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ORIGINAL ARTICLE

Elevation of the renal threshold for glucose is associated with insulin resistance and higher glycated hemoglobin levels

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Keywords
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ABSTRACT

Aims/Introduction: The renal threshold for glucose (RTg) corresponds to a blood glucose level of ~180 mg/dL; however, in hospitals, patients are often encountered who are hyperglycemic, but urine glucose test strip-negative, who remain negative for urine glucose even at blood glucose concentrations >180 mg/dL, implying a high RTg value. In this study, we aimed to identify factors determining high RTg in Japanese patients with type 2 diabetes mellitus.

Materials and Methods: We estimated RTg (eRTg) using urinalysis data from 67 type 2 diabetes mellitus patients for whom the glucose infusion rate (GIR) was determined by hyperinsulinemic-euglycemic clamp. After allocating patients to two groups according to their baseline eRTg (<180 mg/dL or ≥180 mg/dL), we identified the factors affecting eRTg using simple and multiple linear regression analyses.

Results: GIR, glycated hemoglobin (HbA1c), insulin use and dyslipidemia differed significantly between the groups. In simple regression analysis, GIR, HbA1c, body muscle-to-fat ratio and insulin use were significantly correlated with eRTg; and in multiple regression analysis, GIR and HbA1c remained independent negative and positive determinants, respectively, with the contribution of GIR being substantial. In receiver operating characteristic curve analysis, when GIR <5.7 was used as the insulin resistance threshold, the cut-off value of eRTg was 189 mg/dL (P = 0.0001). Furthermore, in receiver operating characteristic analysis using eRTg ≥189 mg/dL, the cut-off value for HbA1c was 8.0% (P = 0.0006).

Conclusions: High eRTg is associated with low GIR and high HbA1c, with GIR making a substantial contribution.

INTRODUCTION

Historically, urine glucose testing has not been used for daily glucose monitoring, because of its lack of accuracy in hypoglycemic or hyperglycemic patients. Instead, the use of self-monitoring of blood glucose is the widely accepted method of daily glucose status monitoring. However, urine glucose testing is still routinely carried out in diabetes outpatient clinics, because it is a valuable source of information, alongside other qualitative tests, such as urine protein, occult blood and ketone bodies. It is generally accepted that when blood glucose concentrations exceed ~180 mg/dL, urinary glucose excretion occurs, and this blood glucose concentration is referred to as the renal threshold for glucose (RTg)[1,2]. However, diabetes patients who do not show positive for urinary glucose on a strip test, even if their blood glucose concentration is >200 mg/dL, which implies a higher RTg value, are quite frequently encountered in daily medical practice.

In the Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study, it was shown that if blood glucose concentrations exceed 200 mg/dL 2 h after glucose loading, the associated mortality rate is approximately twice that of individuals with lower blood glucose values[3], and that a negative result for postprandial urinary glucose excretion in diabetes patients is indicative of a reasonable level of

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glycemic control. However, on this basis, patients who are hyperglycemic, but urine glucose test strip-negative, would be at risk of serious vascular complications being missed if urinalysis alone was carried out. Therefore, negative outcomes of urine glucose testing should be interpreted with caution.

Although many studies have shown that reductions in RTg induced by the use of sodium–glucose cotransporter 2 inhibitors (SGLT2i) ameliorate insulin resistance in type 2 diabetes mellitus patients, few studies have evaluated the association between an increase in RTg and insulin resistance in type 2 diabetes mellitus patients. However, Yue *et al.*[4] recently reported that homeostasis model assessment-insulin resistance (HOMA-IR) is independently associated with high RTg in type 2 diabetes mellitus patients. In the present study, we retrospectively characterized type 2 diabetes mellitus patients with hyperglycemia, but negative urine glucose test strip results, with respect to their level of insulin resistance, assessed using hyperinsulinemic-euglycemic glucose clamp (HEC).

METHODS

Study design

We initially assessed insulin resistance by HEC in a total of 139 patients with type 2 diabetes mellitus on admission to the Jinnouchi Hospital Diabetes Care Center, Kumamoto, Japan, between April 2011 and November 2017. Patients being treated with SGLT2i at the time of the HEC were excluded. Random urine samples collected within the year preceding the HEC were used to evaluate urine glucose excretion (Multistix 10 SG Reagent Strips; [Siemens Healthcare Diagnostics K.K., Tokyo, Japan] and Clinitek Advantus Urine Chemistry Analyzer [Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA]). RTg was estimated as the blood glucose concentration at which the urinary glucose test strip changed from (–) to (1+) (estimated RTg; eRTg; Table 1), as previously reported[5]. If the maximum blood glucose value among those with a urinary glucose test-strip reading of (–) was higher than the lowest blood glucose value among those with a urinary glucose test-strip reading of (1+), we used the former value as the eRTg. We selected only participants for whom the total number of urinary glucose test-strip readings of (–) and (1+) obtained was more than three, and among which there was at least one urinary glucose test strip (–) and one (1+) reading. The number of patients who met our criteria were 67 (46 men, 21 women; age range 31–84 years), and the mean ± standard deviation number of urine samples collected per patient was 11.0 ± 2.4, of which the total number of urinary glucose test-strip readings of (–) or (1+) was 7.0 ± 2.8 [4.9 ± 2.8 for (–) and 2.1 ± 1.3 for (1+)].

Table 1 | Estimation of the renal threshold for glucose

Urinary glucose test strip	(–)	(–)	(–)	(1+)	(1+)	(1+)
Blood glucose (mg/dL): Case 1	158	170	181	185†	203	223
Blood glucose (mg/dL): Case 2	147	152	181†	168	185	190

The blood glucose concentrations that were associated with urinary glucose test strip readings of (–) or (1+) are shown. †The estimated renal threshold for glucose. Typically, the minimum blood glucose value at which the urinary glucose test strip changes from (–) to (1+) is regarded as the estimated renal threshold for glucose (case 1). However, if the maximum blood glucose concentration measured alongside a urinary glucose test-strip reading of (–) was higher than the lowest blood glucose concentration measured alongside a urinary glucose test-strip reading of (1+), we used the former value as the estimated renal threshold for glucose (case 2). (–), Not detectable; (1+), 100–249 mg/dL.

The protocol for this research project was approved by Jinnouchi Hospital Ethics Committee (Approval No. 2018-12-3), and it conformed to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). Informed consent was obtained from all the participants.

Measurement procedures

Blood samples were collected from an antecubital vein immediately after urine collection. Glycated hemoglobin (HbA1c), estimated glomerular filtration rate (eGFR) and other biochemical data were quantified in the hospital laboratory. HbA1c was measured using high-performance liquid chromatography. Serum creatinine concentration (Cr) was measured and the eGFR was calculated using the equation of the Japanese Society of Nephrology: eGFR $(mL/min/1.73m^2) = 194 \times Cr^{-1.094} \times age^{-0.287}$ ($\times 0.739$ for women)[6]. Urinary C-peptide was quantified by enzymatic immunoassay using 24-h pooled urine samples. Body muscle-to-fat ratio was measured using a direct segmental multifrequency bioelectrical impedance analyzer (InBody770; Biospace, Seoul, Korea) as previously reported[7]. Hypertension was defined by a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg or current therapy for hypertension. Dyslipidemia was defined by a high-density lipoprotein cholesterol <40 mg/dL, a low-density lipoprotein cholesterol ≥140 mg/dL, triglyceride concentration ≥150 mg/dL or current therapy for dyslipidemia. The administration of antihypertensive, antidyslipidemic or oral antidiabetic agents, smoking status and alcohol consumption was determined from medical records. The definition used for a drinker was someone who consumed alcohol more than three times a week.

Hyperinsulinemic-euglycemic glucose clamp

Insulin sensitivity was evaluated by an HEC using an artificial pancreas (Nikkiso STG-22 or STG-55; Nikkiso, Tokyo, Japan) after admission, as reported previously[8]. The stable glucose infusion rate (GIR; mg/kg/min) was calculated and used as an index of insulin sensitivity. We started to measure the plasma insulin concentration during the steady state of the HEC after June 2013. The median and interquartile range of the steady state blood glucose (mg/dL) and plasma insulin (μU/mL) concentrations were 96.5 (95.0–99.3; $n = 67$) and 94.9 (77.0–129.0; $n = 44$), respectively.

Statistical analysis

Data were analyzed using JMP 10.0.2 software (SAS Institute Inc., Cary, NC, USA). Categorical variables are presented with their frequency distribution. Relationships between categorical variables were evaluated using the χ^2-test or Fisher's exact test. To identify normality in continuous variables, the Shapiro–Wilk test was used. Continuous variables are summarized as the mean ± standard deviation. If continuous variables were not normally distributed, they were summarized using the median and interquartile range. If a normal distribution was identified, Student's *t*-test was used to compare the groups, and if not, the Mann–Whitney *U*-test was used. Differences between the two groups at baseline were analyzed using unpaired *t*-tests for continuous variables, and Pearson's χ^2-tests for categorical variables. The relationships between eRTg and other variables were assessed using simple linear regression analyses. Correlations between eRTg and these other variables are shown using the standardized regression coefficient (β), which is equivalent to the correlation coefficient (r) for continuous variables and to the correlation ratio (η) for categorical variables. The relationships between eRTg and multiple variables were assessed using multiple linear regression analyses. For these analyses, sex (female = 0, male = 1), habitual smoking (never- or ex-smoker = 0, current smoker = 1), alcohol consumption (≤twice a week = 0, ≥three times a week = 1), hypertension and dyslipidemia (not meeting the above definition = 0, meeting the above definition = 1), and the use of antidiabetic agents (not used = 0, used = 1) were numerically coded. Receiver operating characteristic curve analyses were carried out to calculate the cut-off values of eRTg for individuals with normal insulin sensitivity and for those who were insulin resistant, and cut-off values of HbA1c for eRTg values that were found in individuals with normal insulin sensitivity or insulin resistance. The results were considered statistically significant when $P < 0.05$.

RESULTS

eRTg was determined using the urinalysis data from 67 participants. Their baseline characteristics are presented in Table 2. The mean age of the participants when sampled was 65.5 years, 68.7% were men, their mean body mass index (BMI) was 26.4 kg/m^2, the mean duration of diabetes was 19.1 years, the mean HbA1c value was 8.5%, the mean eGFR was 60.3 mL/min/1.73 m^2 and 52.2% were administering insulin. When the participants were allocated to two groups, according to whether their eRTg values were ≥180 mg/dL or <180 mg/dL (hereafter referred to as high and low eRTg groups, respectively), then the GIR, HbA1c, insulin use and dyslipidemia significantly differed between the groups. However, there were no differences in body weight, BMI, body muscle-to-fat ratio, diabetes duration,

Table 2 | Baseline characteristics of the study participants

Variable	Overall samples (n = 67)	eRTg ≥180 mg/dL (n = 35)	eRTg <180 mg/dL (n = 32)	*P*-value
Age, years (mean ± SD)	65.5 ± 11.7	67.7 ± 9.7	63.2 ± 13.4	0.1179
Sex, male (%)	68.7	65.7	71.9	0.5871
Body weight, kg (mean ± SD)	70.3 ± 13.5	69.7 ± 12.2	70.9 ± 15.0	0.7332
Median body mass index, kg/m^2 (IQR)	26.4 (24.3–29.1)	26.9 (24.7–29.4)	25.9 (23.0–27.7)	0.6774
Median body muscle-to-fat ratio (IQR)	2.38 (1.87–3.03)	2.38 (1.85–2.87)	2.32 (1.94–3.36)	0.3281
Diabetes duration, years (mean ± SD)	19.1 ± 9.2	20.5 ± 7.9	17.5 ± 10.4	0.1878
HbA1c, % (mean ± SD)	8.5 ± 1.5	9.1 ± 1.4	7.9 ± 1.3	0.0007**
eGFR, mL/min/1.73 m^2 (mean ± SD)	60.3 ± 22.9	55.7 ± 19.8	65.3 ± 25.2	0.0844
Hypertension (%)	67.2	77.1	56.3	0.0689
Dyslipidemia (%)	79.1	88.6	68.8	0.0462*
Smoking (%)	16.4	17.1	15.6	0.8670
Alcohol (%)	28.4	28.6	28.1	0.9677
Urine C-peptide (μg/day) (median [IQR])	74.3 (33.5–103.1)	52.4 (31.1–82.2)	72.0 (34.7–110.6)	0.4632
GIR (mg/kg/min)	6.7 ± 2.7	5.6 ± 2.3	7.9 ± 2.6	0.0002**
eRTg (mg/dL)	182.5 ± 29.7	206.8 ± 16.5	158.4 ± 17.0	<0.0001**
Insulin (%)	52.2	71.4	34.4	0.0024**
Metformin (%)	40.3	40.0	40.6	0.9585
Sulfonylurea (%)	31.3	25.7	37.5	0.2989
DPP4i (%)	19.4	20.0	18.8	0.8972
α-Glucosidase inhibitor (%)	20.9	22.9	18.8	0.6796
Thiazolidinedione (%)	9.0	2.9	15.6	0.0675
Glinide (%)	6.0	2.9	9.4	0.2607

Data are presented as mean ± standard deviation, median (interquartile range [IQR]) or %. *P < 0.05, **P < 0.01. DPP4i, dipeptidyl peptidase-4 inhibitor; eGFR, estimated glomerular filtration rate; eRTg, estimated renal threshold for glucose; GIR, glucose infusion rate; HbA1c, glycated hemoglobin.

current smoking status, alcohol consumption, urine C-peptide, metformin use, sulfonylurea use, dipeptidyl peptidase-4 inhibitor use, α-glucosidase inhibitor use or glinide use between the groups.

GIR is a measure of insulin resistance, and is also referred to as the M value. Healthy individuals have been reported to have a GIR >7.0–8.0 mg/kg/min[9,10], and patients with clear insulin resistance have been reported to have a GIR <5.7 mg/kg/min[10]. The high eRTg group had a GIR of 5.6 ± 2.3 mg/kg/min, implying that they were insulin resistant, whereas the low eRTg group had a GIR of 7.9 ± 2.6 mg/kg/min, implying that they were insulin sensitive. These values were significantly different ($P = 0.0002$). HbA1c was higher in the high eRTg group than in the low eRTg group (9.1 ± 1.4% vs 7.9 ± 1.3%, respectively, $P = 0.0007$). This was also reflected in the proportion of participants using insulin therapy, because individuals in the high eRTg group were twice as likely to use insulin as those in the low eRTg group ($P = 0.0024$). Similarly, the prevalence of dyslipidemia was higher in the high eRTg group ($P = 0.0462$).

It has been reported that older adults are more likely to have a high RTg[11]. This is generally considered to be because aging is associated with a reduction in glomerular filtration rate, such that their glucose excretion threshold increases. In the present study, eGFR tended to be lower in the group with high eRTg, but this difference did not reach significance ($P = 0.0844$), and eRTg tended to be higher in older participants, but again this trend was not significant ($P = 0.1179$). It has also been reported that women tend to have a higher RTg[11], but our data were not consistent with this ($P = 0.5871$). In addition, when thiazolidinedione use was low, eRTg tended to be high, but this was also not significant ($P = 0.0675$).

Next, we carried out simple linear regression analysis of the relationships between eRTg and other parameters, including GIR. As shown in Table 3, eRTg significantly negatively correlated with GIR and body muscle-to-fat ratio ($r = –0.5281$, $P < 0.0001$ and $r = –0.3039$, $P = 0.0146$, respectively; Figure 1a,c; Table 3), and positively correlated with HbA1c and the prevalence of insulin use ($r = 0.4395$, $P = 0.0002$ and $\eta = 0.2924$, $P = 0.0163$, respectively; Figure 1b,d; Table 3). There were weak and non-significant relationships of eRTg with dyslipidemia and hypertension.

To identify variables that are independently associated with eRTg, we carried out multiple linear regression analysis (Table 4). Multivariate analysis, adjusted for body muscle-to-fat ratio and insulin use, showed GIR to be negatively, and HbA1c to be positively, associated with eRTg (model 1). Only GIR and HbA1c were independent contributors in any multiple linear regression analysis when the data were adjusted for BMI, hypertension, dyslipidemia and thiazolidinedione use (model 2), or additionally for age, sex, current smoking status and alcohol consumption (model 3). The standardized coefficient (β) was –0.437 for GIR and 0.311 for HbA1c, and the contribution to eRTg elevation was therefore considered to be higher for GIR than HbA1c (Table 5). In multiple regression models including GIR and HbA1c as independent variables, the formula for eRTg was calculated to be $–4.75 \times \text{GIR} + 6.11 \times \text{HbA1c} + 162$, and this accounted for 37% of the variability of eRTg in type 2 diabetes mellitus patients ($R^2 = 0.3675$).

Table 3 | Relationships of estimated renal threshold for glucose with other variables in patients with type 2 diabetes mellitus

Variable	Standardized regression coefficient (β)		*P*-value
	r	η	
Age (years)	0.1612	–	0.1926
Sex (male)	–	–0.1388	0.2625
Body weight (kg)	0.0595	–	0.6327
Body mass index (kg/m^2)	0.1575	–	0.2032
Body muscle-to-fat ratio	–0.3039	–	0.0146*
Diabetes duration (years)	0.1291	–	0.2979
HbA1c (%)	0.4395	–	0.0002**
eGFR (mL/min/1.73 m^2)	–0.1399	–	0.2590
Hypertension	–	0.2388	0.0516
Dyslipidemia	–	0.2284	0.0630
Smoking	–	–0.0034	0.9783
Alcohol	–	–0.0388	0.7555
Urine C-peptide (μg/day)	–0.1370	–	0.2805
GIR (mg/kg/min)	–0.5281	–	<0.0001**
Insulin	–	0.2924	0.0163*
Metformin	–	0.0935	0.4516
Sulfonylurea	–	–0.1580	0.2017
DPP4i	–	0.0646	0.6036
α-Glucosidase inhibitor	–	0.1591	0.1984
Thiazolidinedione	–	–0.1542	0.2128
Glinide	–	–0.0407	0.7439

The correlation coefficients and ratios are shown as *r* and η, respectively. *$P < 0.05$, **$P < 0.01$. DPP4i, dipeptidyl peptidase-4 inhibitor; eGFR, estimated glomerular filtration rate; GIR, glucose infusion rate; HbA1c, glycated hemoglobin.

Furthermore, in receiver operating characteristic analysis, when the GIR was >7.0, the cut-off value for eRTg was 180 mg/dL (area under the curve [AUC] 0.752, sensitivity 0.74, specificity 0.72, $P = 0.0009$; Figure 2a). This is consistent with the commonly stated upper limit of the RTg being 180 mg/dL. Also, when a GIR <5.7 was defined as representing clear insulin resistance, the cut-off value for eRTg was 189 mg/dL (AUC 0.815, sensitivity 0.74, specificity 0.78, $P = 0.0001$; Figure 2b). Conversely, receiver operating characteristic analysis for HbA1c, the other independent factor found to influence eRTg, showed that when the eRTg values were ≤180 or ≥189 mg/dL, the cut-off values for HbA1c were 7.7% (AUC 0.740, sensitivity 0.485, specificity 0.882, $P = 0.0015$; Figure 2c) or 8.0% (AUC 0.769, sensitivity 0.862, specificity 0.554, $P = 0.0006$; Figure 2d), respectively. These data imply that the hyperglycemic patients who are urine glucose test strip-negative are either insulin resistant and/or have HbA1c values ≥8.0%.

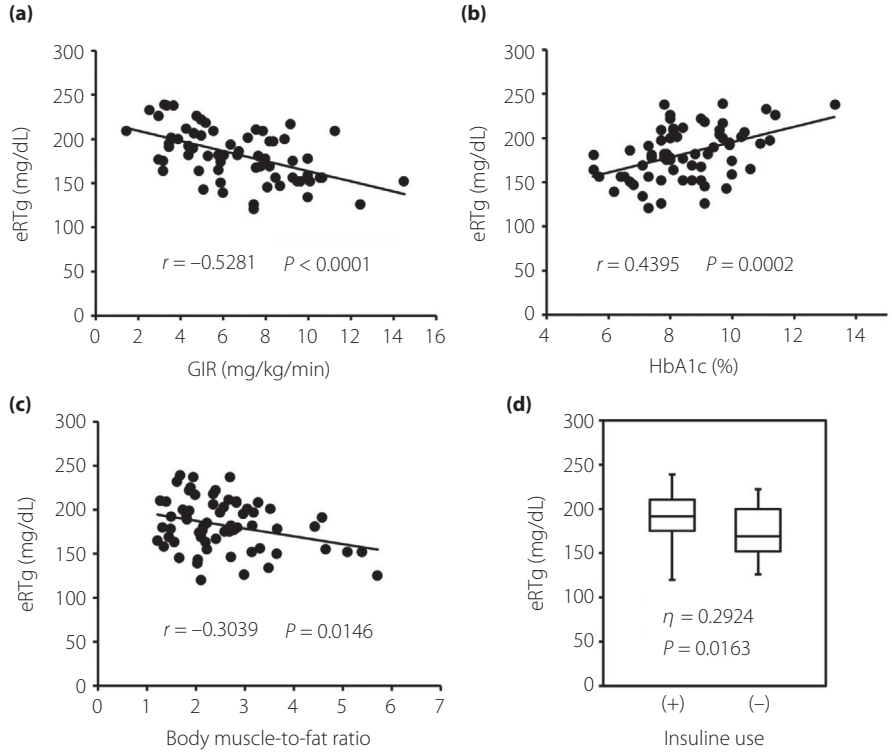

Figure 1 | Regression analyses of the relationships between (a) the estimated renal threshold for glucose (eRTg) and the glucose infusion rate (GIR), (b) glycated hemoglobin (HbA1c), (c) body muscle-to-fat ratio and (d) insulin use. (d) In the box-and-whisker plots, the lines within the boxes represent median values, and the top and bottom lines of the boxes represent the 75th and 25th percentiles, respectively. The top and bottom bars outside the boxes represent the maximum and minimum values, respectively. *r*, correlation coefficient; η, correlation ratio.

Table 4 | Multiple linear regression analysis showing the relationship between the estimated renal threshold for glucose and other variables in patients with type 2 diabetes mellitus

Variable	Model 1 (R^2: 0.3675)		Model 2 (R^2: 0.4051)		Model 3 (R^2: 0.4199)	
	β	*P*-value	β	*P*-value	β	*P*-value
GIR (mg/kg/min)	–0.3979	0.0028**	–0.3843	0.0018**	–0.4761	<0.0001**
HbA1c (%)	0.3000	0.0129*	0.3446	0.0022**	0.2962	0.0063**
Body muscle-to-fat ratio	–0.0427	0.7260	–	–	–	–
Insulin	0.0126	0.9174	–	–	–	–
Body mass index (kg/m^2)	–	–	–0.0363	0.7361	–	–
Hypertension	–	–	0.0647	0.5680	–	–
Dyslipidemia	–	–	0.0681	0.5241	–	–
Thiazolidinedione	–	–	–0.1648	0.1150	–	–
Age (years)	–	–	–	–	0.1520	0.1808
Sex (male)	–	–	–	–	–0.1440	0.2086
Smoking	–	–	–	–	0.2122	0.0708
Alcohol	–	–	–	–	0.1426	0.2031

GIR, glucose infusion rate; HbA1c, glycated hemoglobin. *$P < 0.05$, **$P < 0.01$

DISCUSSION

In the present study, we have characterized patients who are hyperglycemic, but negative for glucose on a urine test strip, who are frequently encountered in diabetes outpatient clinics, with respect to their level of insulin resistance, assessed using a HEC. Our data show that such patients have a higher eRTg,

Table 5 | Multiple linear regression analysis showing the relationships between the estimated renal threshold for glucose, glucose infusion rate and glycated hemoglobin in patients with type 2 diabetes mellitus

	Unstandardized coefficient		Standardized coefficient	95% Confidence interval for *B*		*P*-value	VIF
	B	SEM	β	Lower bound	Upper bound		
Constant	162	21.1	0	120	204	<0.0001	–
GIR (mg/kg/min)	−4.75	1.13	−0.437	−7.01	−2.49	<0.0001	1.094
HbA1c (%)	6.11	2.04	0.311	2.03	10.2	0.0039	1.094

R^2 = 0.3675. The regression equation constructed was estimated renal threshold for glucose = −4.75 × GIR + 6.11 × HbA1c + 162. GIR, glucose infusion rate; HbA1c, glycated hemoglobin; SEM, standard error of the mean; VIF, variance inflation factor.

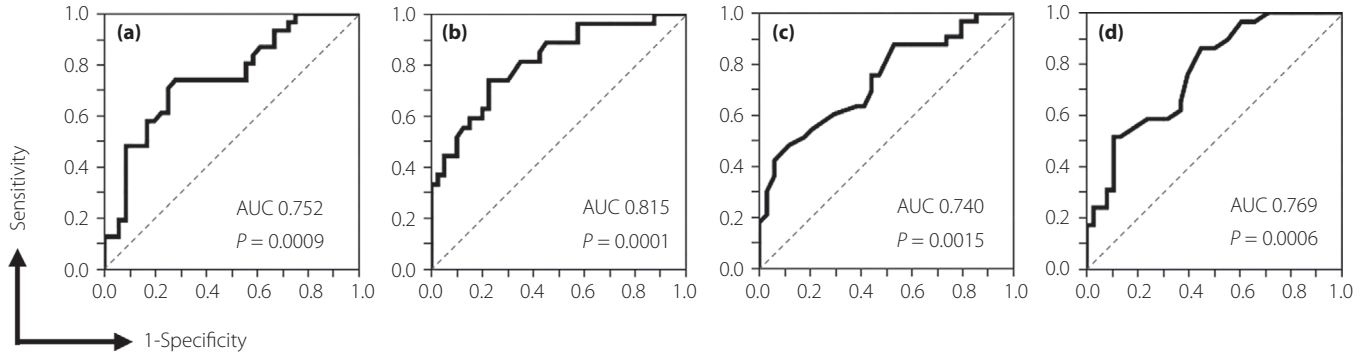

Figure 2 | Receiver-operating characteristic curve analyses were carried out to determine the cut-off values of estimated renal threshold for glucose for individuals with (a) normal insulin sensitivity and (b) insulin resistance, and the cut-off values of glycated hemoglobin for estimated renal threshold for glucose values considered to correspond to (c) normal insulin sensitivity or (d) insulin resistance. The results were considered to be statistically significant when $P < 0.05$. AUC, area under the curve.

and that GIR negatively, and HbA1c positively, contribute to the eRTg value. This implies that a high eRTg is associated with insulin resistance and/or poor glycemic control (HbA1c ≥8.0%). Most of the participants had both insulin resistance and a high HbA1c (for example, an eRTg of 237 mg/dL was associated with a GIR of 3.7 and an HbA1c of 13.3%), but there were also a few participants with higher HbA1c values in the absence of insulin resistance (for example, an eRTg value of 197 mg/dL was associated with a GIR of 8.2 and an HbA1c of 11.2%), because GIR and HbA1c were independent contributors to eRTg. When insulin resistance was defined by a GIR <5.7, the cut-off value for eRTg was 189 mg/dL, which clearly exceeds the normal RTg of 180 mg/dL, and is likely to be present in many patients who are hyperglycemic, but urine glucose test strip-negative.

The cut-off value for HbA1c was 8.0% when an eRTg ≥189 mg/dL was defined as showing insulin resistance, although this value might be adjusted in the future, because the sample size was relatively small in the present study, notwithstanding the identification of statistically significant relationships (Figure 2c,d). Clinically, an HbA1c value of 8.0% is a less stringent goal than is desirable, even if a patient's glycemic target has been set at a higher-than-normal level, because of a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications, extensive comorbid conditions, or long-standing diabetes. It is of interest that the level of eRTg that is associated with insulin resistance corresponds to this treatment goal for HbA1c. Previous clinical trials have shown the relationship between HbA1c and diabetic complications. The Diabetes Control and Complications Trial showed a continuous relationship between HbA1c and the appearance of microangiopathy in type 1 diabetes mellitus patients, with no threshold effect, but with an HbA1c of >8.0%, the risk of retinopathy increased faster[12]. The UK Prospective Diabetes Study assessed patients over a period of 10 years, and found that intensive glycemic control (median HbA1c 7%) reduced the risk of microvascular disease by 25% compared with standard treatment (median HbA1c 7.9%) in patients with type 2 diabetes mellitus[13]. Thus, HbA1c 8.0% can be regarded as a meaningful threshold, above which microvascular complications are more likely to develop or progress. However, at present it is unclear whether a high RTg and diabetic nephropathy

are mechanistically related. Further studies are required to determine whether the pathogenesis of diabetic nephropathy involves alterations to nephron glucose handling.

When participants were allocated to two groups according to their eRTg, then those with dyslipidemia were significantly more likely to be in the high than in the low eRTg group (Table 2). Lipid abnormalities in type 2 diabetes mellitus patients can include hypertriglyceridemia, and low high-density lipoprotein and high small, dense low-density lipoprotein cholesterol concentrations, and are thought to occur secondary to insulin resistance[14]. In addition, simple regression analysis of the relationships between eRTg and anthropometric parameters showed a significant negative correlation between body muscle-to-fat ratio and eRTg (Table 3). Our recent study showed that body muscle-to-fat ratio positively correlates with insulin sensitivity in untreated type 2 diabetes mellitus patients[15]. Although neither dyslipidemia nor body muscle-to-fat ratio significantly correlated with eRTg in the present multiple linear regression analysis, both are associated with insulin resistance. The trends observed are consistent with the notion that eRTg is associated with insulin resistance.

A number of methods have been used for the assessment of insulin resistance, but it is difficult to make an accurate assessment. Of these, HEC is the gold standard method for the assessment of insulin sensitivity. However, it is a costly, time-consuming and invasive method; therefore, simpler approaches are typically used for the estimation of insulin sensitivity. In the clinic, HOMA-IR has been extensively used as a reliable surrogate measure of insulin resistance in patients with type 2 diabetes mellitus[16], because its calculation requires only fasting plasma glucose and insulin values. However, its validity is fundamentally limited when it is applied to individuals with high fasting plasma glucose concentrations or poor insulin secretion. When this measure was originally published, the correlation coefficient between HOMA-IR and HEC was $R_s = 0.88$ in type 2 diabetes mellitus patients treated using diet alone and with no glycosuria at the time[16]. However, the correlation coefficients between log HOMA-IR and HEC were $r = -0.44$ to -0.53 in type 2 diabetes mellitus patients with poorer insulin secretion, high fasting plasma glucose or lower BMI[17], and was $r = -0.60$ in a recent meta-analysis[18]. In the present study, we found the correlation coefficient for eRTg and HEC to be $r = -0.53$, which is comparable to that previously reported for log HOMA-IR. Therefore, it is reasonable to assume that type 2 diabetes mellitus patients with an eRTg >189mg/dL are likely to be insulin resistant. Importantly, the estimation of RTg does not require fasting plasma glucose or insulin concentrations, indicating that the insulin resistance defined by high eRTg could reflect the individual's net insulin resistance, irrespective of plasma insulin concentration, in such patients with adequate insulin sensitivity, but low apparent GIR due to rapid clearance of insulin.

Previous studies have shown that the higher the RTg values diabetes patients have, the more effective the reduction in HbA1c achieved using SGLT2i can be[5]. A meta-analysis of randomized clinical trials of at least 12 weeks' duration that compared the efficacy of an SGLT2i with that of other treatments for type 2 diabetes mellitus showed that the 24-week reduction in HbA1c achieved using SGLT2i was greater in trials enrolling patients with a higher baseline HbA1c[19]. Our group has also reported that patients with an HbA1c of ≥7.7% achieve a significant reduction in body fat percentage when they add an SGLT2i to their treatment regimen[20]. These patients might have high RTg values, and patients with a high eRTg are considered to be most appropriate for SGLT2 inhibition, because larger improvements in blood glucose and/or body fat are achieved.

In general, there is a reduction in glucose utilization in skeletal muscle, liver and adipocytes because of insulin resistance in type 2 diabetes mellitus, and as a result, hyperglycemia persists[21]. Lower glucose availability leads to a compensatory increase in the expression of SGLT2 and glucose transporter 2 in renal tubules[22], creating a vicious cycle in which greater urinary glucose reabsorption exacerbates hyperglycemia[23]. Recent studies have shown that SGLT2 expression is stimulated through tubular insulin receptor signaling in renal tubule-specific insulin receptor-deficient mice[24], and is higher in hyperinsulinemic *db/db* mice[25]. Therefore, in animal models, hyperglycemia and consequent hyperinsulinemia, are drivers of SGLT2 expression. Although this regulation is yet to be shown directly in humans, the upregulation of SGLT2 might be proportional to the severity of insulin resistance, and the increase in RTg secondary to the upregulation of SGLT2 might represent a useful index for the evaluation of insulin resistance in type 2 diabetes mellitus. If a patient is not treated with an SGLT2i, which lowers the RTg[2,26], RTg might reflect the whole-body insulin resistance at the time, and thus be a new marker of insulin resistance in type 2 diabetes mellitus patients, whether they are on dietary therapy alone or taking other medication, including insulin.

There were several limitations to the present study. First, it was carried out retrospectively. Second, few patients had undergone HEC, thus the sample size was small. Third, because the estimation of eRTg relied on the use of test strips, its accuracy might be limited. To determine RTg more accurately, it should be measured using the hyperglycemic clamp method[27] or simultaneous 24-h blood glucose measurement and 24-h urine collection[28].

In conclusion, the present study is the first to show that high eRTg is associated with both low GIR and high HbA1c, with GIR making a substantial contribution. RTg can be estimated by repeated urine glucose assessment using the simple and inexpensive urine test-strip method, and just one urine sample from a hyperglycemic patient that is negative for urine glucose on a test strip might be sufficient to identify insulin resistance. For the treatment of such patients, SGLT2i are most appropriate if a sufficient level of insulin secretion remains. Thus, the test-strip method could represent a valuable tool for the

identification of insulin resistance, and should continue to be used routinely in clinical practice.

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DISCLOSURE

Kunio Hieshima received lecture honoraria from Taisho Pharmaceutical, Co., Ltd. Seigo Sugiyama is on the Speaker's Bureaus of MSD, Inc., AstraZeneca Pharmaceuticals LP, Ono Pharmaceutical Co., Ltd. and Bayer Yakuhin Ltd. Hideaki Jinnouchi has received consultant fees from Sanofi U.S., Novo Nordisk, Inc. and Eli Lilly Japan K.K., and is also on the Speaker's Bureaus of MSD, Inc., Astellas Pharma US, Inc., Sanofi U.S., Novo Nordisk Pharma, Ltd., Taisho Pharmaceutical, Co., Ltd., Daiichi-Sankyo Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Eli Lilly Japan K.K., Boehringer Ingelheim Pharmaceuticals, Inc., Takeda Pharmaceutical Company Ltd. and AstraZeneca Pharmaceuticals LP. All other authors declare no conflict of interest.

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