Abstract. Renal tubular reabsorption of glucose is substantially increased in humans and rats with diabetes mellitus. The influx of luminal glucose is mediated by Na+/glucose cotransporter system and glucose efflux from tubules to interstitium by facilitative glucose transporters (GLUT). In Zucker diabetic rats, GLUT2 protein levels of renal proximal tubules were higher than in control litter mates: 9.67 ± 1.95 versus 4.72 ± 1.55 (P = 0.0073). In the same proximal tubules, diabetes was associated with minor decreases in GLUT1 protein levels: 1.96 ± 0.37 for diabetics and 2.37 ± 0.34 for controls (P = 0.12). Na+/glucose cotransporter system protein levels were similar in both groups, whereas Na+/K+ ATPase levels were slightly decreased in diabetic rats, but the difference was not statistically significant. In this report, it is suggested that in long-term uncontrolled diabetes, GLUT2 transporters are overexpressed in renal tubules. This adaptation promotes low-affinity, high-capacity glucose efflux. The higher number of high K_m GLUT2 ensures that glucose reabsorption is increased by promoting glucose efflux, which could be rate-limiting in the face of hyperglycemia. (J Am Soc Nephrol 8: 943–948, 1997)

Overexpression of GLUT2 Gene in Renal Proximal Tubules of Diabetic Zucker Rats

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The renal reabsorption of glucose is a unidirectional process oriented from urine space to interstitial space (1,2). This trans-epithelial transport function is carried out by two sequential systems of glucose transporters localized to the luminal and basolateral surfaces of renal proximal tubular cells (3–8). The influx of luminal glucose is mediated by Na+/glucose cotransporter system (SGLT), which also elevates intracellular glucose concentration above extracellular levels by energizing glucose influx with the Na⁺ electrochemical gradient (9). Once glucose is concentrated in cells, it diffuses out to the interstitial space across specific facilitative glucose transporters (GLUT) localized in the basolateral membrane (6–8). This step is energized by the outwardly directed glucose chemical gradient generated by coupled Na⁺/glucose influx (1.5). In the proximal convolution, the bulk of glucose efflux occurs through low-affinity, high K_m GLUT2 transporters (10,11), whereas in the late part of proximal tubule, it is mediated by higher-affinity, low K_m GLUT1 transporters (5,12).

Renal tubular reabsorption of glucose is substantially increased in humans and rats with diabetes mellitus, and the changes are proportional to the changes in GFR (13). This higher glucose influx requires glucose efflux to be augmented accordingly. We have reported previously that the basolateral low-affinity GLUT2 gene is overexpressed in Sprague-Dawley rats with streptozotocin-induced diabetes after 3 wk postinduction (14). We now report that the GLUT2 gene is still overexpressed in spontaneously induced diabetic obese Zucker rats after several months. In this study, we used genetically obese Zucker rats after the onset of diabetes as the animal model of diabetes. These rats are characterized by hyperglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemia, and hypercholesterolemia (15–18).

Materials and Methods

Partially inbred obese (400 to 565 g body wt) Zucker male rats ZDF/Drt-fa (Genetic Models, Inc., Indianapolis, IN) were divided into two groups: control and diabetic (four control and eight diabetic littermates were studied, Table 1). The rats were acclimated to a 12:12 light/dark cycle and fed ad libitum standard laboratory rat diet (Ralston Purina, St. Louis, MO). The development of diabetes in some of the littermates was apparent several weeks after birth (Figure 1). The verification of diabetes was achieved by measuring glucose, insulin, and HbA1C levels in serum obtained from tail vein blood drawings at the time of euthanasia 17 wk after birth.

Isolation of Rat Proximal Tubules

Rat proximal tubules were obtained as described previously (5,19). Briefly, the kidneys from anesthetized rats were perfused in situ with 25 ml of ice-chilled Krebs-bicarbonate buffer (KB), which contained (in mM): 150 Na⁺, 5 K⁺, 115 Cl⁻, 25 HCO₃⁻, 1.12 Ca²⁺, 0.7 Mg²⁺, 1 sulfate, 2 phosphate, 5 alanine, 10 acetate, 5.5 glucose, 0.1 myo-inositol, and 0.5 g/liter fatty acid-free bovine serum albumin (BSA). KB was saturated with 95% O₂ and 5% CO₂, pH 7.4. The kidneys were then removed, the medullae excised, and the renal cortices sliced with a Stadie-Riggs microtome. The renal cortical slices were digested in KB with collagenase (type D;2 g/liter) and BSA (40 g/liter) at 30°C in a shaker incubator bath for 50 min. The digested tissue was washed with KB three times by centrifugation (60 × g at 5°C) and loaded in a 37% Percoll gradient (Sigma, St. Louis, MO) generated by centrifugation (240 × g, 5°C, 5 min). The proximal tubules were recovered from the bottom of this gradient.
Table 1. Characteristics of diabetic and control Zucker rats

<table>
<thead>
<tr>
<th>Group*</th>
<th>Rats</th>
<th>Type</th>
<th>Body Weight (g)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (U/ml)</th>
<th>HbA1C (%)</th>
<th>Kidneyb Wt (g)</th>
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<tr>
<td>DM</td>
<td>UA41-318</td>
<td>fa/fa</td>
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<td>340</td>
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<td>12.4</td>
<td>5.355</td>
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<tr>
<td>DM</td>
<td>UA41-319</td>
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<td>1.53</td>
<td>18.1</td>
<td>4.28</td>
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<tr>
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<td>732</td>
<td>4.47</td>
<td>20</td>
<td>3.175</td>
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<tr>
<td>DM</td>
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<td>680</td>
<td>0.45</td>
<td>20.3</td>
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<td>2.26</td>
<td>17.2</td>
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<td>fa/fa</td>
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<tr>
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<td>fa/fa</td>
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<td>0.83</td>
<td>19.2</td>
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<tr>
<td>CON</td>
<td>UA41-322</td>
<td>+/-fa</td>
<td>562</td>
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<td>2.92</td>
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<td>1.475</td>
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<tr>
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<td>132</td>
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<td>CON</td>
<td>UA41-321</td>
<td>+/-fa</td>
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<td>+/-fa</td>
<td>456</td>
<td>135</td>
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<td>5</td>
<td>1.275</td>
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</table>

* DM, diabetes mellitus; CON, control.

** Kidney weight.

** Proximal Tubule RNA and Slot Blot Analysis

Renal proximal tubules were disrupted by vortexing in 4 M guanidinium isothiocyanate, which also contained 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl, and 0.7% β-mercaptoethanol. Total RNA was then extracted with phenol-chloroform-isoamyl alcohol (vol/vol = 1:2:0.0083) and precipitated from the aqueous layer with an equal volume of isopropanol overnight (−20°C) (20). The RNA pellet was dissolved in diethylpyrocarbonate-treated H2O and quantitated by spectrophotometry (260/280 optical density) (20). Twenty micrograms of RNA were loaded on each lane of 1% agarose plus 9% formaldehyde gel, and RNA integrity was checked by visualization of the 18S and 28S ribosomal bands. When ribosomal RNA integrity was verified, mRNA from rat tubules was measured on slot blots according to the manufacturer’s instructions (Schleicher and Schuell, Keene, NH). Twenty micrograms of RNA were dissolved in 100 μl of diethylpyrocarbonate water with 200 μl of 6.15 M formaldehyde plus 5X SSC (1X SSC is 150 mM NaCl and 15 mM sodium citrate, pH 7.0) and then blotted using vacuum on “Nytran” nylon membranes (Schleicher & Schuell, Keene, NH) (21), rinsed under vacuum with 0.2% SDS and 0.1 X SSC at 52°C and exposed to [32P]deoxycytosine triphosphate to a specific activity of 0.5 to 1 × 10⁶ cpm/μg by the oligolabeling method (26). The membranes were washed in 0.2% SDS and 0.1 X SSC at 52°C and exposed to Kodak XAR-5 film at −70°C with a Cronex (DuPont Inc., Wilmington, DE) intensifying screen. Cross hybridizing mRNAs were depicted by autoradiography and then quantitated on the membranes by β-radioactivity scanning (Ambis, San Diego, CA).

** Measurement of GLUT1, GLUT2, and SGLT Proteins by Western Blot

Proximal tubules were homogenized on ice with a glass/Teflon homogenizer (Sigma) in TES-PI buffer, which contained 20 mM Tris-HCl (pH 7.4), 225 mM sucrose, and 1 mM EDTA plus 5 μg/ml pepstatin, 5 μg/ml leupeptin, and 5 μg/ml aprotinin. The resulting tubular homogenate, 100 μg of protein/lane, was solubilized in Laemmli sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis on 1.5-mm slab gels containing 10% polyacrylamide (27,28). The proteins were electrophoretically transferred to nitrocellulose and reacted with specific antibodies (5). Antibody to GLUT1 was raised in rabbits immunized with a synthetic peptide comprising the last 12 amino acids of the COOH end of rat GLUT1 (29) and was purchased from East Acres Biologicals (Southbridge, MA). Antibody to GLUT2 was raised in rabbits immunized with a synthetic peptide containing the last 25 amino acids of rat GLUT2, and it was kindly made available by Drs. David James and Robert Piper (Washington University, St. Louis, MO). Monoclonal antibody specific for SGLT was raised in mouse against the protein purified from pig renal brush border membrane and expressed in hybridoma cells (30); it was kindly made available by Dr. Julia E. Lever (University of Texas, Southwestern, Houston, TX). The cross-reacting proteins were identified by autoradiography of [125I]-protein A label; their apparent molecular weight was calculated from comigration of known standards, and their relative quantity was measured by β-radioactivity scanning.

** Materials

BSA was from ICN Immunologicals (Lisle, IL). Collagenase (type D) was from Boehringer Mannheim (Indianapolis, IN). [32P]Deoxyctosine triphosphate and [125I]-protein A were from Du Pont-New England Nuclear (Boston, MA). Percoll formamide, Denhardt’s solution, SDS, 5 X SSC, guanidinium isothiocyanate, sodium citrate (pH 7.0), sarkosyl, leupeptin, pepstatin, aprotinin, and β-mercaptoethanol were from Sigma (St. Louis, MO). Nitrocellulose and nylon membranes were from Schleicher and Schuell. Rabbit anti-GLUT1 and anti-GLUT2 antibodies were purchased from East Acres Biologicals. The β-scanner was manufactured by Ambis.

** Statistical Analysis

Data are expressed as mean ± SD. Statistical significance was determined by t test for unpaired groups.
Results

In control rats, random serum glucose concentrations were stable during the length of the study. In contrast, in diabetic rats the glucose levels were more than 300 mg/dl, 4 wk after birth and remained elevated for the duration of the study (Figure 1). The mean HbA1C level was 4.93 ± 0.36 (%) in control rats and 18.18 ± 2.64 (%) in diabetic rats, indicating long-term hyperglycemia in diabetic rats (P < 0.0001).

Both control and diabetic animals were obese with mean body weights of 526 ± 50 and 449 ± 39 g, respectively (P = 0.015), whereas mean kidney weight was 1.4 ± 0.1 g for control rats and 2.6 ± 0.9 g for diabetic rats (P = 0.04) (Table 1).

Protein Expression in Kidney

In both groups, the expression of GLUT1, GLUT2, SGLT, and α1-subunit of Na+/K+ ATPase (NKA) proteins in proximal tubules was appraised on immunoblots using specific antibodies. In these studies, tubular proteins were size-fractionated by polyacrylamide gel electrophoresis, immobilized on nitrocellulose filters, and reacted with specific transporter antibodies followed by 125I-protein A. In each subgroup, GLUT1 protein in proximal tubules migrated as a broad band (molecular mass, 43 to 55 kDa) as described previously (7), whereas GLUT2, SGLT, and NKA were more sharply defined at approximate molecular masses of 58, 68, and 97 kDa, respectively.

From these immunoblots, it appeared that diabetes was associated with a minor and nonsignificant decrease of GLUT1 protein levels (Figure 2) and a highly significant increase of GLUT2 protein levels in proximal tubules (Figure 3). These changes in GLUT2 protein levels persisted chronically for up to 6 months after the development of diabetes. SGLT protein levels were similar in both groups (Figure 4), whereas NKA levels were slightly decreased in diabetic rats (Figure 5), but the difference was not statistically significant. The GLUT1 levels were 2.37 ± 0.34 (cpm/mm2) for controls and 1.96 ± 0.37 for diabetics (P = 0.12). GLUT2 levels were 4.72 ± 1.55 for controls and 9.67 ± 1.95 for diabetics (P = 0.0073). SGLT levels were 2.9 ± 0.97 for controls and 2.73 ± 0.49 for diabetics (P = 0.73). NKA levels were 7.38 ± 1.95 for controls and 5.93 ± 1.03 for diabetics (P = 0.24).

mRNA Expression in Kidney

Renal proximal tubular GLUT1 and GLUT2 mRNA levels were normalized to 28S rRNA measured on the same membranes. The data were then expressed as the ratio GLUT
Figure 4. Western blot of SGLT protein in renal proximal tubules. SGLT protein levels were similar in control and diabetic Zucker rats. The transport proteins were detected after size-fractionation of protein (100 µg) by immunoblotting with GLUT1 antibody. Numbers indicate apparent molecular weight in kilodaltons.

Figure 5. Western blot of α1-subunit of Na+/K+ ATPase (NKA) in renal proximal tubules. The protein levels were slightly decreased in diabetic Zucker rats. The proteins were detected after size-fractionation of protein (100 µg) by immunoblotting with NKA antibody. Numbers indicate apparent molecular weight in kilodaltons.

The transport activity of SGLT is reduced in renal membrane vesicles isolated from rats with uncontrolled diabetes (33–35). This finding, in conjunction with the finding of stable levels of SGLT transporters reported herein, suggests that the decrease in transport activity might be secondary to post-translational changes imposed by the diabetic state. However, in whole animals glucose influx through SGLT remains supranormal, most likely because of mass action effects derived from the higher glucose-filtered load (36). Thus, tubular glucose reabsorption, which comprises glucose influx and efflux, is augmented in diabetes (36), even though hyperglycemia has the potential to lower the tubule/interstitium glucose gradient, the energy source for facilitative glucose efflux (7).

In a previous study, we reported that streptozotocin-induced diabetes caused marked reduction in tubular GLUT1 mRNA steady-state levels. In the same tubules from diabetic rats, 2 to 4 wk of diabetes resulted in marked increases in GLUT2 mRNA steady-state levels (14). In this study, we found that in obese Zucker rats diabetes is associated with a highly significant increase of GLUT2 mRNA and protein levels. There also was a minor decrease of GLUT1 protein levels in renal proximal tubules that was not significant. SGLT protein levels did not change significantly, whereas NKA levels decreased slightly in diabetic rats, but the difference was not statistically significant. There was also a substantial increase in the kidney weight of diabetic rats. These changes persisted chronically for up to 3 months after the development of diabetes.

In summary, in uncontrolled diabetes, glucose influx via luminal SGLT is elevated, an event caused mainly by mass action effects due to high filtered load of glucose (36). The higher glucose load presented to the tubule requires that glucose efflux be correspondingly augmented for maintenance of tubule to the interstitium is accomplished by facilitative transporters (GLUT) located exclusively in the basolateral membrane, GLUT2 in early proximal tubule, and GLUT1 in early and late proximal tubule (3–8,12).

The renal tubular reabsorption of filtered glucose is accomplished by two major classes of transporters. Luminal glucose is concentrated in tubules by SGLT located exclusively in the apical membrane and SGLT2 in early proximal tubule and SGLT1 in late proximal tubule (1,31,32). Glucose efflux from mRNA:28S rRNA. Proximal tubular GLUT1 mRNA normalized to 28S rRNA subunit was 0.36 ± 0.05 for controls and 0.33 ± 0.04 for diabetics (P = 0.38). Proximal tubular GLUT2 mRNA normalized to 28S rRNA subunit was 0.01 ± 0.004 for controls and 0.022 ± 0.005 for diabetics (P < 0.009) (Figure 6).

**Discussion**

The renal tubular reabsorption of filtered glucose is accomplished by two major classes of transporters. Luminal glucose is concentrated in tubules by SGLT located exclusively in the apical membrane and SGLT2 in early proximal tubule and SGLT1 in late proximal tubule (1,31,32). Glucose efflux from...
steady-state trans-tubular glucose flux. Overexpression of GLUT2 transporter promotes low-affinity, high-capacity glucose efflux (5, 14, 37). The higher number of high \( K_m \) GLUT2 ensures that efflux is not saturated in the presence of higher glucose concentrations (36). Thus, the adaptive responses designed to maintain a higher trans-tubular glucose flux in diabetes. The signals responsible for the adaptation of glucose transporter are unknown. However, luminal glucose interaction with luminal SGLT appears to be necessary for the activation of GLUT2 expression (38).

Acknowledgments
We thank Dr. Morris Birnbaum for GLUT1 cDNA, Dr. Harvey F. Lodish for GLUT2 cDNA, Drs. David James and Robert Piper for the gift of GLUT2 antibodies, and Dr. Bruce Hirayama for the gift of SGLT antibody. We also thank Dr. Julia E. Lever for providing us with hybridoma cells producing SGLT antibody and Dr. Merrill Benson for growing the hybridoma cells in culture, inoculating mice with the cells, and recovering ascitic fluid. This work was supported by the Veterans Administration and by Grant DK-43640 from the National Institute of Diabetes and Digestive and Kidney Diseases to J. H. Dominguez.

References


