Renal glucose excretion as a function of blood glucose concentration in subjects with type 2 diabetes—results of a hyperglycaemic glucose clamp study

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Abstract

Background. The purpose of this study was to investigate renal glucose excretion as a function of blood glucose concentration and to evaluate the within-subject variability and between-subject variability in subjects with type 2 diabetes.

Methods. Twenty-two subjects with type 2 diabetes [age 58 (12) years, diabetes duration 7 (6) years, endogenous creatinine clearance 117 (38) ml min⁻¹ 1.73 m⁻²; median (inter-quartile range, IQR)] underwent two five-period hyperglycaemic glucose clamp experiments at intervals of 7–21 days. Starting from an initial blood glucose level of 12.2 mmol l⁻¹, subsequent glucose clamp levels were chosen using an algorithm based on urinary glucose concentrations measured at the end of the preceding glucose clamp period. That is, blood glucose was either stepwise decreased or increased depending on whether urinary glucose concentration was above or below 11.1 mmol l⁻¹, respectively.

Results. As expected, increasing the blood glucose from 7.8 to 13.3 mmol l⁻¹ during the glucose clamps resulted in a steep increase of urinary glucose excretion from 0.06 to 0.77 mmol min⁻¹. With decreasing blood glucose, a measurable glucosuria persisted up to a blood glucose level of 7.8 mmol l⁻¹. When defining the (pseudo)threshold for renal glucose excretion (PRTG) as the highest blood glucose level during glucose clamps associated with a concomitant glucose concentration in urine of <2.8 mmol l⁻¹, median (IQR) PRTG was 11.0 (1.1) mmol l⁻¹. The within-subject variability of PRTG, i.e. the difference between two assessments, was low, 0.1 (0.0) mmol l⁻¹ while the between-subject variability of PRTG was high, ranging from 7.7 to 12.2 mmol l⁻¹.

Conclusion. Renal glucose excretion increases in a proportional manner with increasing blood glucose. When decreasing blood glucose to euglycaemic blood glucose levels, glucosuria persists so that the classical concept of a renal threshold for glucose excretion cannot be upheld in subjects with type 2 diabetes.

Keywords: hyperglycaemic glucose clamp; physiology; renal glucose excretion; type 2 diabetes mellitus; urinary glucose

Introduction

At euglycaemic blood glucose concentrations, glucose is freely filtered at the glomerulus and completely reabsorbed at the level of the proximal convoluted tubule. With rising blood glucose the reabsorption of filtered glucose in the proximal convoluted tubule increases until a maximum value is reached. Any further increase in blood glucose (and in the resultant glucose load presented to the proximal tubule) results in the excretion of glucose in urine.

The appearance of glucose in urine is reflected in the concept of a renal threshold for glucose excretion. In some, but not all, textbooks the concept of a renal threshold for glucose excretion is propagated with the threshold specified at ~10 mmol l⁻¹ [1–6]. According to this concept, no glucose should be detectable in urine at subthreshold blood glucose levels. However, researchers have described a basal glucosuria at euglycaemic, i.e. subthreshold blood glucose levels. According to this concept, no glucose should be detectable in urine at subthreshold blood glucose levels. However, researchers have described a basal glucosuria at euglycaemic, i.e. subthreshold blood glucose levels, as early as in 1904—a finding unequivocally in contradiction to the concept of a renal threshold [7–10]. Obviously, the demonstration of a renal threshold depends on the sensitivity of the bio-analytical method employed for the measurement of urinary glucose—so that the question whether or not glucosuria is present at euglycaemic blood glucose levels ought to be reduced to the issue of the lower limit of quantification (LLOQ) of the method used for measuring urinary glucose.
So far, only a few studies have characterized renal glucose excretion as a function of blood glucose in humans [3,6,11,12]. Moreover, apart from one exception [11], studies assessing the relationship between blood glucose concentration and concomitant glucose excretion in humans were not performed under steady-state conditions [3,6,12]—which, from a methodological viewpoint, is a must.

Therefore, we investigated renal glucose excretion in subjects with type 2 diabetes under steady-state conditions employing five-period hyperglycaemic glucose clamps with blood glucose levels ranging from 7.8 to 13.3 mmol l\(^{-1}\). Moreover, we determined the within-subject variability and the between-subject variability of the relationship between blood glucose and renal glucose excretion.

**Subjects and methods**

**Subjects**

Subjects were selected from a pool of subjects with type 2 diabetes, who consented to participate in clinical studies conducted at a contract research organization in Neuss, Germany. Subjects enrolled met the following inclusion criteria: type 2 diabetes according to ADA criteria for at least 6 months [13], age 40–65 years, body mass index (BMI) between 22 and 33 kg m\(^{-2}\) and fasting blood glucose between 7.8 and 16.7 mmol l\(^{-1}\). Subjects were treated with diet alone or by stable regimes of metformin, thiazolidinedione or insulin. Twenty-two men and women with type 2 diabetes of Caucasian race were included in the study. One subject enrolled presented with proteinuria (\(>500 \text{mg dl}^{-1}\) of urinary protein) whereas the remaining 21 subjects showed no signs of renal disease. Clinical, metabolic and renal characteristics of subjects investigated are summarized in Table 1.

The study protocol was approved by the local ethics committee and the study was carried out according to the guidelines of Good Clinical Practice and the Declaration of Helsinki. Subjects gave the consent to participate in the study in writing after a detailed oral and written explanation of the study objectives. The study was conducted in an open-label and prospective manner. Following a screening visit, subjects underwent two hyperglycaemic glucose clamp experiments within an interval of 7–14 days. The experimental procedure on both study days was identical.

**Hyperglycaemic glucose clamps**

Subjects were connected to a Biostator (glucose-controlled insulin infusion system—GCIIS, Life Science Instruments, Elkhardt, IN, USA) allowing for continuous measurement of arterialized venous whole blood glucose concentration. Arterialization of venous blood was achieved by using the heated hand technique with the hand placed in a box in which the air was warmed to \(\sim 55\)°C. The glucose clamp experiments were started by a run-in period during which the subject’s blood glucose was adjusted to an initial hyperglycaemic target level of 12.2 mmol l\(^{-1}\). This was accomplished either by a Biostator controlled i.v. infusion of 20% glucose solution or an individual i.v. infusion of regular insulin—depending on the subject’s initial blood glucose concentration. Once the target blood glucose concentration was reached, a five-period hyperglycaemic glucose clamp was commenced during which an i.v. insulin infusion (Actrapid, NovoNordisk, Mainz, Germany) was given throughout the glucose clamp at a constant rate of 0.4 mU kg\(^{-1}\) min\(^{-1}\). Each of the five consecutive glucose clamp periods lasted for 2.5 h. The subjects were asked to void urine twice during each glucose clamp period; for the first time after 1 h and for the second time towards the end of the glucose clamp period, i.e. after 2.5 h. Invariably, the first blood glucose target level during the experiments was 12.2 mmol l\(^{-1}\). Target levels for the second and all subsequent glucose clamp periods were varied using a fixed algorithm (Figure 1) based on the urinary glucose concentration of the second urine sample collected: if urinary glucose concentration was \(<11.1\) mmol l\(^{-1}\), the target level was raised by 1.1 mmol l\(^{-1}\) (highest permitted glucose level 14.4 mmol l\(^{-1}\)), if it was \(\geq 11.1\) mmol l\(^{-1}\), the target level was reduced by 1.1 mmol l\(^{-1}\) (lowest permitted glucose level 7.8 mmol l\(^{-1}\)). The rationale for choosing a cutoff of 11.1 mmol l\(^{-1}\) was as follows: glucose concentration in urine reflects not only concomitant blood glucose concentration, but varies also with urine flow rate, i.e. the urine volume excreted per unit time. During previous hyperglycaemic glucose clamp experiments (data not shown) the use of a cutoff level of 11.1 mmol l\(^{-1}\) resulted in a constant relationship between blood glucose concentration and urinary glucose concentration. Figure 2 gives an example of a five-period hyperglycaemic glucose clamp.

High urinary flow rates were achieved by an i.v. infusion of 0.15 mmol l\(^{-1}\) saline and additional oral intake of mineral water during the experiments. Glucose infusion rates (GIR) and subject’s blood glucose concentrations were electronically recorded on a minute-to-minute basis by the Biostator. Blood glucose and GIR values were transmitted on-line from the Biostator to an external PC and stored on its hard disk for subsequent analysis. Biostator-based blood glucose measurements were cross-checked by a glucose oxidase reference method (Super GL, Hitado, Delecke-Möhnese, Germany) in regular intervals.

**Table 1.** Clinical, metabolic and renal characteristics of 22 subjects with type 2 diabetes investigated, median (inter-quartile range for the 75th and 25th percentile)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (IQR)</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>58 (12)</td>
</tr>
<tr>
<td>Gender (male/female)(^a)</td>
<td>19/3</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>29.5 (3.7)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>140 (20)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 (17)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol l(^{-1}))</td>
<td>8.6 (2.4)</td>
</tr>
<tr>
<td>Serum triglyceride (mmol l(^{-1}))</td>
<td>1.3 (1.3)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol l(^{-1}))</td>
<td>4.9 (1.3)</td>
</tr>
<tr>
<td>Serum creatinine (µmol l(^{-1}))</td>
<td>64 (18)</td>
</tr>
<tr>
<td>Urea N (mmol l(^{-1}))</td>
<td>3.4 (1.8)</td>
</tr>
<tr>
<td>Proteinuria (yes/no)(^a)</td>
<td>1/21</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate.

\(^a\)Ratio.
Analytical procedures

At the screening visits, venous blood samples were drawn for the determination of serum triglycerides, cholesterol, serum creatinine, urea N and additional safety laboratory parameters, which were measured by standard methods. Urine analysis was performed using a dipstick method (Combur 9, Roche Diagnostics, Mannheim, Germany). Glomerular filtration rate was assessed by endogenous creatinine clearance calculated from serum creatinine concentration and urinary creatinine excretion in 24 h urine samples.

Blood glucose was determined in whole blood during the glucose clamp experiments, both when measured by the Biostator and by the glucose oxidase-based reference method (Super GL, Hitado, Delecke-Möhnesee, Germany) with a LLOQ of 0.6 mmol l\(^{-1}\). Glucose concentration in urine samples was measured using the same device with glucose concentrations above 25 mmol l\(^{-1}\) diluted prior to measurements.

Data analysis

For each subject, the highest mean blood glucose concentration during the glucose clamp experiments which was associated with a concomitant urine glucose concentration \(<2.8\) mmol l\(^{-1}\) was determined. This (pseudo)threshold for renal glucose excretion \((PRT\_G)\) was determined to allow a comparison of our findings to the results of previous studies employing methods for the measurement of urinary glucose with a LLOQ of \(\sim2.8\) mmol l\(^{-1}\) [6,11]. Glucosuria was calculated by multiplying urine glucose concentration by urine flow rate. Data is given as median [inter-quartile range (IQR) for the 75th and 25th percentile] throughout the text.

Results

Blood glucose target levels during the glucose clamp experiments \((7.8, 8.9, 10.0, 11.1, 12.2\) and \(13.3\) mmol l\(^{-1}\)) were precisely met as median (IQR) blood glucose concentrations during corresponding glucose clamp periods were \(7.8 (0.1), 8.9 (0.2), 10.0 (0.2), 11.1 (0.2), 12.2 (0.1)\) and \(13.3 (0.0)\) mmol l\(^{-1}\), respectively. No glucose clamp with a blood glucose target level of \(14.4\) mmol l\(^{-1}\) was performed. Mean urinary flow rate during the glucose clamp periods was \(6.0 (3.8)\) ml min\(^{-1}\).

An increase of blood glucose during the glucose clamps from \(7.8\) to \(13.3\) mmol l\(^{-1}\) resulted in a concomitant increase of glucosuria from \(0.06\) to \(0.77\) mmol min\(^{-1}\) (Figure 3). When lowering blood glucose from \(13.3\) to \(7.8\) mmol l\(^{-1}\) glucosuria persisted; i.e. even at the lowest blood glucose level of \(7.8\) mmol l\(^{-1}\) at least some glucose in the urine was detectable in every sample.

A linear function was fitted to the experimental data resulting in a high coefficient of determination \((R^2 = 0.89)\) (Figure 3). When extrapolating the linear function beyond the lowest blood glucose level employed during the glucose clamp experiments, a renal threshold for glucose excretion of \(\sim7\) mmol l\(^{-1}\) was predicted.

The \(PRT\_G\) of the 22 subjects investigated was \(11.0 (1.1)\) mmol l\(^{-1}\) and ranged between subjects from \(7.7\) to \(12.2\) mmol l\(^{-1}\) of blood glucose (Figure 4). Within subjects \(PRT\_G\) varied only slightly showing a mean difference between the first and second glucose clamp experiment of \(0.1 (0.0)\) mmol l\(^{-1}\).

Stepwise multiple regression analyses were performed with \(PRT\_G\) as dependent variable and age, sex, body mass index (BMI), diabetes duration, glomerular filtration rate (GFR), fasting blood glucose and serum lipids as possible explanatory variables. The results, however, were inconclusive and therefore not reported.
Discussion

This study, employing a five-period hyperglycaemic glucose clamp to provide steady-state conditions over a broad range of blood glucose levels, characterizes renal glucose excretion as a function of blood glucose concentrations in subjects with type 2 diabetes. Glucose excretion in these subjects increased in a proportional manner with increasing blood glucose. Using a sensitive method for the measurement of urinary glucose, even at euglycaemic blood glucose levels, glucosuria could be demonstrated in each of the subjects investigated. Thus, our study showed that the classical concept of a defined renal threshold for glucose excretion cannot be upheld in subjects with type 2 diabetes. In contrast, our data are in line with the hypothesis that a renal threshold for glucose can only be demonstrated when using insensitive bio-analytical methods for the measurement of urinary glucose.

In order to compare our results to the previously published literature specifying a ‘renal threshold’ for glucose excretion in healthy individuals [1,2] or in subjects with type 2 diabetes [4,11] we defined the highest mean blood glucose concentration associated with a concomitant urine glucose concentration <2.8 mmol l\(^{-1}\) as \(\text{PRT}_G\). The chosen cut-off of 2.8 mmol l\(^{-1}\) corresponds to the LLOQ of methods employed for urinary glucose measurement in most of the studies suggesting a renal threshold for glucose excretion [4,11]. Applying this procedure for \(\text{PRT}_G\) assessment, we determined a median \(\text{PRT}_G\) in our study of 11.0 mmol l\(^{-1}\), which is close to the value of 10 mmol l\(^{-1}\) stated in some [5], but not all [9], textbooks and scientific publications.

The excretion of glucose in urine in small amounts at euglycaemic blood glucose levels represents a phenomenon which in the literature has been described as basal glucosuria [7–10]. Basal or physiological glucosuria is independent of blood glucose concentration, urinary flow rates, renal threshold for glucose and maximal rate of tubular glucose absorption [8,10,14]. These features suggest that physiological glucosuria does not reflect the capacity of the active transport system in the proximal tubules, but may very well result from distal tubular leakage [8,10].

It is worth reminding that glucosuria without hyperglycaemia is a hallmark of proximal tubule disorder. Such a disorder may be subclinical and not easy to detect due to discordant markers. Tubular function was not assessed specifically, as the focus of the study was a more clinical one, namely to

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![Fig. 3. Median glucose excretion at different blood glucose concentrations during the glucose clamps performed in 22 subjects with type 2 diabetes. Bold lines represent fitted linear function. Insertion: fitted linear equation and coefficient of determination.](image)

![Fig. 4. Distribution of the highest individual blood glucose concentrations during two hyperglycaemic glucose clamps associated with a concomitant urinary glucose concentration <2.8 mmol l\(^{-1}\) in 22 subjects with type 2 diabetes. White bars indicate results of 1st glucose clamp; black bars indicate results of 2nd glucose clamp. The within-subject variability of \(\text{PRT}_G\) was 0.1 (0.0) mmol l\(^{-1}\) while the between-subject variability of \(\text{PRT}_G\) ranged from 7.7 to 12.2 mmol l\(^{-1}\).](image)
characterize glucose excretion as a function of blood glucose in a comparatively sizeable cohort of subjects by means of a steady-state method, i.e. a multiple-step hyperglycaemic glucose clamp. We, therefore, cannot completely rule out that some of the subjects investigated may have suffered from subclinical tubular lesions which is not a rare finding in an unselected population of patients with type 2 diabetes [15]. However, to explain the glucose excretion at euglycaemic blood glucose levels demonstrated in our study by proximal tubule disorder, would assume that this entity would have been present in all 22 subjects investigated.

We did not explore glucose excretion at low euglycaemic levels representing the fasting state in healthy volunteers or sometimes even in subjects with type 2 diabetes, as the lowest blood glucose target level during glucose clamps was 7.8 mmol l$^{-1}$. Therefore, we cannot completely exclude the possibility that there is a threshold for glucose excretion just below the lowest blood glucose level employed in our study. However, the results of sporadically measured urinary glucose in subjects with type 2 diabetes, presenting in our institute with fasting blood glucose levels between 5 and 8 mmol l$^{-1}$, do not support this hypothesis as, with no exception at least a small amount of glucose could always be detected in urine (data not shown). Nevertheless, to precisely characterize glucosuria as a function of blood glucose in the low euglycaemic range, an adequately designed glucose clamp study is necessary.

Due to the steady state methodology used for the assessment of the relationship between blood glucose concentration and concomitant glucose excretion, PRT$_G$ is necessarily categorized by the blood glucose levels. It is conceded that this represents a limit of our study, but when striving for steady-state conditions this is inevitable.

Repeating the hyperglycaemic glucose clamps with an identical experimental procedure on a second study day revealed a low within-subject variability of the (pseudo)renal threshold. As the relationship between blood glucose and related glucose excretion was characterized over a broad range of blood glucose levels, we conclude from this finding that the individual characteristics of urinary glucose excretion do not vary from day to day. Although this conclusion seems only natural, we, to our knowledge, are the first to demonstrate this low within-subject variability of the (pseudo)renal threshold for glucose excretion.

In contrast to the low within-subject variability, the between-subject variability of the (pseudo)renal threshold was broad in our study. This finding matches the results of a study by Ruhnau and colleagues [11] conducted in subjects with type 2 diabetes, but is in contrast to the results of Mohnike and colleagues [16] who found only a small range of thresholds in subjects with type 2 diabetes. Most probably, this discrepancy may be explained by huge methodological differences between both studies, i.e. a stepwise hyperglycaemic glucose clamp technique employed by Ruhnau and colleagues [11] in contrast to the measurement of urinary glucose concentration during descending blood glucose in context of an insulin challenge employed by Mohnike and colleagues [16]. In any case, clinicians may conclude from our data that it is advisable to determine the individual (pseudo)threshold for glucose excretion in subjects with type 2 diabetes before predciting their metabolic monitoring on urinary glucose measurements. Certainly, such an assessment would require a procedure considering a clinical framework, i.e. a procedure much simpler than the one employed in our study.

In summary, by means of five-period hyperglycaemic glucose clamps we characterized renal glucose excretion as a function of blood glucose in subjects with type 2 diabetes. With rising blood glucose during the glucose clamps renal glucose excretion increased in a proportional manner. When decreasing blood glucose to euglycaemic levels glucosuria persisted so that the classical concept of a renal threshold for glucose excretion cannot be upheld in subjects with type 2 diabetes. When defining a (pseudo)threshold for renal glucose excretion, this study shows that the within-subject variability of such a threshold was low and the range of this (pseudo)threshold between subjects was broad.

Conflict of interest statement. None declared.

References
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