Coordinated Regulation of Fat-Specific and Liver-Specific Glycerol Channels, Aquaporin Adipose and Aquaporin 9

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Plasma glycerol is a major substrate for hepatic gluconeogenesis. Aquaporin adipose (AQAPap/7), an adipose-specific glycerol channel, provides fat-derived glycerol into plasma. In the present study, we cloned the coding and promoter regions of mouse aquaporin 9 (AQP9), a liver-specific glycerol channel. Fasting and refeeding of mice increased and decreased hepatic AQP9 mRNA levels, respectively. Insulin deficiency induced by streptozotocin resulted in increased hepatic AQP9 mRNA. These changes in hepatic AQP9 mRNA were accompanied by those of hepatic gluconeogenic mRNAs and plasma glycerol levels. In cultured hepatocytes, insulin downregulated AQP9 mRNA. The AQP9 promoter contained the negative insulin response element TGTTTTC at −496/−502, similar to the promoter of the AQAPap/7 gene. In contrast, in insulin-resistant db/db mice, AQPap/7 mRNA in fat and AQP9 mRNA in liver were increased, despite hyperinsulinemia, with high plasma glycerol and glucose levels. Glycerol influx in the db/db mice augmented hepatic glucose output. Our results indicate that coordinated regulations of fat-specific AQAPap/7 and liver-specific AQP9 should be crucial to determine glucose metabolism in physiology and insulin resistance. Diabetes 51: 2915–2921, 2002

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he precise mechanism for the common association between obesity and diabetes remains unresolved. Adipose tissue supplies lipolysis-derived free fatty acid (FFA) and glycerol to other organs in various physiological conditions, such as diet restriction and physical exercise (1–4). We and others have reported that accumulation of intra-abdominal visceral fat is closely associated with increased incidence of metabolic complications in obesity (5–9). Higher influx of FFA into the liver, especially from accumulated visceral fat, drives the hepatic synthesis of triglyceride, leading to fatty liver and hypertriglyceridemia (10–12).

Glycerol, another product of lipolysis, is an important substrate for hepatic gluconeogenesis (13–15). As the substrate for hepatic glucose production, glycerol accounts for 90% in the prolonged fasting state and ~50% in the postabsorptive state in rodents (16,17). In humans, it was estimated that 20% of the gluconeogenesis was mediated by glycerol after 60 h of fasting (13,18). Higher influx of glycerol into the liver from the accumulated fat may be relevant to the development of diabetes in obesity. The molecular basis for the secretion and uptake of glycerol has been characterized recently. Aquaporins (AQPs), which are channel-forming integral proteins, function as water channels (19). To date, at least 10 AQPs have been identified (20). Functional studies have distinguished the members of the AQP family into two subgroups: AQPs that are selective water channels and aquaglyceroporins that transport glycerol as well as water (21). We recently cloned a novel adipose-specific glycerol channel, designated aquaporin adipose (AQAPap/7) (22), and reported that this protein functions as a glycerol channel from fat to blood (23–25). Recently, a liver-specific aquaglyceroporin was also identified and named AQP9 (26). The significance and regulation of AQP9 in physiological and pathophysiological conditions have not been elucidated.

In the current study, we demonstrate the inhibitory effect of insulin on the expression of the AQP9 gene in normal liver through the negative insulin response element (IRE) in its promoter region (27), similar to the regulation of AQAPap/7 in adipose tissue (23,24). These coordinated regulations of AQAPap/7 and AQP9 should account for the physiological suppression of glycerol release from fat and gluconeogenesis in liver by feeding. We also show a dysregulated increase of AQP9 in the liver and AQAPap/7 in the adipose tissue of insulin-resistant hyperinsulinemic mice. Higher amounts of glycerol, released by increased function of AQAPap/7, should be taken up into hepatocytes more efficiently via enhanced AQP9, leading to augmentation of hepatic gluconeogenesis. The coordinated changes in two tissue-specific glycerol channels, adipose AQAPap/7 and hepatic AQP9, may determine, at least in part, plasma glucose levels during nutritional alterations as well as in insulin-resistant conditions.

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AQAP, aquaporin; AQAPap/7, aquaporin adipose; DMEM, Dulbecco’s modified Eagle’s medium; FFA, free fatty acid; GlyK, glycerokinase; HSL, hormone-sensitive lipase; IRE, insulin response element; IRS, insulin receptor substrate; PEPCK, phosphoenolpyruvate carboxykinase; RACE, rapid amplification of cDNA ends; STZ, streptozotocin.
RESEARCH DESIGN AND METHODS

Materials and general methods. Plasma glycerol was measured by a fluorometric enzyme method (22–25). FFA was determined using Nescauto NEFA kit (Arzella, Osaka, Japan). Glucose and insulin were measured by a Blood Sugar Glucose Oxidase Perid Method kit (Roche Diagnostic, Tokyo, Japan) and a double-antibody enzyme immunoassay using a Glazyme Insulin EIA kit (Sanyo Chemical Industries, Osaka, Japan) with rat insulin as a standard, respectively.

Animals and cells. All experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Osaka University Graduate School of Medicine. Eight-week-old male ICR (MCH) mice, C57BL/6I, C57BL/KsJ and C57BL/6J mice (db/db) mice were purchased from Clea Japan, Osaka, Japan. The animals were housed in an air-conditioned room with a 12/12 h-dark-light cycle (8:00 a.m.–8:00 p.m.) and acclimated to the new environment for 1 week before the experiment. For experiments on insulin deficiency, streptozotocin (STZ, Sigma Aldrich, Tokyo, Japan) or PBS was administered intraperitoneally (100 mg/kg in 0.05 ml citrate buffer, pH 4.5) into 9-week-old male ICR (MCH) mice. Three days later, both groups of mice were anesthetized with 5 mg/ml pentobarbital sodium to be killed.

For experiments designed to study the effects of fasting and refeeding, male C57BL/6J mice (each group, n = 4) were allowed free access to standard laboratory diet and tap water for 12 h after a 24-h fast. The fasted group was fasted for 12 or 18 h before being killed. The refed group had free access to standard laboratory diet for 12 h after an 18-h fast. For comparison between the observed and control groups, all and C57BL/KsJ (db/db) and C57BL/6J mice on 3 h fasting were used. All mice were phlebotomized from the tail and portal vein.

A rat hepatoma H4IE cell line (passage no. 6) was obtained from Dainippon Pharmaceutical (Osaka, Japan). H4IE rat hepatoma cells were grown as monolayer cultures in low-glucose Dulbecco’s medium (1.0 g/l) supplemented with 10% FCS and incubated in a humidified 5% air–5% CO2 atmosphere at 37°C until they reached confluence (60–70%), when they were collected for analysis (28). H4IE hepatoma cells (preconfluent state) were washed twice with calcium- and magnesium-free PBS and incubated with Dulbecco’s modified Eagle’s medium (DMEM) containing 0.5% fatty acid free BSA (Sigma) for 12 h. After cells were washed twice with PBS, they were incubated in DMEM with 10 nmol/l insulin for 0, 6, or 24 h or with 0, 10, or 100 nmol/l insulin for 24 h. Total RNA was isolated and subjected to Northern blotting for AQP9.

Isolation of mouse AQP9 gene. The primer was designed to amplify the coding region of mouse AQP9, according to the sequence of rat AQP9 (AP016406) (26), which was cloned from rat. Forward and reverse primers were used for specific PCR amplification: 5’-GGCAGAACCCCAAAAGTGGCAGGCTGTC-3’ and 5’-ACACCCACACCATGTTAG-3’. RT-PCR was performed by using the template of mouse liver mRNA. Determination of 5’ and 3’ end of mouse AQP9 by 5’ and 3’ rapid amplification of cDNA ends PCR. For determining the 5’ and 3’ ends of mouse AQP9, the 5’ and 3’ end rapid amplifications of the cDNA ends (5’ and 3’ RACE) were conducted according to the protocol provided by the manufacturer (Marathon cDNA amplification kit; BD Biosciences Clontech Japan, Tokyo, Japan). Using the following primers: first primer 5’-TTCTTCTTGCGTCGTGTTTGTTT-3’ and nested primer 5’-CTTGGGCTTTTCTGCAAGGATAGTTCTCCCACCA-3’, for 5’ RACE; and first primer 5’-GAACGGGAGGAAAACTGATGAAATCACA-3’ and nested primer 5’-AAGAAAAACACGCAAATGCACTCTAG-3’ (italized translation stop codon AGT), and nested primer 5’-CTTGTTGTTTTTCTGCAAGGATAGTTCTCCCACCA-3’, for 3’ RACE, respectively.

Isolation of mouse AQP9 promoter. Cloning of the 5′ flanking region and promoter of the mouse AQP9 gene was performed using the GenomeWalker kit (BD Biosciences Clontech Japan) according to the protocol recommended by the manufacturer, with the following primers: primer 5′-TGGCAGGACTCTGTC-3′, nested primer 5′-AGAGAGGCTGTTCGTGTTTTGCTC-3′, 5′-TTCTTCTTGTGCTGTTTCAAGGACATCCTCA-3′ (italized translation start codon ATG), and nested primer 5′-TCCTTCTTGCGTCGTGTTTGTTT-3′ and first primer 5′-GAACGGGAGGAAAACTGATGAAATCACA-3‘ and nested primer 5′-AGAAAAACACGCAAATGCACTCTAG-3’ (italized translation stop codon AGT), for 3′ RACE, respectively.

Luciferase assay. The mouse AQP9 promoter regions (−667/−1) were amplified from mouse genomic DNA using a Mlu I site-added 5′ primer and XhoI site-added 3′ primers. The mouse AQP9 promoter-luciferase reporter plasmids were constructed by excising the amplified promoter fragment of AQP9 and inserting it into the Mlu I and XhoI site of the control pGL3 basic luciferase expression vector (Promega, Madison, WI). Partial deletion mutant of pGL3 basic luciferase plasmid was constructed using the QuickChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). IRE-deleted constructs (∆IRE) were designed from the wild-type construct by deleting the IRE region (−502/−496). The plasmids for transfection were purified using the EndoFree Plasmid kit (Qiagen, Valencia, CA).

Primary hepatocytes were isolated from nonfasting male Sprague-Dawley rats (approximate body weight 250 g), as described previously (29). Briefly, the prepared hepatocytes were attached to a 12-well plate in DMEM supplemented with 10% FCS and 100 nmol/l triiodothyronine. Four hours later, the medium was replaced with Medium 199 (M199) supplemented with 100 nmol/l dexamethasone, 100 nmol/l triiodothyronine, and 1 nmol/l insulin. For each 12-well culture plate, 50 ng of firefly (Photinus pyralis) luciferase plasmid, construct derived from pGL3-basic luciferase expression vector of a sea pansy (Renilla reniformis) luciferase pRL-SV40 plasmid (Promega), were complexed with LipofectAMINE2000 (Life Technologies, Tokyo, Japan) following the protocol provided by the manufacturer and then used for transfection. The transfection mixture was removed 24 h after transfection, and the cells were maintained in M199 containing the indicated concentration of insulin. The cells were harvested with passive lysis buffer (Promega), and luciferase activity was measured using the Dual Luciferase Assay System (Promega) using the procedure described by the manufacturer.

Construction of cDNA probes and RNA analysis. Mouse AQPap7, AQP9, glycerokinase (GlyK), phosphoenolpyruvate carboxykinase (PEPCK), and hormone-sensitive lipase (HSL) cDNAs were synthesized by RT-PCR as reported previously (22,30). Insulin receptor substrate (IRS)-1 or -2 cDNA probe was described previously (30). Total cellular RNA was extracted using TRIZOL reagent kit (Invitrogen, Tokyo, Japan). Northern blot analysis was performed as described previously (23). Abundance of mRNAs was determined by densitometric analysis using FastScan Scanning Imager (Molecular Dynamics, Buckinghamshire, U.K.) and expressed in arbitrary units.

Immunoblot of AQPap. Immunoblot analysis of AQPap7 protein was performed as reported previously (22). Briefly, the membrane fractions of adipose tissue were obtained from rat perirenal fat pads (40 μg) were loaded and separated on a 12.5% SDS–polyacrylamide gel and transferred onto nitrocellulose transfer membrane (Schleicher & Schuell, Keene, NH). Western blot analysis was performed using antiserum to AQPap7 at a dilution of 1:500. Horseradish peroxidase–coupled donkey anti-rabbit immunoglobulins were used at a dilution of 1:3,000. Detection by chemiluminescence was performed using the ECL system (Amersham Pharmacia Biotech, Tokyo, Japan).

Mouse liver perfusion studies. Mouse liver perfusion studies were performed as described previously, with a minor modification (31,32). After 18 h of fasting, the male C57BL/6J (db/db) and (db+/+m) mice (n = 4, each) were anesthetized with an intraperitoneal injection of 5 mg/ml pentobarbital sodium. The abdominal cavity was opened, and the superior mesenteric artery and bilateral renal arteries and veins were ligated. Then, 0.25 ml (25 units) of heparin was injected into the inferior vena cava, and the liver was perfused via the portal vein at a constant flow rate of 4 ml/min. The perfusate was albumin-free Krebs buffer (in mmol/l: NaCl 120, K HPO O 1.2, MgSO 1.0, H O, 1.2, CaCl O, 1.2, KCl 4.8, CaCl O, 1.7, and Na HCO 3) at 37°C, buffered with 95% O–5% CO gas mixture. The concentration of glycerol infused into the portal vein was increased every 20 min from 200 to 400, 600, and 800 μmol/l. A recovery curve was inserted into the inferior vena cava, and the bile was collected every 10 min after perfusion for 30 min and stored at −80°C for analysis. At the end of perfusion, the whole liver was removed and weighed.

Glucose concentration in the recovery samples was measured using the Blood Sugar Glucose Oxidase Perid Method kit.

Statistical analysis. All data were expressed as mean ± SE. Differences between groups were examined for statistical significance using the Student’s t test. P < 0.05 denoted the presence of a statistically significant difference.

RESULTS

Cloning of the mouse AQP9 gene. To investigate the regulation of AQP9, we cloned mouse AQP9 cDNA from the mouse liver. Figure 1 illustrates the amino acid alignment of AQP9 among mouse, rat, and human. The mouse AQP9 cDNA encodes a 295 amino acid (GenBank accession no. AB 037180) and shows 90% homology to rat AQP9 (GenBank accession no. AF 016406) (26) and 76% homology to human AQP9 (GenBank accession no. XM 012421) (33,34). Hydropathy analysis showed that mouse AQP9 has six putative transmembrane domains, similar to human and rat AQP9. Mouse AQP9 has two Asn-Pro-Ala consensus motifs, characteristic of members of the major intrinsic protein family (20).

Effect of fasting/refeeding on AQP9, GlyK, and PEPCk mRNA expressions. Fasting activates lipolysis and glycerol release from adipose tissue (13). We recently
reported that AQPap mRNA expression in white adipose tissue was enhanced by fasting and suppressed by refeeding and that the changes in AQPap mRNA showed a pattern similar to that of plasma glycerol (23). Glycerol becomes a substrate for gluconeogenesis in the liver that expresses GlyK (35). Figure 2 shows the effects of fasting and refeeding on the regulation of hepatic mRNAs of AQP9 and GlyK. Portal plasma levels of glycerol and FFA increased during fasting and decreased after refeeding (Fig. 2A). In contrast, portal plasma insulin levels decreased during fasting and increased after refeeding (Fig. 2A). The mRNA levels of AQP9 and GlyK increased significantly by fasting and decreased by refeeding, similar to the change in PEPCK mRNA (Fig. 2B) (36), which is known to be downregulated by insulin. The coordinated enhancements of AQPs (22), GlyK, and PEPCK are effective to supply plasma glycerol for hepatic gluconeogenesis in fasting. Similar effects of fasting on these hepatic mRNAs were also seen when fasting started at 0:00 h.

Effect of insulin on AQP9 mRNA expression in mouse liver and H4IIE cells. Next, we examined the regulation of hepatic AQP9 mRNA by insulin (Fig. 3). Hepatic mRNA levels of AQP9 and GlyK were high in insulin-deficient STZ-treated mice, similar to that of PEPCK mRNA (Fig. 3A). Furthermore, insulin downregulated the expression levels of AQP9 mRNA in H4IIE hepatoma cells in dose- and time-dependent manners (Fig. 3B). These results suggested that the AQP9 gene expression is directly downregulated by insulin, similar to PEPCK mRNA.

Promoter of mouse AQP9 gene. The 5′ flanking region of the mouse AQP9 gene was sequenced (GenBank accession no. AB 073723), and transcriptional initiation site of the AQPap/7 gene was determined by 5′RACE using total RNA isolated from mouse liver (Fig. 4A). A search of the promoter region of the AQP9 gene for canonical consensus sequences revealed the presence of several putative binding sites for transcription factors (Fig. 4A). Several binding sites for CCAAT enhancer binding protein and hepatic nuclear factor-3 were identified in the promoter (23).

Negative IRE in the mouse AQP9 gene promoter. In the mouse AQP9 gene promoter, we identified a region similar to the core negative IRE, (T/G)TTTT(T/G) (27,37), which is also found in promoters of various genes, including PEPCK (38) and AQPap/7 (23,24) (Fig. 4B). For
determining whether the putative IRE is a specific region required for insulin-mediated repression of AQP9 transcription, deletion mutant of the IRE in the mouse AQP9 promoter was subcloned into luciferase vectors (Fig. 4C). The wild-type construct containing the native −667/−1 regions showed 45% inhibition of luciferase activity after treatment with insulin (Fig. 4C). In contrast, the construct lacking IRE was completely resistant to the inhibitory effect of insulin on promoter activities as a result of the reduced basal promoter activity. Insulin suppressed the wild-type luciferase activity in rat primary hepatocytes in a dose-dependent manner (Fig. 4D). In the absence of insulin, the wild-type AQP9 promoter produced a higher luciferase activity than the deletion mutant (ΔIRE) promoter. In the presence of insulin, the activity of the wild-type AQP9 promoter was reduced to the level of the mutant promoter, which was not affected by insulin. Next, we examined the point mutation analysis (Fig. 4E). The activity of the wild-type AQP9 promoter was reduced to 55% in the presence of insulin, similar to the results in Fig. 4C. Each mutation in base pair 2 (G→C) and 3 (T→A) of the heptanucleotide sequence completely blocked insulin-sensitive repression of mouse AQP9 transcription (Fig. 4E), similar to the results of mouse and human AQP9 promoters that we recently reported (23,24). These results confirm that the IRE sequence (−502/−496) is required for insulin-mediated repression of mouse AQP9 transcription.
Insulin-mediated suppression on the transcription of the mouse AQP9 gene.

**Metabolic profile and expression of AQPap/7 and AQP9 mRNAs in db/+db+ mice.** The db/+db+ mice were significantly insulin resistant. Plasma glycerol levels in the portal vein were higher in db/+db+ mice. They had a higher concentration of plasma glucose despite severe hyperinsulinemia (Fig. 5A). Figure 5B and C demonstrates the results of Northern blotting of mesenteric fat and liver, respectively. The mRNA and protein amounts of AQPap/7 and HSL mRNA were higher in the mesenteric fat of db/+db+ mice (Fig. 5B), although both are negatively regulated by insulin. The hepatic AQP9 mRNA was also induced despite hyperinsulinemia (Fig. 5C). Hepatic insulin resistance was accompanied by markedly reduced IRS-2 (Fig. 5C), as reported previously (30).

**Hepatic glucose production from glycerol in control and db+/db+ mice.** Glycerol is the main gluconeogenic substrate in prolonged fasting (15-17). Hepatic glucose production from glycerol was compared between db+/db+ and db+/+m mice in the 18-h fasted state using a previously described hepatic perfusion procedure (31,32). Glucose production increased in parallel with the amount of glycerol infused into the portal vein in db+/+m and db+/db+ mice (Fig. 6). Glucose output was significantly higher in the liver of db+/+m mice than db+/m mice. These results indicated that the glycerol-mediated gluconeogenic activity was augmented in the liver of db+/db+ mice (39), where the mRNAs of AQP9 and gluconeogenic enzymes were augmented.

**DISCUSSION**

Glycerol is one of the major factors that determine plasma glucose (40). During fasting, hepatic glucose output is the main source of plasma glucose, and glycerol taken up from plasma becomes a major substrate for hepatic gluconeogenesis (14). However, adipose tissue is the major source of plasma glycerol (13). Therefore, effective systems are required during fasting that enhance glycerol release from adipose tissue and glycerol uptake into the liver. With regard to the glycerol transport proteins, three aquaglyceroporins are known: AQPap/7, AQP3, and AQP9 (20,22). To date, AQPap/7 is the only aquaglyceroporin expressed in adipose tissues, and AQP9 is the only aquaglyceroporin expressed in the liver (22). It is conceivable that during fasting, AQPap/7 in fat is essential for the supply of glycerol to the liver and that AQP9 is critical for hepatic uptake of glycerol for glucose production.

Insulin suppresses the amount of AQPap/7 mRNA in adipose tissue (23) and AQP9 in liver (current study). We also identified similar negative IREs in the promoter regions of AQPap/7 (23,24) and AQP9 genes (current study). We identified the potential IRE sequence (−591/−597; TGTTTTC) in the promoter region of the human AQP9 gene (33). These results indicate that the gene expressions of AQPap/7 and AQP9 are regulated coordinately by the plasma concentrations of insulin in accordance with the nutritional condition, such as fasting and refeeding. These regulations should increase or decrease the supply of glycerol and glucose in plasma by fasting or refeeding, respectively.

In hepatic gluconeogenesis, AQP9, which is localized at the sinusoidal plasma membrane (41), serves as a channel for glycerol uptake. GlyK converts the incorporated glycerol into the phosphorylated form to be used for gluconeogenesis, and PEPCK is one of the limiting enzymes to drive the glycerol-independent gluconeogenic pathway (2). In the normal liver, the mRNAs for all of these proteins
are downregulated by insulin (27,37). During fasting, the low concentration of plasma insulin coordinately and effectively enhances all of these mRNAs to drive hepatic gluconeogenesis. However, under pathological conditions of insulin resistance in the db/db mice, high mRNA expression levels of hepatic AQP9, GlyK, and PEPCK mRNAs and of adipose AQPap/7 were found despite the high concentration of plasma insulin. Dysregulated increase of AQPap/7 and hepatic AQP9 may underlie the pathophysiology of hyperglycemia in severe insulin resistance. Our recent studies indicated that the dysregulated increase of AQPap/7 in adipose tissues causes higher plasma glycerol in insulin-resistant rodents (22). The liver of insulin-resistant db/+db+ mice exhibited a high gluconeogenic activity by using glycerol as a substrate (Fig. 6). Hepatic insulin resistance may be accounted for by the significantly low levels of IRS-2. We previously demonstrated that chronic hyperinsulinemia in lipodystrophy and ob/ob mice was associated with low hepatic IRS-2, which resulted in increased mRNAs of gluconeogenesis, such as glucose-6-phosphatase and PEPCK (30). There were no apparent changes in adipose mRNAs for IRS-1 and IRS-2 between obese and lean mice (data not shown). In the previous articles, IRS-1–mediated insulin signaling was shown to be deteriorated in the adipose tissue of insulin-resistant animals, with relatively normal IRS-2–mediated pathway (42,43). We previously showed that insulin’s inhibition of AQPap/7 was mediated by a phosphatidylinositol 3-kinase–dependent manner (23). Taken together, the increase of AQPap under insulin resistance may be caused by the decreased IRS-1–mediated insulin signaling in the adipose cells. In the present study, it is conceivable that reduced hepatic IRS-2 in db/+db+ mice resulted in disturbance of insulin signaling, which led to an increase in AQP9 and hepatic glycerol uptake. In the insulin-resistant state, both the level of plasma glycerol and the glucose production associated with glycerol input were increased, which might lead to the more severe hyperglycemia. Coordinated augmentation of AQPap/7 and AQP9 in insulin resistance should increase the supply of fat-derived glycerol as a substrate for hepatic gluconeogenesis, which aggravates hyperglycemia.

Figure 7 represents a summary of the working model based on the results of our previous (21–25) and present studies. In the physiological feeding state, high plasma insulin coordinately suppresses the AQPap/7 mRNA for glycerol release from fat and AQP9 mRNA for glycerol uptake into the liver, through the negative IRE in AQPap/7 and AQP9 gene promoters. In contrast, in the insulin-resistant state, reciprocal increases of AQPap/7 and AQP9 despite hyperinsulinemia lead to more utilization of the fat-derived glycerol for hepatic glucose production and release. Identifying the responsible transcriptional factors and associated proteins involved in the transcriptional regulation of AQPap/7 and AQP9 genes and defining the precise mechanism that modifies the amounts and activities of these proteins by insulin should provide pivotal insight for the design of new pharmaceutical strategies to combat insulin resistance syndrome.

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REFERENCES


43. Esposito DL, Li Y, Cama A, Quon MJ: Tyr(612) and Tyr(632) in human insulin receptor substrate-1 are important for full activation of insulin-stimulated phosphatidylinositol 3-kinase activity and translocation of GLUT4 in adipose cells. *Endocrinology* 142:2833–2840, 2001