnosed as having DM, and 89 (8.5% of total) were diagnosed as having IFG. Of the 43 newly diagnosed DM, 6 had both abnormal FPG (≥7.0 mmol/L) and HbA1c levels (≥6.5%), 23 had abnormal FPG only, and 14 had abnormal HbA1c only.

Men had significantly higher BMI and waist circumference than women. The percentages of men with a history of smoking and hypertension were significantly higher than those of women, and men had significantly higher SBP and DBP than women. Although men had significantly higher levels of FPG, percentage with a history of DM, and newly diagnosed DM or IFG than women, there were no significant differences in the levels of HbA1c and fasting serum insulin between men and women. The percentage of women with a history of dyslipidemia was significantly higher than that of men, and women had significantly higher total cholesterol and high and low density lipoprotein cholesterol than men, but men had significantly higher triglyceride than women. Men had significantly higher levels of creatinine and hemoglobin than women, but there was no significant difference in the level of eGFR between men and women.

Table 1 – Clinical characteristics of 1046 participating residents (Study 1).

Factors	Men (n = 346)	Women (n = 700)	p-Value
Age (years)	61 [54, 64]	59 [52, 64]	0.056
Body mass index (kg/m^2)	23.3 [21.4, 25.3]	22.0 [20.2, 24.3]	<0.001
Body mass index ≥ 25, n (%)	99 (28.6)	141 (20.1)	0.003
Waist circumference (cm)	83 [78, 89]	80.5 [74, 86]	<0.001
Systolic blood pressure (mmHg)	127 [116, 138]	123 [113, 133]	<0.001
Diastolic blood pressure (mmHg)	78 [70, 86]	72 [65, 80]	<0.001
History of smoking, n (%)	248 (71.7)	67 (9.6)	<0.001
Present smoking, n (%)	87 (25.1)	29 (4.1)	<0.001
History of hypertension, n (%)	108 (31.2)	153 (21.9)	0.001
History of dyslipidemia, n (%)	30 (8.7)	90 (12.9)	0.050
History of diabetes, n (%)	19 (5.5)	20 (2.9)	0.038
Newly diagnosed as diabetes mellitus, n (%)	21 (6.4)	22 (3.1)	0.029
Diagnosed as impaired fasting glucose, n (%)	44 (12.7)	45 (6.4)	<0.001
Glycated hemoglobin (%)	5.5 [5.2, 5.7]	5.5 [5.2, 5.7]	0.524
Glycated hemoglobin (mmol/mol)	36.6 [33.2, 38.8]	36.6 [33.2, 38.8]	0.524
Fasting plasma glucose (mmol/L)	5.4 [5.0, 6.0]	5.1 [4.8, 5.6]	<0.001
Fasting serum insulin (μU/mL)	4.9 [3.2, 7.8]	5.1 [3.6, 7.7]	0.153
High sensitivity C-reactive protein (mg/L)	0.33 [0.15, 0.77]	0.28 [0.13, 0.58]	0.005
Creatinine (mg/mL)	0.81 [0.73, 0.89]	0.62 [0.56, 0.70]	<0.001
Estimated glomerular filtration rate (mL/min/1.73m^2)	75.4 [67.6, 84.9]	75.3 [67.1, 85.2]	0.698
Total cholesterol (mg/mL)	201 [176, 222]	212 [189, 233]	<0.001
Triglyceride (mg/mL)	98 [70, 143]	84 [62, 116]	<0.001
High density lipoprotein cholesterol (mg/mL)	60 [50, 73]	68 [58, 80]	<0.001
Low density lipoprotein cholesterol (mg/mL)	117 [98, 134]	123 [103, 143]	<0.001
Hemoglobin (g/L)	151 [142, 158]	134 [127, 141]	<0.001

Data are shown as median [first-quartile, third-quartile], mean ± SD, and number.

3.2. *Study 2*

Table 2 shows the characteristics of the Study 2 participants (173 who received OGTT). Of the 173, 28 (16.2%) were newly diagnosed with DM, 63 (36.4%) had IGT, 9 (5.2%) had IFG, and 73 (42.2%) were NGT. Of the 173, 105 had FPG levels between 5.5 and 6.0 mmol/L at the health examination. From the results of OGTT, 9 of 105 (8.6%) were newly diagnosed DM and 38 of 105 (36.2%) had IGT. Because the number of participants with IFG was too small for comparison with other groups, we categorized 173 participants into DM, prediabetes (IGT and IFG), and NGT groups.

3.3. *Influence of sex, age and eGFR on the UMI level of the NGT group*

To evaluate the influence of sex, age, and eGFR on the UMI level, we analyzed the data of the NGT group (33 men and 40 women).

Table 2 – Clinical characteristics of 173 residents who received oral glucose tolerance test (Study 2).

Factors	DM (n = 28)	Prediabetes (n = 72)	NGT (n = 73)
Men/women	12/16	34/38	33/40
Age (years)	63 [60, 65]	62 [59, 66]	62 [56, 64]
Fasting plasma glucose (mmol/L)	6.44 [5.93, 7.04][a]	5.69 [5.34, 6.11][a]	5.39 [5.06, 5.61]
1 h-plasma glucose (mmol/L)	13.95 ± 1.67[a]	11.28 ± 2.10[a]	8.25 ± 2.11
2 h-plasma glucose (mmol/L)	13.01 [12.13, 15.07][a]	8.81 [8.06, 9.65][a]	6.00 [5.11, 6.69]
Fasting UMI (mg/g Cr)	21.9 [13.8, 32.4][a]	18.0 [13.4, 27.2]	16.5 [12.8, 25.7]
2 h-UMI (mg/g Cr)	113.4 [84.3, 162.3][a]	46.5 [26.5, 73.9][a]	27.2 [19.2, 40.8]
Δ-UMI (mg/g Cr)	97.1 [60.8, 136.7][a]	23.6 [8.6, 50.7][a]	7.7 [2.3, 18.8]
Glycated hemoglobin (%)	6.21 ± 0.49[a]	5.88 ± 0.35[a]	5.56 ± 0.26
Fasting serum insulin (μU/mL)	6.2 [4.0, 8.1][b]	4.7 [3.2, 7.3]	4.2 [3.1, 5.9]
Serum insulin 1 h postprandial (μU/mL)	34.9 [29.6, 43.8][a]	41.6 [29.2, 63.4]	50.2 [34.4, 65. 5]
Serum insulin 2 h postprandial (μU/mL)	59.2 [41.0, 85.0][a]	48.3 [34.9, 74.2][a]	31.8 [23.1, 44.0]
Fasting urinary albumin (mg/g Cr)	12.4 [5.1, 29.6]	8.7 [3.7, 16.9]	6.1 [3.3, 15.6]
Urinary albumin 2 h postprandial (mg/g Cr)	8.7 [4.7, 21.9]	6.4 [3.2, 12.8]	5.7 [3.6, 11.0]

Data are shown as median [first-quartile, third-quartile], mean ± SD, and number.
DM, diabetes mellitus; NGT, normal glucose tolerance; UMI, urinary myo-inositol; Cr, creatinine; Δ-UMI was defined as the difference between fasting UMI and 2 h-UMI.
[a] $p \leq 0.005$ (vs. NGT).
[b] $p \leq 0.05$ (vs. NGT).

The fasting UMI levels (median [first quartile, third quartile]) of women were significantly higher than those of men, 21.9 [13.9, 35.0] and 14.1 [11.1, 20.3] mg/g Cr, respectively ($P = 0.002$). However, the 2 h-UMI and Δ-UMI levels of women, 31.1 [20.6, 40.9] and 7.4 [3.4, 17.2] mg/g Cr, respectively were not significantly higher than those of men, 23.1 [16.1, 42.3] and 8.7 [1.7, 20.3] mg/g Cr, respectively ($P = 0.075$ and 0.951, respectively).

The average ages of men and women (median [first quartile, third quartile]) of the NGT group were 63 [59,67] and 62 [56,63] years, respectively. To evaluate the influence of age, we divided 74 NGT participants into 10 year cohorts (≤49 years, 50–59 years, 60–69 years, and 70–79 years). There was a significant difference in the age distribution of the fasting UMI level ($P = 0.009$), but there was no significant difference in the 2 h-UMI ($P = 0.060$) or Δ-UMI level ($P = 0.742$). For fasting UMI, a significant difference was observed only between the ≤49 years and 70–79 years cohorts ($P = 0.026$).

The average eGFR levels (median [first quartile, third quartile]) of the NGT group were 74.2 [66.5, 80.9] mL/min/1.73 m^2. The correlation coefficient between eGFR and fasting UMI was −0.106, that between eGFR and 2 h-UMI 0.051, and that between eGFR and Δ-UMI 0.200. No influence of eGFR on UMI was found in the NGT group.

3.4. *Influence of micro-albuminuria on the UMI level*

The data of the Study 2 participants were analyzed to evaluate the influence of micro-albuminuria on the UMI level. As shown in Table 2, the levels of fasting and 2 h postprandial urinary albumin tended to increase as glucose intolerance worsened, but there was no significant difference among the three groups. In the NGT group (33 men and 40 women), the correlation coefficient between fasting urinary albumin and fasting UMI was 0.005, and that between 2 h postprandial urinary albumin and 2 h-UMI was −0.005. For the 173 Study 2 participants, the values were 0.012 and 0.003, respectively. No influence of urinary albumin on UMI was found.

3.5. *Variations and correlations of Δ-UMI, 2 h-UMI, and HbA1c*

Table 2 shows that the Δ-UMI, 2 h-UMI, and HbA1c levels of the DM and prediabetes groups were significantly higher than those of the NGT group and that the values tended to increase as glucose intolerance worsened.

Δ-UMI had a significant positive correlation to FPG ($r = 0.518$, $P < 0.001$) and HbA1c ($r = 0.389$, $P < 0.001$), and HbA1c had a significant positive correlation to FPG ($r = 0.586$, $P < 0.001$). 2 h-UMI had a significant, positive correlation to Δ-UMI ($r = 0.896$, $P < 0.001$). The positive correlation coefficient between 2 h-UMI and FPG was 0.477 ($P < 0.001$), and between 2 h-UMI and HbA1c was 0.380 ($P < 0.001$).

3.6. *ROC analyses of the utility of Δ-UMI, 2 h-UMI, and HbA1c in the diagnosis of DM*

ROC analyses were performed on HbA1c, 2 h-UMI, and Δ-UMI to determine their usefulness in making a diagnosis of DM. Fig. 2 shows the ROC curves for the diagnostic accuracy of HbA1c (Fig. 2a), 2 h-UMI (Fig. 2b), and Δ-UMI (Fig. 2c). From these curves, the optimal threshold values for predicting DM were 6.3% for HbA1c, 84.2 mg/g Cr for 2 h-UMI, and 51.1 mg/g Cr for Δ-UMI. Using the above cutoff levels for HbA1c, Δ-UMI, and 2 h-UMI, the sensitivities of Δ-UMI (82.1%) and 2 h-UMI (79.3%) were higher than that of HbA1c (48.3%), and the negative predictive values (NPV) of Δ-UMI (96.2%) and 2 h-UMI (95.6%) were higher than that of HbA1c (90.1%). The specificities and the positive predictive values (PPV) of Δ-UMI (86.2% and 53.5%, respectively) and 2 h-UMI (89.0% and 59.0%, respectively) were similar to those of HbA1c (92.5% and 56.0%, respectively). The AUC values for Δ-UMI (0.903) and 2 h-UMI (0.891) were higher than that for HbA1c (0.785), but not significantly ($P = 0.061$ and 0.082, respectively).

3.7. *ROC analyses of the utility of Δ-UMI, 2 h-UMI, and HbA1c in the diagnosis of glucose intolerance*

ROC analyses were done for HbA1c, 2 h-UMI, and Δ-UMI to determine their ability to detect glucose intolerance, including IFG, IGT and DM. Fig. 3 shows the ROC curves for the diagnostic accuracy of HbA1c (Fig. 3a), 2 h-UMI (Fig. 3b), and Δ-UMI (Fig. 3c). From these curves, the optimal threshold values for predicting glucose intolerance were 6.0% for HbA1c, 42.2 mg/g Cr for 2 h-UMI, and 25.4 mg/g Cr for Δ-UMI. Using the above cutoff levels, the sensitivities of Δ-UMI (61.0%) and 2 h-UMI (68.3%) were higher than that of HbA1c (49.0%), and the NPVs of Δ-UMI (62.5%) and 2 h-UMI (64.0%) were higher than that of HbA1c (56.6%). The specificity and PPV of Δ-UMI (89.0% and 88.4%, respectively) were similar to those of HbA1c (91.9% and 89.3%, respectively), but those of 2 h-UMI (78.1% and 81.2%, respectively) were inferior to those of HbA1c. The AUC values for Δ-UMI (0.798) and 2 h-UMI (0.763) were equivalent to that for HbA1c (0.785).

4. Discussion

In this study, three major findings were obtained. First, both the Δ-UMI and 2 h-UMI levels increased as the degree of glucose intolerance worsened in the Study 2 participants who had an OGTT, agreeing with previous studies [16,17]. In these previous studies, the participants were healthy volunteers. In this study, because the Study 2 participants had FPG levels between 5.5 and 6.9 mmol/L and thus were considered to be at high risk of DM, we were able to show the potential utility of UMI in clinical practice. Second, both Δ-UMI and 2 h-UMI were more effective than HbA1c in detecting the DM and glucose intolerance of the Study 2 participants. For detecting DM, our ROC curves showed the optimum cutoff values to be 51.1 mg/g Cr for Δ-UMI and 84.2 mg/g Cr for 2 h-UMI. For detecting glucose intolerance, the values were 25.4 mg/g Cr for Δ-UMI, and 42.2 mg/g Cr for 2 h-UMI. Finally, because the data for 2 h-UMI showed that it has power equivalent to Δ-UMI, we feel that 2 h-UMI would be a useful option for screening for glucose intolerance. UMI is a newer test than HbA1c and there are few clinical studies that show its ability to detect DM and glucose intolerance, thus its detection criteria remain to be clarified. The HbA1c level reflects the mean plasma glucose level over 2–3 months. Some studies have shown that the relative

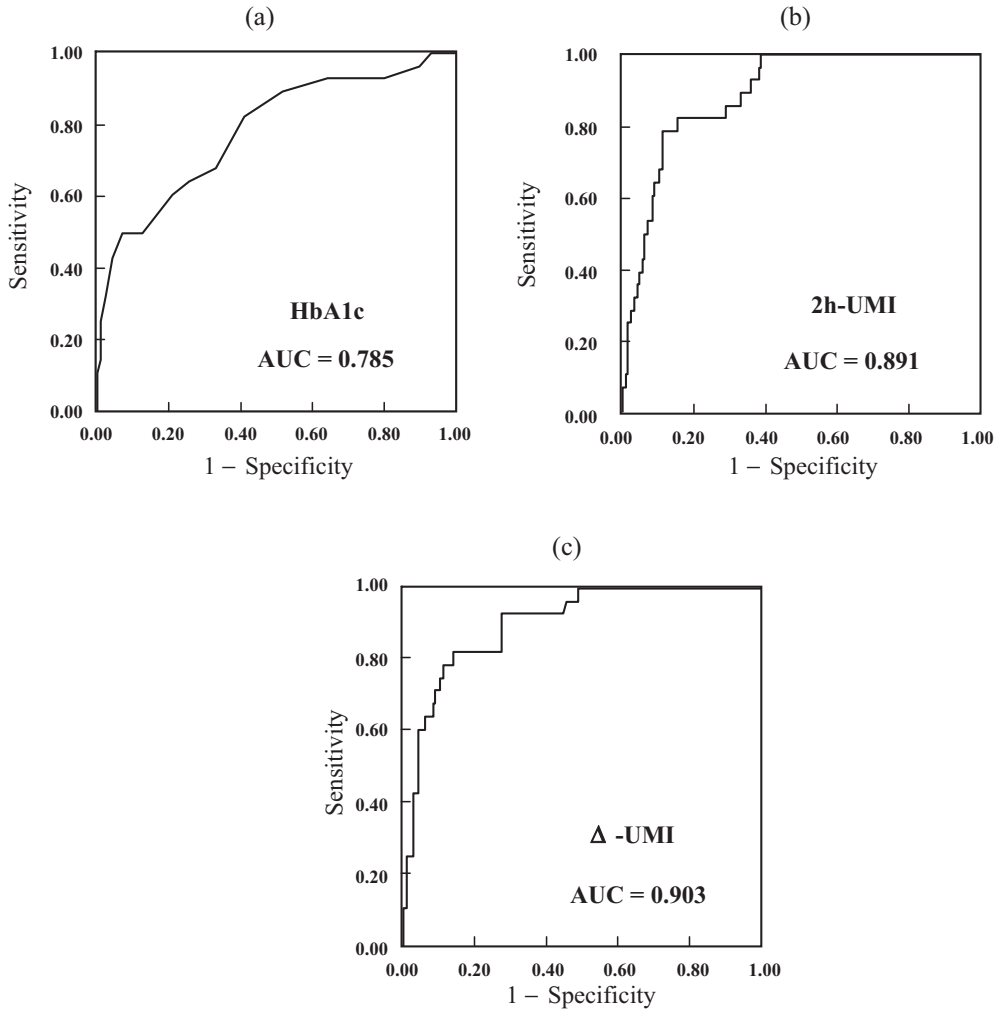

Fig. 2 – ROC analysis of the utility of HbA1c (a), 2 h-UMI (b), and Δ-UMI (c) for diagnosing type 2 diabetes mellitus. The AUC values for HbA1c (a) (0.785, 95% confidence interval (CI): 0.668–0.869), 2 h-UMI (b) (0.891, 95% CI: 0.823–0.935), and Δ-UMI (c) (0.903, 95% (CI): 0.830–0.946). ROC, Receiver operating characteristic; AUC, area under the curve; HbA1c, glycated hemoglobin; 2 h-UMI, 2 hours postprandial urinary myo-inositol; Δ-UMI, the difference between 2 h-UMI and fasting UMI.

contributions of fasting and postprandial glucose levels differ according to HbA1c levels [27,28]. Thus, the HbA1c level does not always reflect fluctuations in plasma glucose levels. OGTT is one of the most precise diagnostic tools for detecting glucose intolerance, however, giving an OGTT to all persons who may have glucose intolerance is expensive and requires considerable time and effort. Measuring UMI still requires fasting and a wait of two hours after glucose ingestion. Because urine is used for the sampling of UMI, it is non-invasive and most likely a less burdensome test for both examinee and examiner, and it can be done for many people simultaneously. We believe that the measurement of UMI for persons with IFG is useful for detecting postprandial hyperglycemia.

Myo-inositol is a form of nine inositol isomers and present in all plant and animal tissues. It is obtained from food at approximately 900–1000 mg/day and is completely absorbed from the gastrointestinal tract. It is also synthesized at about 2000 mg daily in the kidneys, and more than 98% of that is reabsorbed and filtered by the glomeruli [11,29]. The concentration of myo-inositol in blood is maintained at a certain level by incorporation into cells and by excretion, reabsorption, and oxidization in the kidneys, and the concentration of intracellular myo-inositol is several orders of magnitude higher than that in blood. Myo-inositol plays an important role in signaling and is a secondary messenger in a number of biological processes [30,31]. Myo-inositol is extremely stable in urine and can be preserved for a few days at room temperature or for two weeks in a refrigerator without special treatment. It is known that UMI levels are higher in individuals with diabetes [12–14] or chronic renal diseases [32,33] than in healthy individuals. However, because the myo-inositol concentrations in serum and urine are quite low, it was previously difficult to measure. An easy-to-use, highly sensitive enzymatic cycling method of measuring UMI concentration [17] has been available for clinical use in Japan since 2008. It costs about $7 or €6 for one measurement, much lower than an OGTT. Although the methods for measuring UMI are not yet standardized across

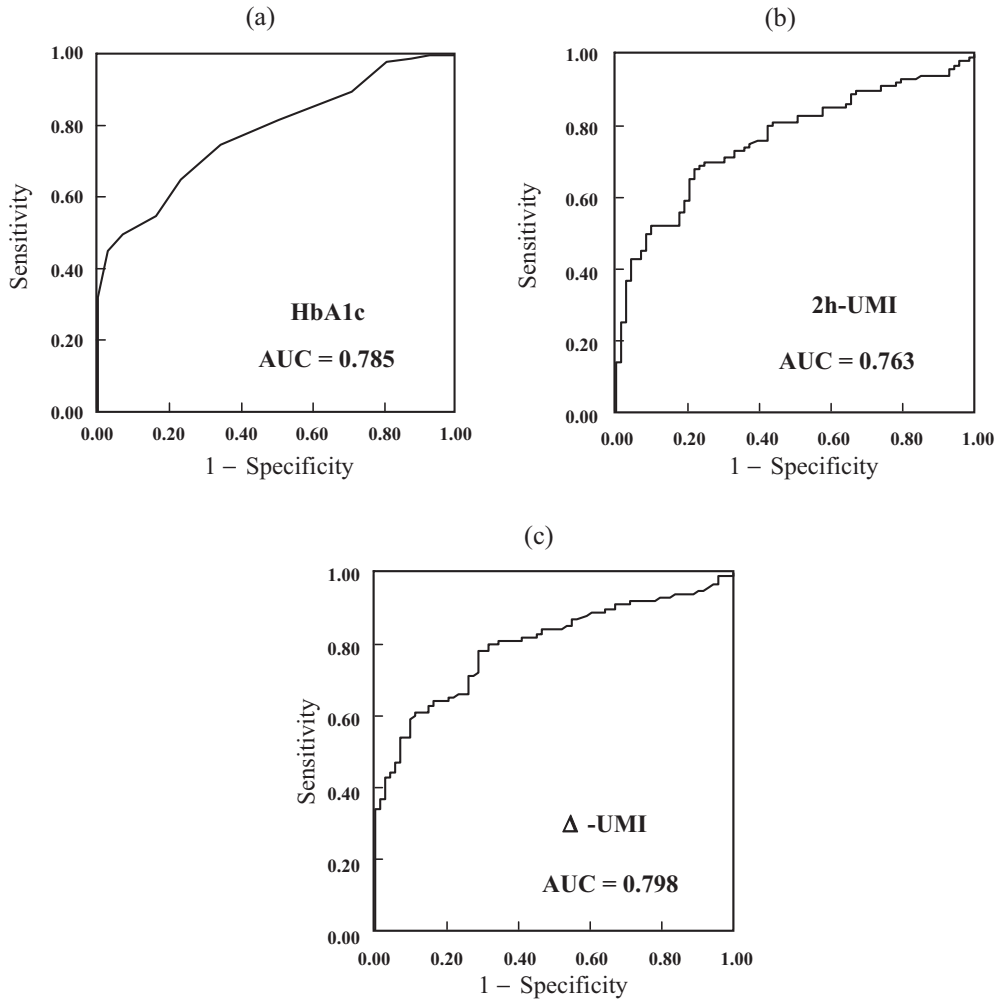

Fig. 3 – ROC analysis of the utility of HbA1c (a), 2 h-UMI (b), and Δ-UMI (c) for diagnosing glucose intolerance. The AUC values for HbA1c (a) (0.785, 95% confidence interval (CI): 0.712–0.843), 2 h-UMI (b) (0.763, 95% CI: 0.685–0.826), and Δ-UMI (c) (0.798, 95% (CI): 0.724–0.856). ROC, Receiver operating characteristic; AUC, area under the curve; HbA1c, glycated hemoglobin; 2 h-UMI, 2 h postprandial urinary myo-inositol; Δ-UMI, the difference between 2 h-UMI and fasting UMI.

the world, the precision of UMI measurement can be assured by use of a general-purpose biochemical auto-analyzer.

Recently, a study of the mechanism of how UMI excretion increases in individuals with hyperglycemia has shown that hyperglycemia itself directly regulates myo-inositol homeostasis and increases UMI excretion [34], thus we can easily detect hyperglycemia by measuring UMI from urine. In agreement with previous studies, the Δ-UMI and 2 h-UMI levels of the participants with DM and glucose intolerance in our study were elevated. We found that UMI would be a useful marker for screening glucose intolerance, particularly postprandial hyperglycemia. In accordance with the mechanism of UMI excretion increase, the UMI level reflects the current status of plasma glucose, while the HbA1c level reflects the mean plasma glucose level over 2–3 months [19]. Thus, both Δ-UMI and 2 h-UMI are more sensitive than HbA1c for detecting current glucose intolerance.

Although HbA1c may be influenced by sex, age, anemia or hemoglobinopathies, renal failure, and many other factors [9,10,35,36], UMI is reported to be influenced by chronic glomerulonephritis and chronic renal failure [32,33]. Thus, we excluded participants whose eGFR level was under 60 mL/min/1.73 m^2 or who had macro-albuminuria. In our study, we found no influence of sex, age or micro-albuminuria to the Δ-UMI and 2 h-UMI levels. Although the fasting UMI levels of women were significantly higher than those of men in the NGT group, the FPG levels of women were lower than those of men. None of the factors tested had an influence on fasting UMI.

The reason for the equivalence of 2 h-UMI and Δ-UMI for detecting DM, IGT and IFG may be that there was no significant difference in fasting UMI among the four groups. This can also be explained by the same mechanism of UMI excretion increase [34]. The 173 participants of Study 2 had FPG levels between 5.5 and 6.9 mmol/L, thus their fasting UMI was not elevated. Generally, the marker we use for glucose intolerance is an elevated FPG level. It has been reported that among Asians, including Japanese, many people tend to have only postprandial hyperglycemia in the early stage of glucose intolerance [37,38]. One of the reasons is that the pathology of glucose intolerance in Asians does not arise mainly from

obesity or insulin resistance, as in Europeans and Americans, but from impaired insulin secretion in lean persons [39]. Therefore, it is difficult to detect the early stage of glucose intolerance using only the FPG level, particularly among Asians. However, many large scale epidemiological studies, such as the Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe study (DECODE) and the Funagata study, have reported that large glucose fluctuations are associated with macrovascular diseases, even in pre-diabetic stages such as IGT [3,4]. Because both 2 h-UMI and Δ-UMI reflect the current status of postprandial hyperglycemia, both 2 h-UMI and Δ-UMI are useful for the screening of the early stages of glucose intolerance. In this study, although the PPVs of 2 h-UMI for detecting DM and glucose intolerance were similar to those of HbA1c, the sensitivities of 2 h-UMI for detecting them were significantly higher than those of HbA1c. The AUC value of 2 h-UMI for detecting DM was higher than that of HbA1c. For detecting glucose intolerance it was equivalent to that of HbA1c. The 2 h-UMI would seem to be a valuable screening option because it has more power for detecting DM and glucose intolerance than does HbA1c. Thus, measuring UMI would be useful for narrowing down the number of persons for whom it is necessary to have an OGTT.

There are some limitations to this study. One is that the relationships between the UMI and FPG levels were unclear because the FPG levels of the participants given OGTT were between 5.5 and 6.9 mmol/L. Further investigation with a wider range of FPG levels will be necessary. Another limitation is that all of the participants were Japanese. Further studies of a global nature will be necessary. In this study, OGTT was given about three months after the health examination. During these three months, it is possible that the participants made changes to their lifestyle. The diagnoses of glucose intolerance by OGTT may not be precisely the same as if we had done the OGTT at the time of the health examination. Furthermore, although WHO guidelines recommend that OGTT be given to individuals with FPG levels between 6.1 and 6.9 mmol/L, we also gave OGTT to our participants with FPG levels between 5.5 and 6.0 mmol/L, because individuals with FPG levels between 5.5 and 6.0 mmol/L have been shown in recent studies to be at high risk of glucose intolerance. Our results showed that about 40% of our participants with an FPG level between 5.5 and 6.0 mmol/L had glucose intolerance. Finally, because our study was cross-sectional we do not have any evidence of the association between the UMI level and the risk for diabetic complications.

In conclusion, although the OGTT will continue to be useful and necessary, measuring the UMI level is a valuable option for the non-invasive screening for glucose intolerance at the early stage in individuals with mild fasting hyperglycemia. The measurement of 2 h-UMI is also helpful. UMI is useful as an alternative marker for the mass screening for dysglycemia.

Conflict of interest

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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