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A diabetic patient with glucosuria

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Abbreviations
T2D: type 2 diabetes *mellitus*; FRG: Familial Renal Glucosuria; RT_G: renal threshold for glucose excretion; T_{mG}: transport maximum for glucose; GCR: glucose to creatinine ratio (spot urine); UGE: urinary glucose excretion (24h collection); SGLT2: Na/glucose co-transporter 2; *SLC5A2*: SGLT2 coding gene.

CASE PRESENTATION

FMSB, an individual with type 2 diabetes *mellitus* (T2D), was referred to our *Consulta de Doenças Renais Hereditárias* at Hospital de Curry Cabral by his nephrologist, due to a disproportionate amount of glucosuria in light of his metabolic control. Aged 58, T2D was diagnosed when he was 45 and his body mass index 31. Glucosuria had always been detected in spot urine (from 100 to 1000 mg/dl), in spite of fasting plasma glucose concentrations never exceeding 150 mg/dl. Apart from an albuminuria of 125 mg/g of creatinine (in a spot urine) and 145 mg/day (in a 24h urine collection), there was no evidence of end-organ damage. Equally, there was no over-excretion of other solutes reabsorbed in the proximal tubule, therefore excluding the Debré-de Toni-Fanconi syndrome. In short, urinary excretion of uric acid, phosphate and urea were all within normal range, and there was no evidence of renal bicarbonate wasting. In sum, the patient presented to us with glucosuria in the absence of a generalized proximal tubular dysfunction.

The patient was requested to perform an oral glucose tolerance test and we evaluated patient's glucose to creatinine ratio (GCR) at 0' as well as at 120' after patient ingested 75 g of glucose. The results are displayed in Table 1.

Table 1

Laboratory evaluation at baseline (0) and at 2 hours (120´) after ingesting 75 g of glucose

Time (min)	0'	120'
Plasma		
Creatinine (mg/dl)	0.74	–
eGFR (ml/min/1.73m^2)	102	–
Hb A1c (%)	6.2	–
Glucose (mg/dl)	126	265
Spot urine		
Glucose (mg/dl)	1354	5551
Creatinine (mg/dl)	156	87
GCR (mg/g)	8679	63804

eGFR: estimated glomerular filtration rate using CKD-EPI equation; Hb A1c: glycated hemoglobin; GCR: glucose to creatinine ratio.

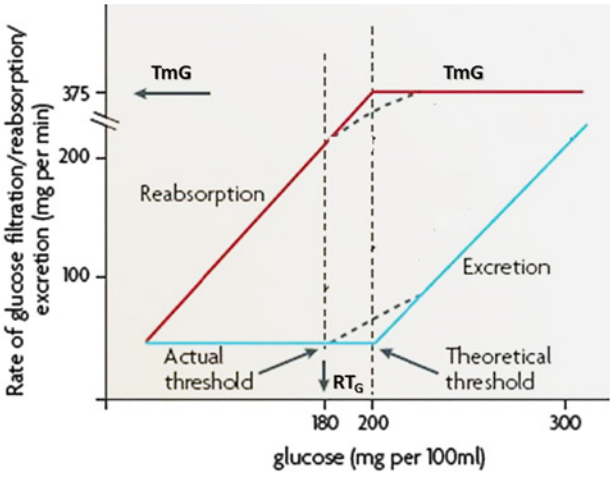
WHY IS THIS DIABETIC PATIENT DISPLAYING GLUCOSURIA?

In the setting of isolated glucosuria found in this diabetic patient, the following general conditions were considered: the patient has (1) hyperglycemic (diabetic) glucosuria, (2) orthoglycemic (normoglycemic) glucosuria or (3) both.

To address the issue, one has to understand the mechanism of renal excretion of solutes (such as glucose) that are freely filtered by the glomerulus and reabsorbed by the tubule. This was initially accomplished in the 1950s by performing renal glucose titration studies (Figure 1, adapted from reference [1]). As glucose is infused and the amount of glucose increases in the proximal lumen, the tubule proportionally increases its reabsorption (red line), therefore preventing the appearance of glucose in the final

Figure 1

Renal Titration studies: Maximal Transport for Glucose (T_{mG}) and Renal Threshold for Glucose excretion (RT_G). Red line represents reabsorption (top line); Blue line represents excretion (bottom). For details see text. (Adapted from reference [1]).



urine. At some point during the raising of plasma glucose concentrations, the maximal tubular glucose transport capacity is exceeded (T_{mG}) and no further glucose can be reabsorbed. From then onwards, all the glucose filtered in excess will appear in the urine (blue line). The plasma glucose value at which, under physiological glomerular filtration rates, glucosuria supervenes, defines the renal threshold for glucose excretion (RT_G). The change from reabsorption to excretion is not abrupt, and even before T_{mG} is attained, some glucose will start to spill in the urine, causing the observed *splay*. The RT_G was recently reassessed in light of the availability of pharmacological inhibition of SGLT2, which is the Na/glucose transporter responsible for the tubular reabsorption of 90% of the filtered glucose[2]. Using stepped hyperglycemic clamp procedures (SHCP), it was found that RT_G in control individuals was 171 mg/dl and 196 mg/dl in individuals with T2D[3]. So, under physiological conditions, glucosuria is only apparent once plasma glucose concentration exceeds 180-200 mg/dl. Due to this up-regulation of SGLT2 in T2D, RT_G in our patient is expected to be ~200 mg/dl. But we have observed that in fasting conditions, the plasma glucose concentration of 126 mg/dl is well below the expected RT_G and, accordingly, cannot justify the GCR of 8679 mg/g. This value increases even more after ingesting 75 g of glucose, when, at 120' and with a plasma glucose of 265 mg/dl, an amazing and disproportionate GCR of 63804 mg/g is reached.

Therefore, an orthoglycemic condition (glucosuria in the absence of hyperglycemia, i.e. renal glucosuria) superimposed on T2D must be considered. Pharmacological SGLT2 inhibition (SGLT2i) is being increasingly used as novel class of oral antidiabetic agents. The abovementioned study using SHCP showed that under SGLT2 pharmacological inhibition, the RT_G was reduced to 21 mg/dl and this was the major pharmacodynamic effect of SGLT2i. But our patient was prescribed vildagliptin and metformin and had never taken SGLT2i. In diabetic patients displaying renal glucosuria, apart from the pharmacological effect of SGLT2i, a particular form of Maturity Onset Diabetes of the Young, MODY 3, one of the autosomal dominantly inherited forms of non-insulin-dependent diabetes caused by mutations in the gene *TCF1* encoding the transcription factor HNF1α[4], should be considered. Defective renal glucose resorption in several families with MODY 3, including in some euglycemic members, has been described, reflecting the fact that HNF1α binding sites are present in the promoter region of the *SLC5A2* gene, the gene coding for SGLT2. HNF1α deficient individuals are expected to have lower SGLT2 expression in the kidney, leading to the observed defective tubular glucose reabsorption[5,6]. But, once again, our patient did not meet the MODY criteria, namely a familial background suggesting a mendelian form of diabetes, and this hypothesis was not pursued.

But, of course, whenever orthoglycemic glucosuria is considered, Familial Renal Glucosuria (FRG) is the most plausible diagnosis, even in a diabetic patient. FRG, a co-dominantly inherited phenotype, is caused by mutations in the SGLT2 coding gene[6-9]. SGLT2, the Na/glucose co-transporter member 2, is predominantly expressed in the early segment of the proximal tubule where it accounts for the reabsorption of most of the glucose[2]. We have shown that non-diabetic heterozygous FRG individuals have a 24h urinary glucose excretion (UGE) < 10 g/1.73m^2, while homozygous and compound heterozygous (i.e., bearing mutations in both copies of the *SLC5A2* gene) display UGE ≥ 10 up-to 180 g/1.73m^2[6]. The UGE reflects not only the disruption of the transport kinetics of SGLT2 but also the plasma glucose excursions throughout the day. The authors have previously documented that mutations in SGLT2 lead to UGE by reducing the RT_G value: homozygous individuals display RT_G values of 17 mg/dl (not really different from what was found for SGLT2i), while in heterozygous, a RT_G of 89 mg/dl was found[10]. In the latter analysis we even included another T2D individual heterozygous for a SGLT2 mutation and she was found to have a slightly higher value for RT_G when compared

to non T2D SGLT2 heterozygous, 128.6 mg/dl vs 83.5 mg/dl, in accordance with the upregulation of renal glucose reabsorption in T2D as described by DeFronzo using the SHCP previously mentioned.

Finally, one must acknowledge that glomerular filtration rate (GFR) is a major determinant in renal glucose excretion. To be precise, the Y axis in Figure 1 should display not plasma glucose concentration but the product of plasma glucose concentration x GFR, because the amount of glucose being offered to the proximal tubule depends also on the amount of plasma being filtered per unit of time. What are the implications of this? Firstly, in the case of hyperfiltration, as frequently seen in nephrotic syndrome patients, the amount of glucose filtered by the glomerulus may exceed the tubular capacity for reabsorption with ensuing glucosuria at physiological plasma glucose levels; Secondly, and as opposed, in cases of moderate GFR impairment it makes no sense in prescribing SGLT2i: if glucose is not being filtered in sufficient amounts there is no point in inhibiting its' later tubular reabsorption aiming at getting significant glucosuria.

FOLLOW-UP, UNANSWERED QUESTIONS AND RECENT DEVELOPMENTS

The *SLC5A2* coding region was Sanger sequenced in the author's lab and a heterozygous sequence variation consisting of Val for Ile substitution at residue 433 (p.Ile433Val; c.1297 G>A) in SGLT2 was found. The allele was validated according to standard guidelines (beyond the scope of this case report) as a *missense* mutation responsible for the phenotype of renal glucosuria.

There remains, however, some areas of uncertainty concerning this case. In this particular setting, where it may be difficult to evaluate how much glucosuria is attributable to hyperglycemia and how much to disruption of the tubular transport of glucose, the determination of the RT_G would be of interest. In our previous work we have used a rather cumbersome procedure that included timely collected blood samples and urine collection for a period of 240' after the patient had ingested a mixed meal for a tolerance test[10]. In the current case report, we have only compared the GCR in a spot urine in fasting conditions and after ingesting 75 g of glucose, and so, we cannot establish definite conclusions regarding the RT_G value. Under orthoglycemic conditions it is assumed that the GCR is roughly equivalent to the amount of UGE in a 24 h period. But in diabetic individuals, plasma glucose excursions may confound this assumption. The patient also has an albuminuria of 125 mg/g of creatinine and is therefore at risk for the development of overt diabetic nephropathy. In light of the recent published work on the slowing of kidney disease progression by SGLT2i, the patient might benefit from such a class of antidiabetics[11]. But the fact that he already bears a mutation in half of the expressed SGLT2 transporters makes the pharmacodynamics of SGLT2i unpredictable.

Within our cohort of FRG individuals, there are a few in whom we were unable to detect mutations in SGLT2. In an international collaborative study, homozygous mutations were identified in MAP17 in one such individual, therefore establishing the basis for genetic heterogeneity in FRG. MAP17 is a protein upregulated in several human carcinomas, but in normal tissues it is only significantly expressed in the human kidney, precisely at the brush-border of proximal tubular cells where it is known to bind the NaPi-IIa cotransporter[12]. In the abovementioned collaborative study, MAP17 was also characterized as an accessory protein for the activity of SGLT2[13]. We pursued that line of work in our lab, and we have just published evidence that MAP17, by co-localizing and co-immunoprecipating *in vitro* with SGLT2 in cellular heterologous expression systems, is also a binding partner for SGLT2[14].

In the case we have just presented, the physiological and molecular pathways discussed as well as the recent developments briefly presented all reflect our long-standing curiosity about the pathogenesis underlying Familial Renal Glucosuria. FRG was the starting point of a journey that began with the clinical evaluation of patients, went through the exploration of geno-phenotype correlations and has now entered the molecular cell biology field, aiming at dissecting the molecular mechanisms behind glucose transport in the proximal tubule. Somewhere along the way, the pharmaceutical industry realized that FRG provided the best possible model in the safety and efficacy of targeting UGE as a *novel* therapeutic target in T2D. For us, going from bedside to bench has been a truly amazing ride.

Disclosure of potential conflicts of interest: none declared

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