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Enhanced Expressions of Sodium-Glucose Cotransporters in the Kidneys of Diabetic Zucker Rats

Niloofar M. Tabatabai, Ph.D.[a,c,*], **Mukut Sharma, Ph.D.**[b,c], **Samuel S. Blumenthal, M.D.**[b,c], and **David H. Petering, Ph.D.**[d]

a *Division of Endocrinology, Metabolism and Clinical Nutrition, Medical College of Wisconsin, Milwaukee, WI, USA*

b *Division of Nephrology, Medical College of Wisconsin, Milwaukee, WI, USA*

c *Kidney Disease Center, Medical College of Wisconsin, Milwaukee, WI, USA*

d *University of Wisconsin-Milwaukee, Milwaukee, WI, USA*

Abstract

Diabetes-mediated changes in mRNA expressions of kidney glucose transporters SGLT1 and 2 were investigated in Zucker rats. SGLTs expressions in pre-diabetic obese rats were similar to leans. SGLT1 and SGLT2 levels in diabetic obese rats was 1.6 ($P < 0.03$) and 4.8 ($P < 0.002$) folds higher than age-matched leans, respectively.

Keywords

Diabetes; kidney; glucose transporter; SGLT; NaPi-2; mRNA

Introduction

Diabetic nephropathy is the leading cause of end-stage renal disease [1]. Functional changes in the proximal tubule (PT) are important in the development of diabetic nephropathy [2]. PT cells isolated from the urines of type 2 diabetic patients showed increased glucose uptake and elevated levels of the glucose transporter, SGLT2 [3].

Glucose is filtered out of blood at the glomerulus but it is completely re-absorbed by PT cells [4]. SGLT1 and SGLT2 are sodium-dependent glucose transporters that are expressed on the apical side of these cells; SGLT2 re-absorbs bulk of the filtered glucose while SGLT1 transports the remainder [4]. Absorbed glucose then diffuses into the blood via the basolaterally located facilitative glucose transporters, GLUTs [4].

Zucker obese (ZO) rat is a model of type 2 diabetes and has been used to study diabetic nephropathy [5,6]. Elevated serum concentrations of glucose and insulin are present by 14 weeks of age [7]. Signs of kidney damage, manifested by increasing albuminuria, appear at 14

*Corresponding Author: Niloofar M. Tabatabai, Ph.D., Medical College of Wisconsin, Kidney Disease Center, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226-0509, USA, Phone: (414) 456-8452, Fax: (414) 456-6312, Email: ntabatab@mcw.edu.

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to 21 weeks [7]. In this study, we used the kidneys from the same rats in the above study [7] to examine the mRNA expressions of SGLT1 and SGLT2 before and after the onset of diabetes. We show that the kidney mRNA levels of both SGLT1 and SGLT2 are elevated in response to diabetes but mRNA levels of sodium-phosphate transporter (NaPi-2) (8) remained the same.

Methods

Semi-quantitative RT-PCR

Male ZO (Crl:ZUC-*Lepr*^{fa}) and their age-matched lean (ZL) controls had been used to isolate kidney total RNA [7]. Semi-quantitative RT-PCR was performed as described previously in detail [9]. In brief, kidney total RNA (2μg) from ZO (*N*=4) and ZL (*N*=4) rats at 7 weeks of age and from ZO (*N*=6) and ZL (*N*=6) rats at 21 weeks of age were used to prepare cDNA samples, which were used as template in PCR. Amplification of S15 cDNA encoding small ribosomal subunit protein was performed for normalization [9]. Amplification cycle was 94 °C, 30 sec; 56 °C, 30 sec; 72 °C, 55 sec. Number of amplification cycles and the expected sizes of the PCR products are shown in Table 1. PCR products were subjected to gel electrophoresis, and the intensities of stained PCR bands were quantified. Ratio intensity of PCR band of SGLT or NaPi-2 to S15 was calculated, and the result was expressed as mean ± SEM for each group.

Results

SGLT mRNA expression

The mRNA levels of SGLT1 in the kidneys of 7 week old pre-diabetic ZO rats were the same as the control ZL rats (Fig. 1A & B). While mRNA level of SGLT2 in ZO rats was slightly higher than the age-matched ZL, this difference was not statistically significant ($P = 0.4$, Fig. 1B). Overall, mRNA expressions of SGLT1 and 2 were the same in the ZO and the ZL groups.

Figure 2 shows the expression of SGLTs mRNA in the kidneys of the 21 weeks old rats. SGLT1 mRNA level in kidneys of diabetic ZO rats was 1.6 folds higher than its expression in ZL ($P < 0.03$). An even larger difference between the two groups was observed in mRNA expression of SGLT2, which was 4.8 folds higher in the kidneys of ZO than the ZL rats ($P < 0.002$, Fig. 2B).

NaPi-2 mRNA expression

To determine whether the increases in SGLT1 and 2 levels were specific, we also examined mRNA levels of sodium-phosphate transporter, NaPi-2, which is also expressed on the luminal surface of the PT cells [8]. NaPi-2 expression in the kidneys of 7 weeks old non-diabetic ZO rats were the same as the age-matched leans (Fig. 3). Although NaPi-2 mRNA level was 1.8 folds higher in the 21 weeks old ZO than the ZL rats, this difference was not statistically significant ($P = 0.1$).

Discussion

Diabetic nephropathy has been associated with increased renal intracellular glucose levels [10]. A limited number of studies have addressed the expression of SGLTs in diabetes. SGLT1 mRNA level was 20% higher and SGLT2 was 36% higher in the kidneys of the alloxan-induced diabetic rats [11]. In the streptozotocin-induced diabetic rats, the mRNA level of SGLT2 remained unchanged [12].

We studied SGLTs mRNA expressions in the kidneys of Zucker rats, a model of type 2 diabetes. The 7 weeks old ZO rats were not diabetic [7]. At this age, renal mRNA levels of both SGLT1 and 2 were similar in ZO and ZL groups (Fig. 1). However, by 21 weeks of age, when ZO rats

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had diabetes and were proteinuric [7], their renal expressions of SGLT1 and SGLT2 were 1.6 and 4.8 folds higher than in the ZL, respectively (Fig. 2). Our results are consistent with the increased SGLT2 expression in PT cells from diabetic patients [3]. We found that NaPi-2 mRNA expression was unaffected by diabetes (Fig. 3). However, Bickel et al. [13] reported a decrease in NaPi-2 protein level in diabetic ZO rats. Taken together, we suggest that diabetes may enhance NaPi-2 protein degradation.

Enhancements in renal SGLT1 and SGLT2 gene expressions can be due to diabetes-related factor(s) present in glomerular filtrate. In hyperglycemia, PT cells are exposed to higher concentrations of filtered glucose [4]. Ovine *SGLT1* promoter activity is enhanced by glucose [14], and this glucose responsive site, HNF-1α, is also conserved in the human and rodent promoters [15,16]. Also, diabetic nephropathy is manifested by increased albumin filtration [2,7]. *In vitro* exposure of cultured rabbit PT cells to albumin increased protein levels of SGLT1 and SGLT2 [17]. SGLT2 expression has been suggested to be up-regulated in response to ANG II [18,19], which is also elevated in diabetes [7,19]. We suggest that the mRNA of expressions of SGLT1 and SGLT2 are increased in the diabetic kidney in association with proteinuria, and this increase may enhance the re-absorption of glucose resulting in tubular damage.

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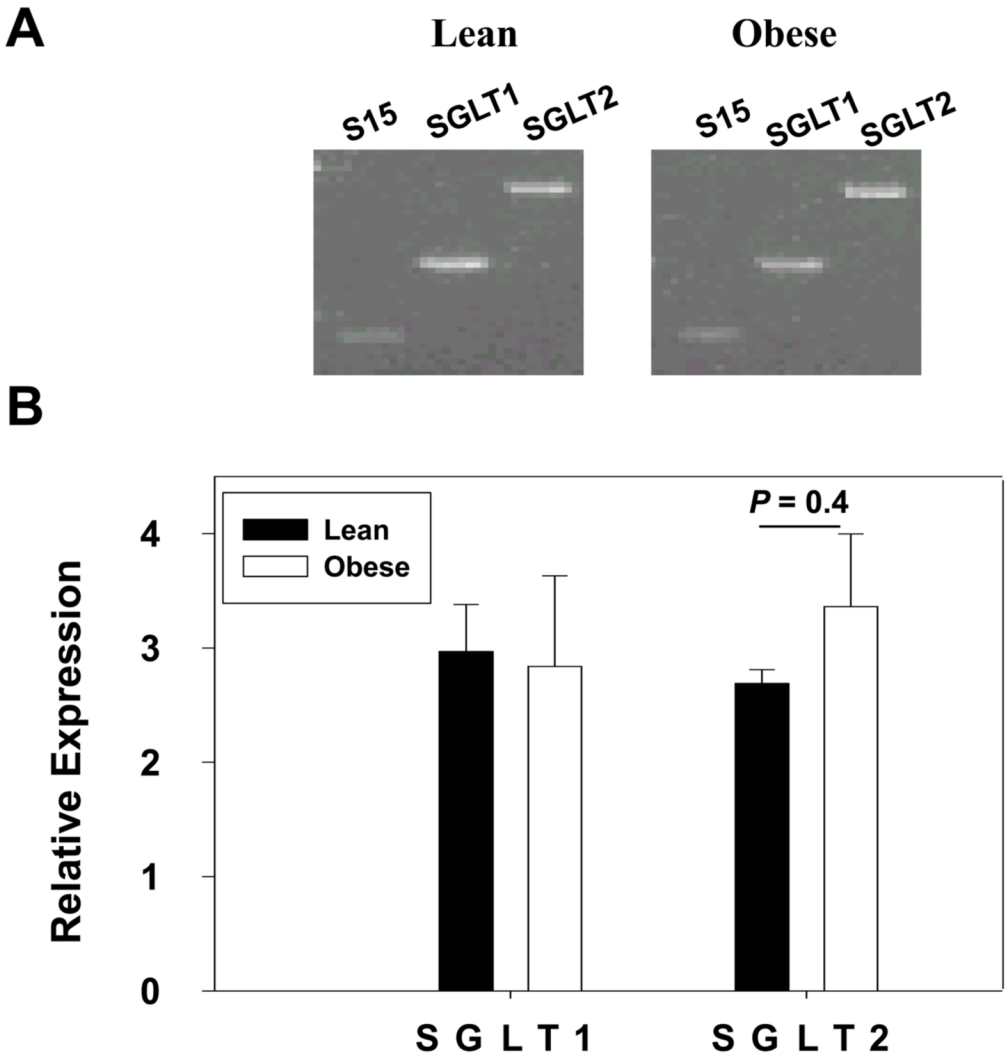

Figure 1. SGLT expression in kidneys of 7 wk old rats

A., Kidney RNA from 7 weeks old ZL and ZO rats were used in RT-PCR (*N* = 4 of each group). **B.,** The mean ratio intensities of SGLTs to S15 was determined for ZL (black bars) and ZO (white bars) rats.

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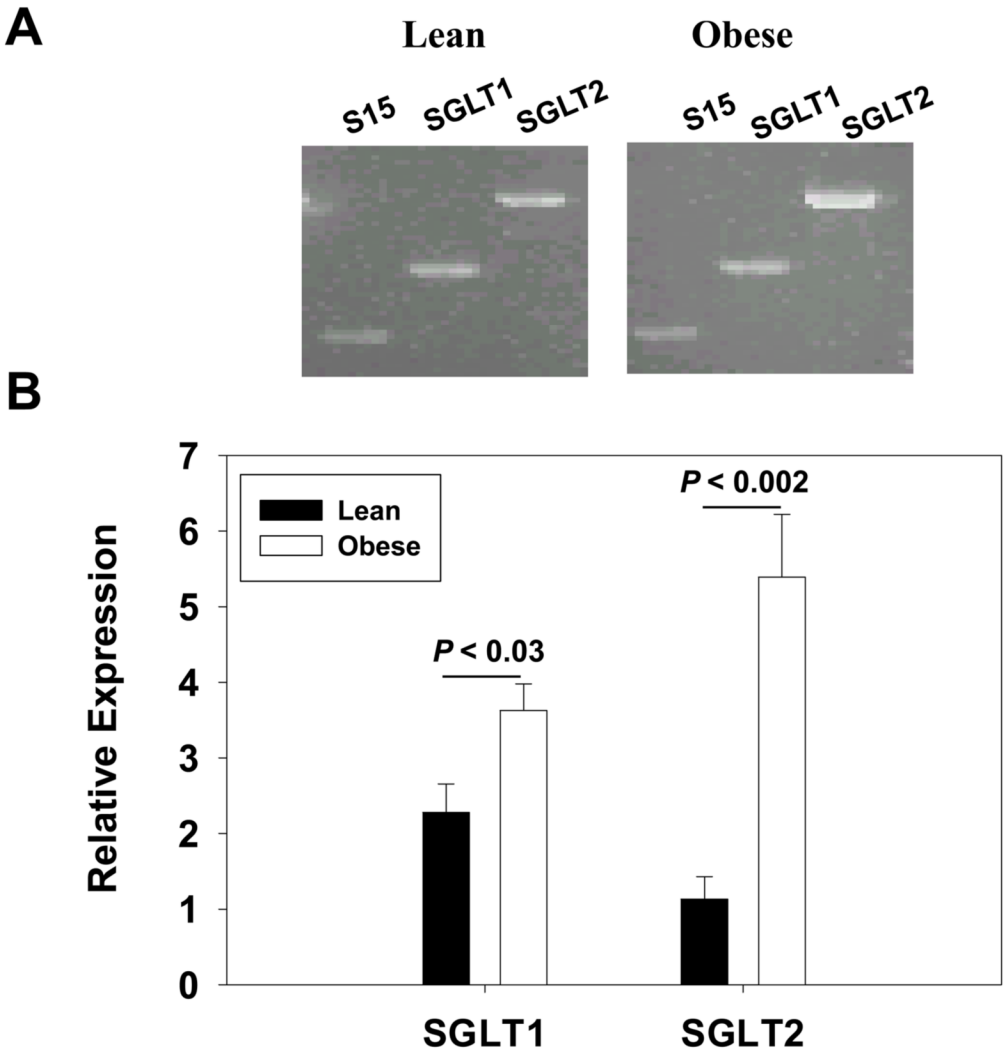

Figure 2. SGLT expression in kidneys of 21 wk old rats

A., Kidney RNA from 21 weeks old ZL and ZO rats were used in RT-PCR (*N* = 6 of each group). **B.,** The mean ratio intensities of SGLTs to S15 was determined for ZL (black bars) and ZO (white bars) rats.

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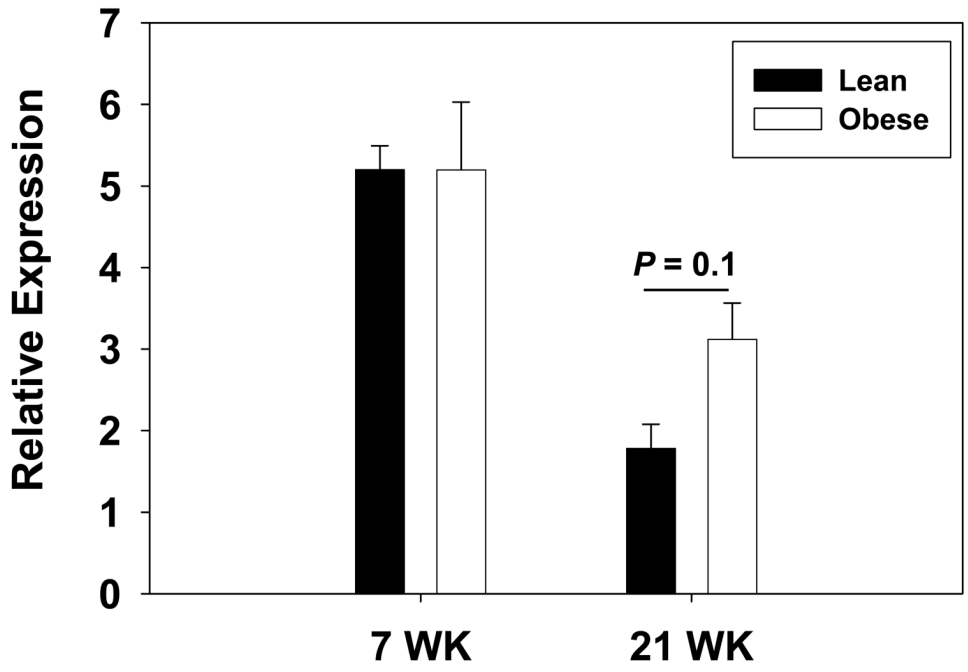

Figure 3. NaPi-2 expression in kidneys of 7 and 21 wk old rats

Kidney RNA samples from either 7 weeks old or 21 weeks old ZL and ZO rats were used in RT-PCR. The mean ratio intensity of NaPi-2 to S15 was determined for ZL (black bars) and ZO (white bars) rats.

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TABLE 1

PCR primers and amplification cycles

Gene	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	# of PCR Cycles7 wk, 21 wk	PCR Size(bp)
SGLT1	GACATATCAGTCATCGTCATC	TGTGATGGTGTAAAGGGCGGTG	30, 25	501
SGLT2	AGGATCCAGCTGTTGGCA	ACGGGGCACAAAGAGT	25, 32	707
NaPi-2	GTTGCCTCCTTCAACATCC	GCTCAATACTGATCACACCC	22, 24	556
S15	TTCCGCAAGTTCACCTACC	CGGGCCGGCCATGCTTTACG	25, 25	361

wk, weeks old; bp, base pairs