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The effects of thiazolidinedione treatment on the regulations of aquaglyceroporins and glycerol kinase in OLETF rats

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Abstract

Aquaporins (AQPs) that transport glycerol in addition to water are classified as aquaglyceroporins (AQP3, 7, 9). AQP7 in the adipose tissue and AQP9 in the liver may coordinately contribute to the increase in hepatic gluconeogenesis in states of insulin resistance. Thiazolidinedione (TZD) has been shown to increase adipose AQP7 and induce glycerol kinase (GlyK) which is nearly absent in adipocytes. In the present study, we analyzed both GlyK and AQP gene expression in adipose and hepatic tissues, and AQP3 in kidneys from Long-Evans Tokushima Otsuka (LETO), Otsuka Long-Evans Tokushima Fatty (OLETF), and rosiglitazone (RSG)-treated OLETF (RSG-OLETF) rats. We also evaluated AQP9 protein expression in cultured human hepatoma cells treated with oleic acid, Wy14643, or RSG. A 2-week RSG treatment increased AQP7 mRNA levels in the mesenteric fat, but not in the epididymal fat of OLETF rats. Rosiglitazone treatment markedly increased GlyK expression in both fat depots, with a greater increase in the mesenteric fat. The magnitudes of GlyK induction by RSG were greater than that of AQP7 in both adipose tissues ($P < .05$, each). AQP9 and GlyK levels in the liver were not affected by RSG treatment in OLETF rats. Oleic acid and Wy14643 upregulated AQP9 protein expression in cultured human hepatoma cells in a dose-dependent manner. AQP3 mRNA levels tended to increase in the outer medulla of the RSG-OLETF rats. These results indicate that in the adipose tissue TZD has an important role in the glycerol metabolic pathway through the regulation of AQP and GlyK, especially by GlyK induction. Free fatty acids may directly enhance glycerol availability in the liver via the upregulation of AQP9 levels. Renal AQP3 may be related to the fluid retention caused by TZD.

1. Introduction

It is well recognized that elevated plasma free fatty acid (FFA) levels associated with obesity are a critical intermediate in the pathophysiology of type 2 diabetes [1-3]. In contrast, glycerol, another product of lipolysis, has drawn less attention [4-6]. As a substrate for hepatic glucose production, glycerol contributes a significant proportion to hepatic gluconeogenesis during the fasting and postabsorptive period [4,7,8]. Because the adipose tissue is a major source of plasma glycerol, a higher influx of glycerol into the liver from the accumulated fat may be relevant to the development of diabetes in obesity [4].

In parallel with recent advances in aquaporin (AQP) research, 3 AQPs (AQP3, 7, 9) were classified as aquaglyceroporins after being reported to transport glycerol in addition to water [9,10]. A unique form of nephrogenic diabetes insipidus in AQP3 null mice indicates that AQP3-mediated water transport across the basolateral membrane of renal collecting duct epithelium is important in the formation of concentrated urine [11,12]. Whether fluid retention occurs if renal AQP3 expression increases still remains unknown. AQP7, the only AQP in adipose tissue, plays an important role in the secretion of glycerol from the adipose tissue [13-15]. AQP9, identified mainly at

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Table 1
Rat primers used in RT-PCR

Gene	Primers (forward, reverse)	Amplicon size (bp)	References
AQP3	5′-AAACTAGCAGCTCAAGGGAAC-3′ 5′-TCCCACCCCTATTCCTAAAAC-3′	367	[24]
AQP7	5′-ATGGCCGGTTCTGTGCTG-3′ 5′-TCTAAGAACCCTGTGGTGG-3′	810	[25]
AQP9	5′-CCAAGATGCCTTCTGAGAAG-3′ 5′-CCACTACATGATGACACTGAGC-3′	897	[26]
Glycerol kinase	5′-GGAGACCAGCCCTGTTAAGCT-3′ 5′-GTCCACTGCTCCCACCAATG-3′	101	[21]
β-Actin	5′-CAGATCATGTTTGAGACCTT-3′ 5′-CGGATGTCMACGTCACACTT-3′	509	[25]

the basolateral plasma membrane of hepatocytes, transports water, glycerol, and a variety of other small uncharged solutes [16].

It is conceivable that during fasting, AQP7 in fat is essential for the supply of glycerol to the liver and that AQP9 is critical for the hepatic uptake of glycerol for glucose production. Although insulin suppresses the amount of AQP7 in adipose tissue [17] and AQP9 in liver [18], the coordinated upregulation of AQP7 and AQP9 expression in conditions of insulin resistance may increase the supply of fat-derived glycerol as a substrate for hepatic gluconeogenesis and, thereby, aggravate hyperglycemia [18].

However, thiazolidinedione (TZD), which has an insulin sensitizing action via the stimulation of peroxisome proliferator–activated receptor γ (PPARγ) and is now being used clinically to treat type 2 diabetes, further increases AQP7 expression in adipocytes [19]. The effect of PPARγ stimulation on hepatic AQP9 expression remains unknown. Despite the upregulation of AQP7 by TZD, glycerol release from RSG-treated adipocytes is not increased; rather, it is significantly decreased [20]. How could such a paradox occur? Guan et al [21] have made a surprising observation: TZDs markedly induce GlyK in adipocytes. Before this report, researchers believed that adipocytes lacked GlyK, and, therefore, glycerol derived from lipolysis could not be reused as a substrate for triglyceride (TG) synthesis in adipocytes [22,23]. This unsuspected induction of GlyK by TZDs could switch the internal milieu of the adipocyte to a state of increased TG synthesis, glycerol recycling, and reduced glycerol release despite AQP7 upregulation.

Previous studies have focused on GlyK and AQP7 independently. Further in vivo studies focusing on the coordinated regulation of GlyK and the aquaglyceroporins including AQP9 are required. To evaluate the changes and relative significance of GlyK and AQPs in the glycerol metabolic pathway in mediating the therapeutic effects of TZD, we analyzed GlyK and aquaglyceroporin gene expression in adipose and hepatic tissues from LETO, OLETF, and RSG-treated OLETF (RSG-OLETF) rats. We also evaluated AQP3 expression in experimental group kidneys and AQP9 protein expression in cultured human hepatoma (HepG2) cells treated with oleic acid, Wy14643, and RSG.

2. Materials and methods

2.1. Animal preparation

All experimental procedures were conducted in accordance with the Guide for Animal Experimentation (College of Medicine, Cheju National University, Republic of Korea). Twelve male OLETF and 6 male control LETO rats aged 4 weeks were obtained from the Tokushima Research Institute of Otsuka Pharmaceutical Co (Tokushima, Japan) and maintained in an animal facility with ventilation, controlled temperature (22°C ± 2°C), and a 12-hour light/dark cycle (lights on at 8:00 AM). All animals were housed in the same plastic cages (n = 3 per cage) and were fed ad libitum with standard chow diet and tap water.

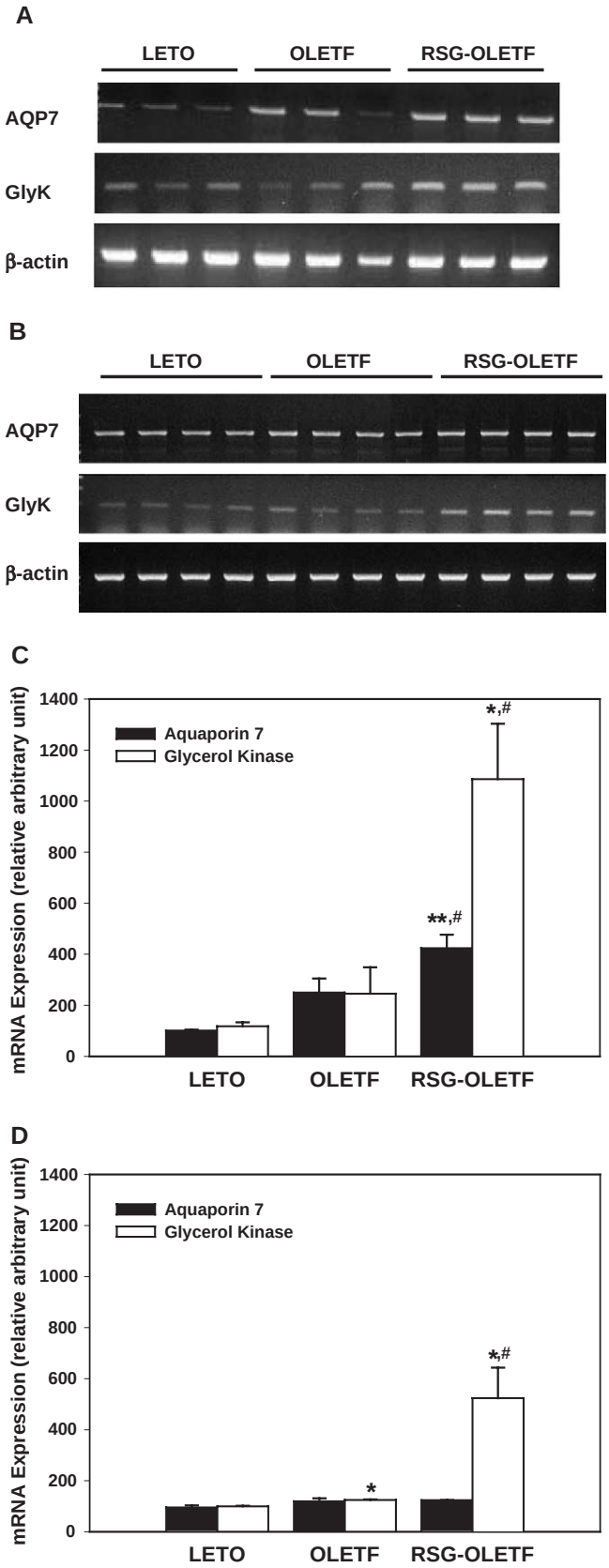
2.2. The effect of RSG administration on aquaglyceroporin expression in the liver, adipose tissue, and kidney

To examine the effect of RSG treatment on the expression of aquaglyceroporins and GlyK in OLETF rats, a total of 12 OLETF rats were divided into 2 groups (n = 6 per group) at 22 weeks of age. Rats were kept on standard rat food or rat food supplemented with 0.01% (wt/wt) RSG (GlaxoSmithKline Pharmaceuticals, Herts, UK) for 2 weeks. Long-Evans Tokushima Otsuka rats of the same age (n = 6 per group) were used as the control group. On the final day of the treatment, the food or food supplemented with RSG was withdrawn from the cages at midnight. Ten hours later, the rats were euthanized by decapitation, and the trunk blood was collected in prechilled EDTA tubes (Vacutainer; BD Biosciences, Franklin Lakes, NJ). Both kidneys, the liver, and the mesenteric and epididymal fat pads were rapidly removed and snap-frozen with liquid nitrogen. Frozen samples were kept at −70°C until analyzed. mRNA expression of the aquaglyceroporins and GlyK in removed tissues was analyzed by reverse transcriptase–polymerase chain reaction (RT-PCR).

Table 2
Body weight and biochemical data of the experimental groups

Parameters	Experimental groups		
	LETO	OLETF	RSG-OLETF
Body weight (g)	488.0 ± 5.3	598.3 ± 13.8*	607.3 ± 17.9
Plasma glucose (mg/dL)	96.0 ± 3.0	117.5 ± 6.2**	104.2 ± 5.3***
FFA (μEq/L)	447.6 ± 120.1	589.0 ± 58.6	379.0 ± 83.3***
Plasma insulin (μU/mL)	92.7 ± 17.8	219.1 ± 36.6**	55.8 ± 10.3****

Values are means ± SE (n = 6, each group).
RSG-OLETF indicates OLETF rats given standard diet containing RSG (0.01%) for 2 weeks.
* $P < .01$ vs LETO group.
** $P < .05$ vs. LETO group.
*** $P < .05$ vs OLETF group.
**** $P < .01$ vs OLETF group.



2.3. Blood/plasma analyses

Whole blood was centrifuged at 3000 rpm (Sorvall RT 6000 D; Sorvall, Newton, Conn) at 4°C for 20 min for plasma separation. Plasma glucose levels were determined with a Beckman Glucose Analyzer II (Beckman-Coulter, Fullerton, Calif). Plasma insulin levels were assayed with a radioimmunoassay using a commercial kit (Linco, St Charles, Mo). Free fatty acid (FFA) was determined using a NEFA-zyme Eiken kit (Eiken, Tokyo, Japan).

2.4. Isolation of total RNA and RT-PCR

Total RNA was extracted from tissues with Trizol reagent following the supplier's protocol. Three to 5 μg of total RNA was treated with amplification grade DNAse I and then reverse-transcribed using an avian myeloblastosis virus RT system (Promega, Madison, Wis). RNA was first incubated for 10 min at 70°C. The reaction mixture included the reverse transcription buffer, 25 mmol/L $MgCl_2$, 10 mmol/L deoxynucleotide triphosphates, avian myeloblastosis virus RT, RNasin ribonuclease inhibitor, and random primers in a final volume of 95 L. This mixture was added to the total RNA and incubated for 10 minutes at room temperature and then for 1 hour at 42°C. Heating to 95°C for 5 minutes stopped the reaction. AQP and GlyK cDNAs were amplified using the PCR with specific primers (Table 1). Polymerase chain reaction products were electrophoresed in 1% agarose gels, and the bands were visualized by ethidium bromide staining. cDNA was quantified by IMAGER and 1D MAIN (Bioneer, Seoul, South Korea). mRNA expression of all genes reported was normalized to the β-actin expression level.

2.5. Cell culture and AQP9 immunoblotting

HepG2 cells were obtained from Korean Celline Bank (Seoul, South Korea), grown in low-glucose (1.0 g/L) Dulbecco's minimal essential medium (supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum) in a humidified 95% air–5% CO_2 atmosphere incubator at 37°C until they reached preconfluence (60%-70%) and then collected for analysis. Two days after plating in 6-well plates, cells were serum starved for 24 hours. After the cells were washed twice with PBS, they were incubated in Dulbecco's minimal essential medium with 0, 10, and 100 μmol/L RSG, with 0, 10, and 50 μmol/L Wy14643 (Calbiochem, San Diego, Calif),

Fig. 1. Effect of RSG treatment on AQP7 and GlyK mRNA expressions in mesenteric (A, C) and epididymal adipose tissues (B, D). RSG-OLETF rats were administered RSG (0.01% w/w) in normal rat chow diet for 2 weeks. Representative RT-PCR products of AQP7 and GlyK in the mesenteric (A) and epididymal fat (B) are shown. Abundance of mRNAs was determined by densitometric analysis. Data were normalized using β-actin mRNA and expressed as a percentage of the value obtained from control chow-treated LETO rats. Each bar represents means ± SE (n = 6). *P < .05, **P < .01 vs LETO group. $^{\#}P$ < .05, $^{\#\#}P$ < .01 vs OLETF group.

or with 0, 250, and 500 μmol/L oleic acid (Sigma-Aldrich, St Louis, Mo) for 24 hours. Oleic acid was complexed to bovine serum albumin in a 3:1 molar ratio. In pilot studies, there was no evidence of cell toxicity after 24 hours of incubation with oleic acid at concentrations up to 0.5 mmol/L. Cell toxicity was assessed by cell morphology, trypan blue exclusion, and total protein mass. After the culture plate cells were lysed using the RIPA solution (Chemicon, Temecula, Calif), the cellular lysates were harvested and centrifuged at 10 000 × *g* at 4°C for 5 minutes. The supernatants were taken and the protein concentrations were measured using a BCA protein assay kit (Pierce, Rockford, Ill). The proteins (40 μg) were loaded and separated on a 12.5% sodium dodecyl sulfate–polyacrylamide gel and transferred onto a nitrocellulose transfer membrane (Schleicher & Schuell, Keene, NH). Western blot analysis was performed using the antiserum to AQP9 (Alpha Diagnostics, San Antonio, Tex) at a dilution of 1:500. Horseradish peroxidase–coupled donkey antirabbit immunoglobulins were used at a dilution of 1:3000. Detection by chemiluminescence was performed using the ECL system (Amersham, Arlington Heights, Ill). After exposing the nitrocellulose sheets to Kodak XAR film, the autoradiographs were scanned and quantified by densitometry using the Molecular Analyst software (Bio-Rad, Hercules, Calif).

2.6. Statistical analysis

The results are expressed as means ± SE. The significance of the differences between the mean values of the groups was evaluated by Student *t* test or analysis of variance when appropriate. $P < .05$ was considered statistically significant for all tests.

3. Results

3.1. Physiological data

Plasma glucose and FFA levels measured after 2 weeks of treatment were significantly decreased in RSG-treated OLETF rats compared with nontreated OLETF rats ($P < .05$, each) (Table 2). There was a tendency for RSG-treated rats to gain more weight compared with control OLETF rats; however, the differences were not significant. OLETF rats were also significantly hyperinsulinemic compared with normal control LETO rats ($P < .05$), and the hyperinsulinemia of OLETF rats was markedly ameliorated by 2 weeks of RSG administration ($P < .01$).

3.2. Effects of RSG treatment on aquaglyceroporin and GlyK mRNA expression in the adipose tissue of OLETF rats

To evaluate the changes in AQP7 and GlyK expression in the glycerol metabolic pathway and the effect of TZD treatment on that pathway under a condition of insulin resistance, both AQP7 and GlyK mRNA expressions were analyzed in the mesenteric and epididymal adipose tissues from the experimental rats. AQP7 mRNA tended to increase by 2.5-fold in the mesenteric fat from OLETF rats compared with LETO rats (249.9% ± 55.0% vs 100.8% ± 4.0%, $P = .07$). Rosiglitazone treatment of OLETF rats caused a significant further increase in AQP7 mRNA expression in the mesenteric fat compared with untreated OLETF rats (423.5% ± 52.7% vs 249.9% ± 55.0% of the abundance in LETO, $P < .05$) (Fig. 1A). These changes were not significant in the epididymal fat (Fig. 1B).

Glycerol kinase expression in OLEFT rats showed a similar trend with AQP7 expression in the mesenteric fat (Fig. 1A). A small increase in GlyK mRNA expression was detected in the epididymal fat from OLETF rats compared with LETO rats (124.5% ± 3.3% of the abundance in LETO, $P < .05$) (Fig. 1B). Rosiglitazone treatment markedly increased GlyK mRNA expression in both the mesenteric and epididymal adipose tissues (1085.9% ± 216.9% and 523.8% ± 119.8% of the abundance in LETO, respectively) (Fig. 1).

The magnitude of GlyK induced by RSG was considerably greater than that of AQP7 in both adipose tissues

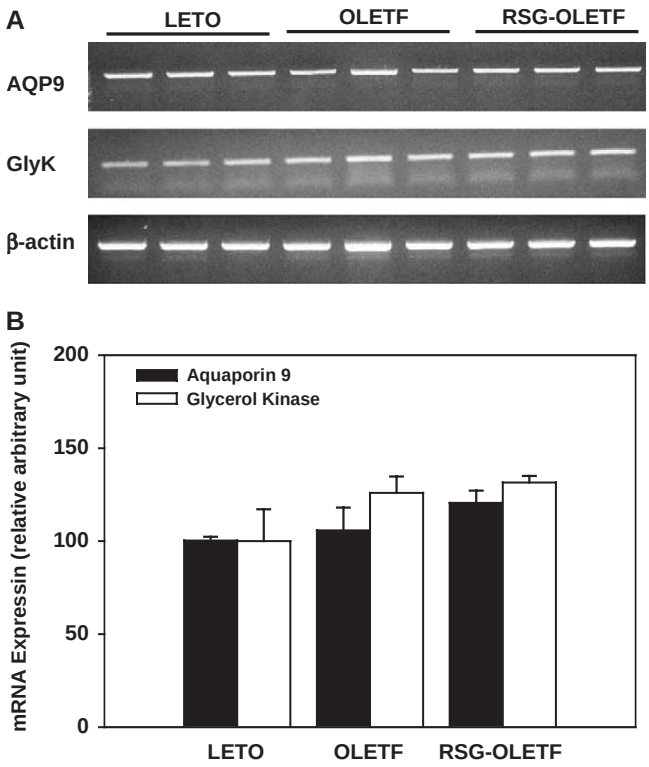


Fig. 2. Effect of RSG treatment on AQP9 and GlyK mRNA expressions in the hepatic tissue. RSG-OLETF rats were administered RSG (0.01% w/w) in normal rat chow diet for 2 weeks. Representative RT-PCR products of AQP9 and GlyK in the hepatic tissue are shown (A). Abundance of mRNAs was determined by densitometric analysis. Data were normalized using β-actin mRNA and expressed as a percentage of the value obtained from control chow-treated LETO rats. Each bar represents means ± SE ($n = 6$) (B).

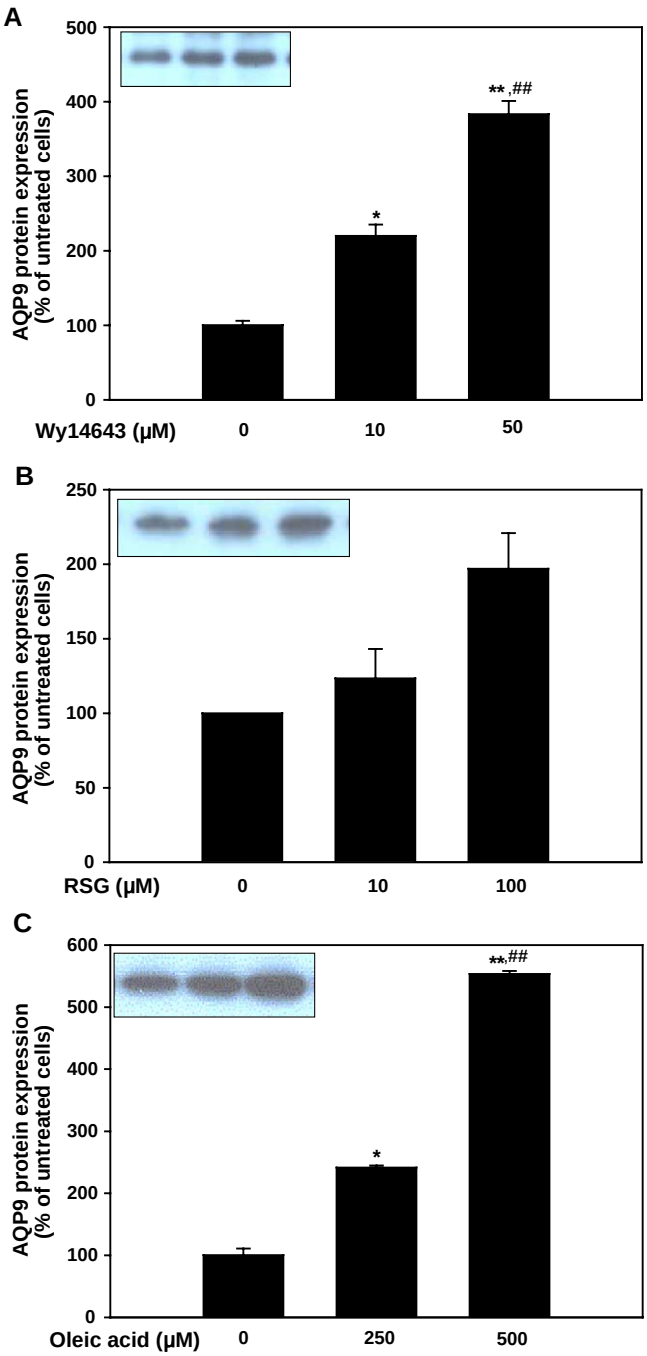


Fig. 3. Effects of Wy14643 (A), RSG (B), and oleic acid (C) on AQP9 protein expression in HepG2 cells. Cells were incubated with indicated agents at various concentrations. Twenty-four hours after incubation, the cells were lysed and used for Western blot analyses. Representative blots (inlet) and quantitative densitometric analyses are shown. Data are expressed as percentages of the value obtained from untreated HepG2 cells. The immunoblot results shown are representative of 3 separate experiments with the same protocol. Data are means ± SE. $^{*}P < .05$, $^{**}P < .01$ vs untreated HepG2 cells; and $^{\#}P < .05$, $^{\#\#}P < .01$ vs the cells incubated with the lower dose of an indicated agent by Student t test.

($P < .05$, each), and the magnitude of GlyK induced by RSG was significantly greater in the mesenteric fat than in the epididymal fat ($P < .05$).

3.3. Effects of RSG on aquaglyceroporin and GlyK mRNA expression in the liver from rats

AQP9 mRNA expression in the hepatic tissue showed no significant difference between OLETF rats and LETO rats. AQP9 in the liver was not upregulated by RSG treatment. Glycerol kinase expression in hepatic tissues showed no significant difference between the experimental groups (Fig. 2).

3.4. In vitro regulation of AQP9 protein expression in human HepG2 cells by Wy14643, RSG, and FFA

In the preliminary Western blot analyses for AQP9 of human HepG2, using rabbit antirat AQP9 IgG, a single clear band at the 29-kDa position was confirmed. Fig. 3 shows the effect of Wy14643, RSG, and oleic acid on AQP9 protein expression in HepG2 cells. Wy14643, a synthetic PPARα ligand, consistently upregulated AQP9 protein expression in HepG2 cells. Rosiglitazone tended to induce AQP9 protein expression in HepG2 cells. However, differences in the effect of different incubating doses of RSG were not significant. We also evaluated the effects of FFA, a significant mediator of insulin resis-

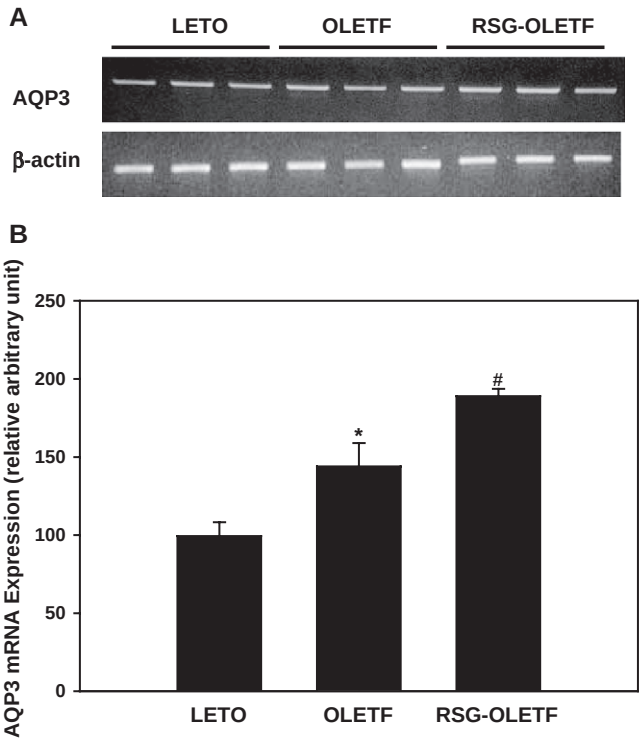


Fig. 4. Abundance of AQP3 mRNA in the medulla of OLETF rats treated with RSG. RSG-OLETF rats were administered RSG (0.01% w/w) in normal rat chow diet for 2 weeks. Representative RT-PCR products of AQP3 in the outer medulla are shown. Abundance of mRNAs was determined by densitometric analysis. Data were normalized using β-actin mRNA and expressed as a percentage of the value obtained from control chow-treated LETO rats. Each bar represents means ± SE ($n = 6$). $^{*}P = .075$ vs LETO group by Student t test. $^{\#}P < .05$ vs LETO group, $P = .088$ vs OLETF group by Student t test.

tance, on the expression of AQP9 in HepG2 cells. Incubation with 250 or 500 μmol/L oleic acid for 24 hours augmented the amount of AQP9 protein in a dose-dependent manner. In the cultured hepatoma cells, dexamethasone also increased the expression of AQP9, whereas glycerol itself had no effect on AQP9 protein levels (not shown).

3.5. Effects of RSG on AQP3 gene expression in the kidney

Because aquaglyceroporins have relatively broad solute specificity and sequence homology [9], and edema is a well-known adverse effect of TZD [27], we explored the effects of TZD treatment in OLETF rats on the expression of AQP3, another aquaglyceroporin expressed strongly in the kidney. Because the AQP3 transcript and protein are expressed particularly in the cortical and outer medullary collecting duct [28], we analyzed the renal cortex and outer medulla. AQP3 mRNA tended to increase in the outer medulla of the OLETF rat kidney compared with LETO rats (144.8% ± 14.9% vs 99.9% ± 8.9%, $P = .075$). RSG-OLETF rats also showed a tendency toward increased AQP3 gene expression compared with untreated OLETF rats (188.9% ± 4.6% vs 144.8% ± 14.9% of the abundance in LETO, $P = .088$) (Fig. 4). There was no difference in AQP3 gene expression in the renal cortex between the experimental groups (data not shown).

4. Discussion

The principal findings of the present study are as follows: (1) 2 weeks of RSG treatment markedly increased the abundance of GlyK mRNA in the mesenteric and epididymal adipose tissues of OLETF rats, with greater expression in the mesenteric fat. (2) The magnitude of AQP7 induction by RSG was different according to the deposit site of the adipose tissue, significant induction in the mesenteric fat, but not in the epididymal fat. (3) The magnitude of GlyK induction by RSG was considerably greater than that of AQP7 in both fat depots. (4) PPARα agonist and oleic acid upregulated AQP9 protein expression in cultured human hepatoma cells.

The present study indicates that the undesirable increase of adipose AQP7 in states of insulin resistance can be overcome through a marked induction of GlyK using TZD. Although both AQP7 and GlyK were upregulated by TZD in mesenteric adipose tissue from OLETF rats, the magnitude of GlyK induction was more than twice that of AQP7. Furthermore, in the epididymal fat of OLETF rats, TZD significantly induced GlyK mRNA expression, but had no effect on AQP7 expression. Although we did not measure the activity of GlyK in the adipose tissue, the mRNA level of GlyK was reported to correlate with its activity [21,29]. These results suggest that TZD exerts its antidiabetic action in the adipose tissue through the recycling of glycerol in addition to its various well-known actions including the enhancement of glyceroneogenesis [20]. In adipocytes, these TZD-related effects result in the decreased release of FFA from the adipose tissue [20,30].

AQP7 in the mesenteric fat and AQP9 in the liver were reported to be upregulated in db+/db+ mice [18]. However, in the present study, these AQPs were not significantly upregulated in both the adipose tissue and liver from OLETF rats, although AQP7 in the mesenteric fat tended to increase. This may be due to differences in species or in the severity of insulin resistance.

Location of the fat depot may also determine the magnitude of AQP7 and GlyK expression, and tissue response to TZD. The marked increase in GlyK induction by TZD in the epididymal fat of OLETF rats was similar in degree to that of other animal models previously reported [21]; however, studies on mesenteric fat are lacking. In the present study, the magnitude of GlyK induction by RSG was significantly greater in the mesenteric fat than in the epididymal fat. To our knowledge, the present study represents the first report that shows TZD has a more significant effect on GlyK than on AQP7 in adipose tissues, especially in the mesenteric fat, of OLETF rats.

One limitation of the present animal study was that the effects of TZD administration were not evaluated in LETO rats, a normal control group of OLETF rats. This was because of their limited availability. However, RSG was previously shown to induce the GlyK gene in adipose tissues of lean mice and lean Sprague-Dawley rats [21].

AQP9 in the liver was not significantly affected by TZD in the present study. A relatively lower expression of PPARγ in the liver than in the adipose tissue may explain this result [31]. Although AQP3 was reported to be expressed in hepatocytes, its functional role in the liver has yet to be determined [32]. In the present study, AQP3 mRNA expression was upregulated in the liver from OLETF rats compared with LETO rats. Rosiglitazone treatment did not affect the abundance of AQP3 mRNA in the liver (data not shown). Further studies on the degree of AQP3 expression, its localization, and its functional role in hepatocytes are required.

To evaluate the factors that modulate the expression of AQP9 in hepatocytes, we treated cultured HepG2 cells with oleic acid, Wy14643, and RSG. Free fatty acid promotes diabetes partly by stimulating hepatic gluconeogenesis and glucose output [3]. Oleic acid, a monounsaturated FFA, upregulated AQP9 protein expression in HepG2 cells in a dose-dependent manner. Also, Wy14643 consistently augmented the amount of AQP9 protein. Because fatty acids cause insulin resistance and are able to activate gene expression via PPARs [33,34], AQP9 induction by oleic acid and Wy14643 may be important.

Although AQP3-mediated water transport across the basolateral membrane of renal collecting duct epithelium

is important for the formation of concentrated urine [11,12], whether fluid retention occurs if renal expression of AQP3 increases has yet to be determined. Considering that AQP3 and AQP7 are both aquaglyceroporins and that AQP7 is upregulated by TZD, edema and fluid retention caused by TZD treatment in type 2 diabetics may be related to renal AQP3. Renal expression of PPARγ, a target of RSG, was confirmed in the medulla of kidneys from male Sprague-Dawley rats [35]. Therefore, we evaluated the effect of TZD on renal AQP3 expression. In the present study, AQP3 mRNA tended to increase in the outer medulla of kidney from RSG-OLETF rats. In accordance with this result, it was recently reported that a 3-day TZD treatment in male Sprague-Dawley rats increased the whole kidney protein abundance of AQP3 [36]. These findings suggest that AQP3 partly contributes to the development of edema in TZD-treated diabetic patients.

In conclusion, our results indicate that, by regulating AQP7 and GlyK expression in adipose tissues, TZD has an important role in the glycerol metabolic pathway. Glycerol kinase induction is more important than AQP7 upregulation in mediating these effects. Free fatty acid may directly enhance glycerol availability in the liver by upregulating AQP9. Renal AQP3 may be related to the fluid retention caused by TZD.

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