Changes in Renal Glucose Transporters in an Animal Model of Metabolic Syndrome

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Key words
- metabolic syndrome
- glucose transporters
- SHR
- monosodium glutamate

Abstract

Considering the similarity between structural, hemodynamic, and functional changes of obesity-related renal disease and diabetic nephropathy, we hypothesized that renal glucose transporter changes occur in obesity as in diabetes. The aim of the work was to evaluate GLUT1 and GLUT2 in kidneys of an animal model of metabolic syndrome. Neonate spontaneously hypertensive rats (SHR), n = 15/group, were treated with monosodium glutamate (5 mg/g) (MetS) for 9 days and compared with saline-treated Wistar-Kyoto (C) and SHR (H) rats. Lee index, systolic arterial pressure (SAP), glycemia, insulin resistance, triglycerides, and HDL cholesterol were evaluated at 3 and 6 months. Medullar GLUT1 and cortical GLUT2 were analyzed by Western blot. MetS vs. C and H rats had the highest Lee index ($p < 0.001$) and insulin resistance (3-months C: 4.3 ± 0.7, H: 3.9 ± 0.9, MetS: 2.7 ± 0.6; 6-months C: 4.2 ± 0.6, H: 3.8 ± 0.5, MetS: 2.4 ± 0.6 %·min^{-1}, $p < 0.001$), similar glycemia, and the lowest HDL-cholesterol at 6-months ($p < 0.001$). In the MetS and H rats, SAP was higher vs. C at 3-months ($p < 0.001$) and 6-months (C: 151 ± 15, H: 190 ± 11, MetS: 185 ± 13 mm Hg, $p < 0.001$) of age. GLUT1 was ~13× lower ($p < 0.001$) at 3-months, reestablishing its content at 6-months in MetS group, while GLUT2 was 2× higher ($p < 0.001$) in this group at 6-months of age. Renal GLUT1 and GLUT2 are modulated in kidney of rats with metabolic syndrome, where obesity, insulin resistance and hypertension coexist, despite normoglycemia. Like in diabetes, cortical GLUT2 overexpression may contribute for the development of kidney disease.

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Introduction

There is increasing support for the idea that obesity per se can initiate and accelerate progression of kidney disease [1]. Overweight and obesity in adolescents were associated with a significantly increased risk for all-cause treated end-stage renal disease [2]. In obese patients, structural (glomerulomegaly, podocyte hypertrophy, increased mesangial matrix, and mesangial cell proliferation) and hemodynamic (high glomerular filtration rate, renal plasma flow, and filtration fraction) changes are frequent, although microalbuminuria is less common [3,4]. Abnormalities in plasma glucose and lipid concentration, caused by obesity, may contribute to glomerular basement thickening, even if the level seen in overt diabetes is not achieved [5]. Moreover, high levels of bioactive substances, such as cytokines may also be involved in the pathogenesis of obesity-related renal disease [6], including the possible involvement of the renal sympathetic nervous system [7].

Increased expressions of renal glucose transporters, such as cortical glucose transporter 1 (GLUT1) of mesangial cells [8,9] and cortical glucose transporter 2 (GLUT2) of S1 tubular cells [9,10], participate in the development and progression of diabetic nephropathy. Diabetes-induced GLUT2 overexpression [11,12] and the further rise in it that can be caused by hypertension [9] may promote, in addition to hyperglycemia, a further elevation in the interstitial renal glucose concentration, and more glucose is taken up by mesangial cells through GLUT1. Changes in GLUT1 and GLUT2 expression result in excessive uptake of glucose by mesangial cells; high intracellular glucose levels are involved in the key pathways that lead to glomerulosclerosis, a concept that is supported by in vitro studies identifying GLUT1 as the predominant glucose transporter in mice [13] and human mesangial cells [14].

Considering the similarity between structural, hemodynamic, and functional changes of obesity-related renal disease and diabetic nephropathy,

it is tempting to speculate that renal glucose transporter changes also occur in obesity, as in diabetes. Thus, the aim of this study was to evaluate the expression of renal glucose transporters in an animal model of metabolic syndrome – spontaneously hypertensive rats (SHR) neonatally treated with monosodium glutamate, which together with hypertension and obesity develop insulin resistance.

Materials and Methods

All animals were bred and kept under standard laboratory animal house conditions at the Animal Production and Research Unit of Fundação Estadual de Produção e Pesquisa em Saúde do RS, Brazil. The study was approved by the Research Ethics Committee of Instituto de Cardiologia do RS, Brazil. Animals received standard rat chow and water ad libitum, and were maintained in controlled 12-h light/dark cycle (6:00 AM/6:00 PM) and 20–25 °C temperature conditions.

Starting at day one of life, neonate male spontaneously hypertensive rats (SHR) received subcutaneous administration of monosodium glutamate (MSG, Sigma®) diluted in saline solution (0.9% NaCl), 5 mg/g/day, for 9 days (MetS, n = 10), only saline solution (H, n = 10) or only saline solution in the Wistar-Kyoto rats (C, n = 10) for the same period. At 21 days of life, the animals were weaned and placed into plastic boxes (4 animals/box). They were weighed, their nasoanal length measured periodically, and Lee index was calculated [15].

At 3 and 6 months of age, the blood pressure of the animals was measured, and the day after they were subjected to an insulin tolerance test, followed by kidney perfusion, with Hanks' buffer, removal, and storage at −70 °C for further analysis of GLUT1 and GLUT2 protein content. Blood was collected for glucose, triglycerides, and high density lipoprotein cholesterol (HDL-C) analysis. The final procedures were performed under anesthesia (ketamine: 100 mg/kg; xylazine: 10 mg/kg).

The insulin tolerance test was performed using human insulin (Humulin, São Paulo, Brazil). After 3-h of food restriction, animals were anesthetized, and 0.75 U/kg body-weight of insulin was injected by vein. Glycemia was measured in blood collected from the tail by Accu-check strips system (Roche, Mannheim, Germany) before insulin injection (zero) and 4, 8, 12, 16, and 20 min after. The glucose decay constant rate (kITT) was calculated as described [16].

For systolic blood pressure, the animals were anesthetized to place a polyethylene catheter (PE-10) inside the femoral artery. The cannula was filled with saline solution and positioned inside the abdominal aorta, through the left femoral artery. The next day the arterial cannula was connected to a pressure transducer, linked by a channel selector to the CODAS analog-digital board in a microcomputer. Systolic blood pressure was evaluated from 20-min basal recordings, in the conscious animal.

Fasting glycemia was measured, from a blood sample collected from the tail, by Accu-check strips (Roche, Mannheim, Germany). Triglycerides and HDL-C concentrations were analyzed using commercial kits (Labtest®, Lagoa Santa, Brazil).

Renal outer cortex and outer medulla were dissected, processed by sonication (Unique, Indaiatuba, SP) in specific buffer, and centrifuged at 3000 *g* for 15 min. The supernatant was centrifuged at 12000 *g* for 20 min , and the pellet was suspended. Western blot was performed as previously described [9]. Briefly, equal amounts of membrane protein (150 µg) were subjected to SDS-PAGE (10%) gel electrophoresis, transferred to nitrocellulose membrane and incubated with the specific antibody (#07-1401 and #07-1402, Millipore, Billerica, USA). The immunoblots were revealed by chemiluminescence using the ECL kit (GE Healthcare, New York, USA). After that, the membranes were reprobed to detect β-actin. Blot intensity was quantified by optic densitometry using Scion Image software and the values of GLUT1 and GLUT1 were corrected by their respective β-actin value, normalized to the control value in each gel.

Results were expressed as arbitrary units by protein loaded (AU/protein loaded) and presented as mean ± standard deviation, compared by 2-way analysis of variance (ANOVA), followed by Bonferroni's post-hoc ($p < 0.05$).

Results

Table 1 shows the general characteristics of the animals. MetS rats had the highest Lee index since 3 months of age, showing their obesity condition. Glycemia was slightly higher in H and MetS rats as compared to C rats, at 3 months of age, as well as at 6 months; these values were, however, far lower than usually observed in diabetic individuals. The kITT was lower in MetS rats, as compared to both C and H, at 3 and 6 months, indicating the presence of insulin resistance. Systolic artery pressure was similarly higher in H and MetS groups as compared to the C group ($p = 0.048$), at 3 months (H vs. C, $p = 0.004$; MetS vs. C, $p < 0.001$) and 6 months of age (H vs. C e MetS vs. C, $p < 0.001$). No differences were observed between H and MetS. The highest levels of triglycerides and lowest levels of HDL-C were observed in MetS rats, as compared with C and H groups at 6 months, but not at 3 months of age.

Table 1 General characteristics of studied animals at 3 and 6 months of age.

	3 Months			6 Months		
	C	H	MetS	C	H	MetS
Lee index	0.78 ± 0.12	0.84 ± 0.14	1.23 ± 0.17*†	1.19 ± 0.11‡	1.29 ± 0.25‡	1.44 ± 0.17*†‡
Glycemia (md/dl)	62.9 ± 2.6	83.1 ± 2.9*	78.8 ± 2.3*	63.0 ± 2.9	78.6 ± 3.1*‡	73.0 ± 2.3*‡
kITT (%·min^{-1})	4.3 ± 0.7	3.9 ± 0.9	2.7 ± 0.6*†	4.2 ± 0.6	3.8 ± 0.5	2.4 ± 0.6*†
SAP (mm Hg)	130 ± 13	177 ± 18*	171 ± 15*	151 ± 15‡	190 ± 11*‡	185 ± 13*
HDL-C (mg/dl)	50 ± 4	53 ± 3	56 ± 2	54 ± 5‡	50 ± 3	33 ± 6*†‡
Triglycerides (mg/dl)	49 ± 9	48 ± 12	50 ± 16	60 ± 7	61 ± 13	123 ± 19*†‡

C: Wistar-Kyoto rats that did not receive any treatment; H: spontaneously hypertensive rats that did not receive any treatment; MetS: spontaneously hypertensive rats that received MSG during the neonatal period (n = 5 in all groups); kITT: Constant rate of decrease of the blood glucose concentration; SAP: Systolic arterial pressure; HDL-C: High density lipoprotein cholesterol

Two-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test: *$p < 0.05$ vs. C; †$p < 0.05$ vs. H, same time; ‡$p < 0.05$ vs. 3 months, same group

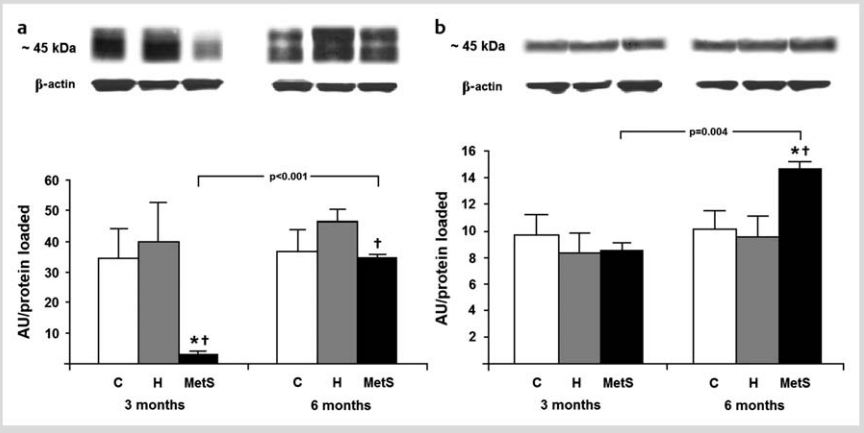

Fig. 1 Renal glucose transporter protein expression. The Western blot bands and quantitative analyses corrected by their respective β-actin value are represented. Panel **a** Medullary GLUT1; Panel **b** Cortical GLUT2. C: Wistar-Kyoto rats, no treatment; H: SHR, no treatment; MetS: SHR that received MSG; n = 5 in all groups. Two-way analysis of variance (ANOVA), Panel **a** ($p < 0.001$) and Panel **b** ($p < 0.001$), followed by Bonferroni's post hoc test: *$p < 0.05$ vs. C; †$p < 0.05$ vs. H, at the same time.

Medullary GLUT1 and cortical renal GLUT2 expression analysis are shown in **Fig. 1**. At 3 months, there was a reduction of ~90% in the medullary GLUT1 expression in kidney from MetS rats as compared to C and H animals ($p < 0.001$ for both comparisons). At 6 months there was a reduction of this glucose transporter in MetS rats as compared to H rats (25% and $p = 0.007$), and no difference was observed when it was compared to C rats. Moreover, medullary GLUT1 expression of the MetS group at 6 months was elevated 13-fold ($p < 0.001$) when compared to the same group at 3 months of age.

Cortical renal GLUT2 expression was similar among groups at 3 months of age, but it was increased 1.5-fold in the MetS group at 6 months as compared to C and H animals ($p < 0.001$ for both comparisons). Moreover, cortical renal GLUT2 expression in MetS rats at 6 months was 76% higher when compared to animals from the same group at 3 months of age ($p = 0.004$).

Discussion and Conclusions

This study demonstrates that renal glucose transporters (GLUT1 and GLUT2) are both modulated in kidneys of rats with metabolic syndrome, where visceral obesity, insulin resistance, and hypertension all coexist, although no hyperglycemia was detected.

Cortical GLUT2 was overexpressed in the kidney of 6-month old MetS rats, as it has been described in the kidney of diabetic [10] and hypertensive rats [12]. *Slc2a2* gene, which encodes the GLUT2 protein, is positively regulated by glucose concentration in liver [17] and kidney [18]. However, glucose homeostasis (glycemia and insulin resistance) of MetS rats was similarly regulated at 3 and 6 months of age, suggesting that GLUT2 overexpression observed only in 6-month-old MetS rats was determined by other concomitant mechanisms. Inflammation could also be related to altered GLUT2 expression, as it is clearly demonstrated for GLUT4 protein in adipocytes [19]; however, the inflammatory condition also seems to be similar in 3- and 6-month-old MetS [20]. Finally, a possible causal mechanism of GLUT2 overexpression could be related to hyperlipidemia, established only in 6-month-old MetS animals [20]. Hyperlipidemia is able to induce oxidative stress [21,22], to which is ascribed the capability of increasing the transcriptional activity of hepatocyte nuclear factor-3 [23]. HNF-3β is an important enhancer of *Slc2a2* gene, and is related to renal GLUT2 overexpression in diabetic rats [18]. Furthermore, the sterol response element-binding protein-1c (SREBP-1c) is another enhancer of the *Slc2a2* gene [24], which can be regulated by changes in lipid metabolism. Thus, hyperlipidemia is a strong candidate to induce GLUT2 overexpression in 6-month-old MetS rats.

Another contribution to GLUT2 overexpression may be related sympathetic hyperactivity, characteristic of SHR [12] and also shown when MSG treatment is associated [25]. Therefore, in addition to hyperlipidemia, the increased sympathetic activity is also a candidate to explain the GLUT2 overexpression in 6-month-old MetS rats.

Finally, in addition to the proposed transcriptional upregulation of the *Slc2a2* gene, as it has been currently described in diabetic rats [10], we can not discard the possibility of a post-transcriptional regulation. Regarding that, we should consider potential changes: 1) in the poly-A tail size of *Slc2a2* mRNA, pointing out, the longer the poly-A tail, the more efficient is the mRNA translation, a regulation that was described for *Slc2a4* gene expression in skeletal muscle of obese rats [26]; and 2) in the expression of small regulatory RNA-143 (miR-143), which has been described as overexpressed in tissues of obese rats [27], and has the *Slc2a2* gene as a target [28]. Besides, considering that we are measuring GLUT2 protein in a plasma membrane enriched fraction, we must also consider the possibility of changes in the intracellular traffic of the protein, increasing its insertion into the tubular brush border membrane, as it was described in kidney of diabetic rats [29].

Renal medullary GLUT1 was drastically reduced 3 months after MSG injection, rising to levels of control rats at the age of 6 months. Differential regulation of glucose transporter expression during development was already described for GLUT4 in adipose tissue of MSG mice, where it was increased at 2–4 months of age, and subsequently decreased at 7 months of age [30], but in that case, converse modulation of insulin sensitivity was observed, which could be responsible for the regulation. It has been proposed that an important mechanism that regulates renal tubular GLUT1 is the intracellular glucose disposal [10]. Thus, we can suppose that during development of the metabolic syndrome (at 3 months of age), an increased cellular glucose offering may be repressing the GLUT1 expression in the S3 segment of the proximal tubule. However, as the GLUT2 content increases in the early segment S1 of the proximal tubule, glucose reabsorption increases, reducing the tubular glucose disposal in the S3 segment, thus recovering (increasing) GLUT1 expression. Importantly, decreased GLUT1 expression was reported in diabetic rats [10], in which GLUT2 expression is markedly increased;

however, in this case, despite increased glucose reabsorption in segment S1, the high glucose filtered rate guarantees high glucose disposal to tubular cells of downstream segment of the nephron. Finally, it is important to show that changes in medullary GLUT1 expression, which is related to S3 segment tubular cells, where only residual glucose reabsorption occurs, was not clearly associated with significant tubular dysfunction yet, as has extensively been shown for changes in GLUT2 expression [18].

In conclusion, GLUT2 overexpression observed in renal cortex of animals with metabolic syndrome is a new finding, possibly related to the visceral obesity, insulin resistance, hypertension, and inflammation of this condition. This GLUT2 overexpression occurs irrespectively of the absence of hyperglycemia, and may contribute to the development of metabolic syndrome-related kidney disease.

Conflict of Interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

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