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Review

Aquaporins and glycerol metabolism

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Abstract

The discovery of aquaporin (AQP) has made a great impact on life sciences. AQPs are a family of homologous water channels widely distributed in plants, unicellular organisms, invertebrates, and vertebrates. So far, 13 AQPs have been identified in human. AQP3, 7, 9, and 10 are subcategorized as aquaglyceroporins which permeabilize glycerol as well as water. Many investigators have demonstrated that AQPs play a crucial role in maintaining water homeostasis, but the physiological significance of some AQPs as a glycerol channel is not fully understood. Adipose tissue is a major source of glycerol and glycerol is one of substrates for gluconeogenesis. This review focuses on recent studies of glycerol metabolism through aquaglyceroporins, and briefly discusses the importance of glycerol channel in adipose tissues and liver.

Keywords: Aquaporin; Adipocyte; Glycerol; Gluconeogenesis; Obesity; Lipolysis

Contents

1. Introduction

In adipocytes, lipogenesis and lipolysis are observed in response to whole body energy balance. Adipose tissue stores triglyceride (TG) in case of excess of nutrition, while adipose tissue provides free fatty acid (FFA) and glycerol to the energy expenditure organs by hydrolyzing TG under starvation and/or

Abbreviations: AQP, aquaporin; BAT, brown adipose tissue; FFA, free fatty acid; GLUT4, glucose transporter 4; HSL, hormone sensitive lipase; TG, triglyceride; WAT, white adipose tissue

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sympathetic nerve-activation state [1]. With regard to the history of human beings, starvation is a great matter of life or death. Adipocytes play a crucial role in energy supply under starvation to maintain energy homeostasis, and contribute to the survival of human beings during long starvation periods. However, at the present day, over-nutrition and lack of exercise cause over-accumulation of fat, especially in industrial countries [2–4]. This socio-environmental change causes and increases life-style-related diseases, such as diabetes, hyperlipidemia, hypertension, and atherosclerosis, that are commonly recognized as metabolic syndrome [5,6]. Fat accumulation, especially intra-abdominal fat deposits, is considered to be located in the pathological upstream of metabolic syndrome [7–10]. Consistent with the concept of metabolic syndrome, many investigators have tried to clarify the underlying mechanism of obesity. To find a novel therapy for metabolic syndrome, it is necessary to focus on the biology and science of adipocyte, that authors named as "Adiposcience". Recent progress in this field shows that the pathogenesis of metabolic syndrome is associated with adipocyte dysfunction [11].

Adipocytes hydrolyze TG and rapidly liberate FFA and glycerol into the circulation. It is presumed that glycerol channel in adipocytes prevent acute rise in intracellular osmotic pressure when glycerol production is rapidly increased during lipolysis. However, the underlying mechanism responsible for glycerol release from adipocytes remains elusive. We identified aquaporin (AQP) 7 from the human adipose tissue cDNA library in 1997 [12].

In this review, we assess research conducted on adipose-specific glycerol channel AQP7 and liver-specific glycerol channel AQP9. We also discuss the relation between glycerol metabolism and AQPs, focusing mainly on analysis of AQP7-deficient mice.

2. Characteristics of adipocytes

Adipose tissue is considered an energy storage organ where lipogenesis and lipolysis occur in response to whole body energy balance (Fig. 1). Adipocytes have a unique feature; lipid droplets occupy a large part of the intracellular region, while the nucleus and cytosome are located in the periphery. In comparison, the nucleus is located in the center of cell in other tissue cells. Thus, adipocytes are morphologically characterized by TG accumulation.

β-cells in the pancreas secret insulin in response to a rise in plasma glucose concentration at feeding state. Insulin acts on adipose tissues as well as skeletal muscles, transfers glucose transporter 4 (GLUT4) to plasma membrane, and takes glucose into cell [13]. In addition, insulin activates lipoprotein lipase (LPL) located on the cell surface of the vascular endothelium. LPL removes fatty acids from intestine-derived chylomicron (CM) and liver-derived very low-density lipoprotein (VLDL), and then fatty acids are taken into adipocytes [14]. In adipocytes, glycerol-3-phosphate (glycerol-3-P) converted from glucose and fatty acids are esterified into TG. Fatty acid binding protein (FABP) [15], fatty acid translocase (FAT) [16,17], and fatty acid

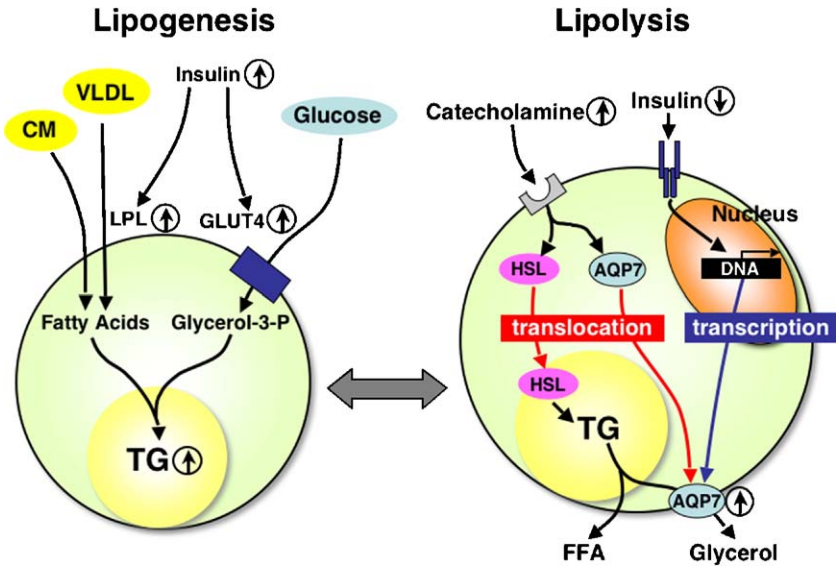

Fig. 1. A model illustrating lipogenesis and lipolysis in adipocytes. Under lipogenic conditions, insulin binds to the insulin receptor located on the surface of adipocytes and transfers glucose transporter 4 (GLUT4) to the plasma membrane, and takes glucose into the cell. Intracellular glucose is converted to glycerol-3-phosphate (Glycerol-3-P). Insulin also activates lipoprotein lipase (LPL) located on the cell surface of the vascular endothelium. Activated-LPL removes fatty acids from intestine-derived chylomicron (CM) and liver-derived very low-density lipoprotein (VLDL), and then fatty acids are taken into adipocytes. Fatty acids and glycerol-3-P are esterified into triglyceride (TG). Under lipolytic conditions, sympathetic nerves are activated and catecholamines are increased. Catecholamines stimulate adrenergic receptors located on the surface of adipocytes. Activation of adrenergic receptors translocates hormone sensitive lipase (HSL), which is a key enzyme hydrolyzing TG to FFA and glycerol, to the lipid droplets, while related stimuli moves AQP7 to the plasma membrane. FFA and glycerol are released into the bloodstream and utilized for thermogenesis and gluconeogenesis, respectively. Moreover, AQP7 mRNA levels are elevated by the decrease of insulin signaling cascade. Thus, long-term regulation of AQP7 is under the control of insulin while short-term regulation is under catecholamines. These two different regulatory pathways of AQP7 assure the efficient release of glycerol from adipocytes under fasting conditions.

transporter protein (FATP) [17,18] are recognized as fatty acid transporters in adipocytes.

In contrast to the feeding state, exercise and/or fasting induces lipolysis in adipocytes [19]. Fasting stimulates sympathetic nerves and elevates catecholamines, such as adrenaline and noradrenalin, which in turn stimulate adrenergic receptor located on the surface of adipocytes. Activation of adrenergic receptor results in converting adenosine 5′-triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Elevation of intracellular cAMP activates hormone-sensitive lipase (HSL) by phosphorylation. Phosphorylated-HSL hydrolyzes TG to FFA and glycerol, and both are released into the bloodstream. FFA and glycerol are utilized for thermogenesis and gluconeogenesis, respectively.

3. Adipose-specific glycerol channel; AQP7

3.1. Molecular cloning and tissue distribution of AQP7

We analyzed the gene expression profile of human visceral and subcutaneous fat to clarify the molecular mechanism of obesity-related diseases [20]. We identified a novel complementary DNA (cDNA) belonging to the AQP family during this analysis, and named it aquaporin adipose (AQPap) because its mRNA is expressed abundantly in adipose tissues and adipocytes [12]. AQPap is a human counterpart of AQP7 that was independently cloned from rat testis by another group at the same time [21]. Fig. 2 demonstrates the tissue distribution of AQP7 in mice. AQP7 is highly expressed in white adipose tissue (WAT), brown adipose tissue (BAT), and testis. Furthermore, a weak expression of AQP7 is also observed in the heart, skeletal muscles, and kidneys. As shown in Fig. 2, the transcript of testis AQP7 forms short length in comparison with the other organ AQP7, which is accounted for the different lengths of the untranslated region of cDNA locating at 3′-end [22].

3.2. Functional analysis and regulation of AQP7 in adipocytes

AQP7-expressing *Xenopus* oocytes gain water and glycerol permeability [23]. This gain of function is inhibited by $HgCl_2$ and its inhibition is recovered by 2-mercaptoethanol, similar to the other AQPs. This result indicates that AQP7 can be subcategorized as an aquaglyceroporin, which enhance permeation of glycerol, in addition to water. In mammals, AQP3, 7, 9, and 10 are considered to belong to aquaglyceroporin family at present. Interestingly, *Escherichia coli* has one aquaporin (AqpZ) and one aquaglyceroporin (GlpF) [24]. These findings indicate that glycerol channel is required even in *E. coli* and that glycerol is an essential substance for living.

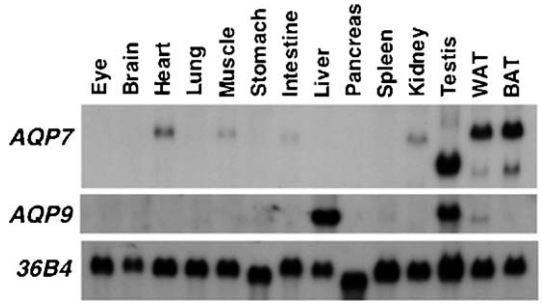

Fig. 2. Tissue distributions of mouse AQP7 and AQP9. Shown is northern blotting analysis of various tissues. Ten micrograms of total RNA was electrophoresed on 1% agarose/formaldehyde gel and transferred to nylon membranes. The membrane was hybridized with the indicated cDNA probe labeled with [α-^{32}P]dCTP. The hybridized membrane was exposed to X-ray film. WAT, white adipose tissue; BAT, brown adipose tissue.

AQP7 mRNA expression increases in parallel with adipocytes differentiation of 3T3-L1 cells. Glycerol release into the media also increases in parallel with AQP7 mRNA levels in differentiating 3T3-L1 adipocytes [23]. Peroxisome proliferator activated receptor γ (PPARγ) is a master regulator of adipocytes differentiation and regulates several adipose-specific genes at the transcriptional level. PPARγ forms a heterodimer with retinoic acid X receptor α (RXRα), and binds to peroxisome proliferator response element (PPRE) site. The PPRE site is identified in the promoter region of AQP7 gene based on analysis of the mouse AQP7 gene. Heterodimers of PPARγ and RXRα bind to the PPRE site of AQP7 promoter and up-regulates AQP7 mRNA expression in adipocytes [25]. Furthermore, administration of thiazolidinediones (TZDs), which are insulin-sensitizing agents and exogenous PPARγ ligands, increase AQP7 mRNA levels in 3T3-L1 adipocytes and in adipose tissues of mice. The precise mechanism of TZD in ameliorating insulin resistance is not fully understood. To clarify whether the TZD-induced adipose AQP7 is related to the amelioration of insulin resistance, requires further studies in the future.

AQP7 mRNA levels are reduced by feeding and increased by fasting in parallel with plasma glycerol levels [23]. These nutrition-related changes in AQP7 and plasma glycerol are the opposites of plasma insulin levels. In fact, in 3T3-L1 adipocytes, insulin suppresses AQP7 mRNA levels in a dose- and time-dependent manners. The insulin negative response element (IRE) is identified in the promoter region of AQP7 gene [22]. This result indicates that AQP7 mRNA expression is closely regulated by insulin at the transcriptional level. Furthermore, glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), which are key enzymes of gluconeogenesis, also contain IRE in their promoter regions. Insulin also suppresses the mRNA levels of G6Pase and PEPCK. Taken together, plasma glycerol levels are partly determined by insulin through adipose AQP7, and suggests that adipose AQP7 may be associated with glucose metabolism.

In 3T3-L1 adipocytes, AQP7 is localized at the periphery of the nucleus in steady state. However, AQP7 translocates to the plasma membrane following adrenaline stimulation, which induces lipolysis [23]. Adrenaline does not affect AQP7gene expression. Adrenaline elevates intracellular cAMP levels through adrenergic receptor and then activates protein kinase A (PKA). Interestingly, similar results are observed in AQP2, which is a key water channel of kidney. Briefly, AQP2 exists in the principal cells of the renal collecting duct. Immunogold electron microscopy studies showed that very little of AQP2 protein is found in the apical

membrane of collecting duct principal cells, but most of AQP2 protein exists in the membranes of intracellular vesicles. However, AQP2 protein relocates to the apical plasma membrane when collecting duct cells are stimulated by vasopressin, an anti-diuretic hormone released from the brain [26]. Vasopressin binds to V2 receptor at the basolateral membrane of the renal collecting duct causing activation of a G-coupled adenylylcyclase cascade that results in phosphorylation of AQP2 by PKA [27]. The phosphorylation site of AQP2 is located at residue 256 on the C-terminus of its protein. Phosphorylated-AQP2 moves to the apical plasma membrane. These results suggest that AQP7, as well as AQP2, may be phosphorylated by PKA under the activation of adrenergic receptor. Database analysis indicates the six prospective sites of PKA phosphorylation in both human and mouse AQP7. It is necessary to determine the phosphorylation site of AQP7 in the future.

Fig. 1 right panel shows a model illustrating the regulation of AQP7 at fasting state. Collectively, catecholamine stimuli translocate HSL to the lipid droplets and moves AQP7 to the plasma membrane. Phosphorylated-HSL hydrolyzes TG to fatty acids and glycerol in cooperation with perillipin, which is another key molecule in lipolysis. Moreover, reduced insulin signal results in up-regulation of AQP7 mRNA levels. Finally, glycerol produced by hydrolysis in adipocytes is released into the bloodstream through AQP7.

3.3. Human genetic mutation in AQP7 gene

Human AQP7 gene mutation was reported in searching 160 Japanese subjects [28]. In that study, three types of missense mutation were identified; R12C (a C→T substitution at nucleotide 206 in exon 3 led to substitution of arginine with cysteine at position 12, which resides in the N-terminal cytoplasmic domain): 1 subject, V59L (a G→C substitution at nucleotide 347 in exon 4 caused substitution of valine with leucine at position 59, which resides in the 1st bilayer-spanning domain): 13 subjects, G264V (a G→T substitution at nucleotide 963 in exon 8 led to substitution of glycine with valine at position 264, which resides in the 6th bilayer-spanning domain): 6 subjects. Functional analysis using *Xenopus* oocytes shows that the permeability of water and glycerol is disturbed in oocytes expressing G264V mutant protein while those expressing R12C or V59L mutant proteins retain water and glycerol permeability. G264V mutation is located in 6th bilayer-spanning domain. Structural analysis of AQP1 shows that the conserved GxxxGxxxG motif in the 3rd and 6th bilayer-spanning domain is important for functional conformation of AQP family protein; glycine can be sometimes replaced by alanine in the motif [29,30]. In the 6th bilayer-spanning domain of human AQP7, A260, G264, and G268 form this motif. Functional defect in the G264V mutant might be caused by disturbance of this motif. The subjects with homozygous mutation of G264V showed a similar exercise-induced rise in plasma noradrenalin compared to healthy volunteers whereas the increase in plasma glycerol was apparently disturbed during exercise [28]. This result indicates that AQP7 may be a crucial molecule for maintaining plasma glycerol levels in vivo. However, obesity and diabetes were not observed in subjects with homozygous mutation of G264V. Further analysis of human AQP7 gene and/or frequency of AQP7 mutation in subjects with the metabolic syndrome should be performed in the future.

3.4. Role of AQP7 in other organs

Our group discovered AQP7 during genomic analysis of human adipose tissue, and at the same time another group identified AQP7 from the rat testis [21]. AQP7 is also expressed in human sperms. Lack of AQP7 expression in sperms is observed in several male infertile subjects. Other studies demonstrated that sperm AQP7 is associated with the capacity of sperm movement [31]. Immunohistochemical analysis shows that AQP7 is also expressed in the proximal tubules of the kidney. AQP7-deficient mice exhibit hyperglycerourea, suggesting that AQP7 is involved in glycerol re-absorption in the kidney [32].

As shown in Fig. 2, skeletal muscles express small amounts of AQP7. Recent immunohistochemical studies using anti-AQP7 antibody demonstrated immunoreactivity at the myofiber surface of type 1 and type 2 fibers in human muscles and of type 2 fibers in mouse muscles [33]. The role of AQP4 has been extensively investigated in skeletal muscles [34,35]. AQP4 is reduced in the skeletal muscle of patients with Duchenne dystrophy [36,37], of mdx mice [38,39], and of patients with amyotrophic lateral sclerosis [40]. The nerve activity is thought to control the expression of AQP4 in skeletal muscles. While the function of AQP4 has been clarified in skeletal muscles, the physiological role of AQP7 in skeletal muscles has remained unclear. Fig. 2 also demonstrates that heart expresses AQP7. Fatty acids and/or glucose are widely recognized as energy source in the heart. Why does AQP7 exist as a glycerol channel in the heart? What is the role of glycerol in the heart? There are currently no published studies on the functional role of AQP7 in the heart. Further investigations of skeletal muscle and/or heart AQP7 should be performed in the future.

4. Liver-specific glycerol channel; AQP9

4.1. Molecular cloning and tissue distribution of AQP9

AQP9 was independently identified in human leukocytes [41] and rat liver [42,43]. Northern blotting analysis identified human AQP9 expression in liver, leukocyte, lung, and spleen [41]. In rat, AQP9 mRNA is found in liver, testis, brain [42], and lung [43]. As in Fig. 2, AQP9 is also highly expressed in mouse liver and testis. In hepatocytes, immunohistochemistry showed that AQP9 is localized at the sinusoidal plasma membrane [44].

4.2. Functional analysis and regulation of AQP9 in liver

Rat AQP9-expressing *Xenopus* oocytes exhibit water and glycerol permeability [41]. A series of studies on *Xenopus*

oocytes also demonstrated that rat AQP9 permeates urea, mannitol, sorbitol, and uracil. In consistent with this finding, another group found that rat AQP9 is permeable to water, glycerol and urea [43]. These results indicate a broad selectivity of rat AQP9. However, another group found the AQP9-induced permeability to be restricted to water and urea in human [41]. Thus, there are conflicting results regarding AQP9-induced permeability between rat and human. Future studies could provide further information based on the physiological function of AQP9 and can be designed to generate and analyze AQP9 knockout mice, and/or analyze human genetic mutation in AQP9 gene.

Arsenic trioxide uptake was examined with yeast expressing mammalian AQP7 or AQP9 as well as in oocytes expressing these proteins [45]. The results of these studies showed that AQP7 and AQP9 may be the major routes of arsenite uptake into mammalian cells. Arsenic trioxide is uncharged at neutral pH values and is very toxic. Clinical features of greatest concern in arsenic poisoning include hepatocellular damage and hepatocellular carcinoma.

Intra-abdominal visceral fat accumulates mainly in the mesentery. The anatomical distribution of intra-abdominal visceral fat indicates that substances released from the visceral fat directly flow into the liver via the portal vein. FFA derived from visceral fat during lipolysis elevates liver acyl-coenzyme A synthetase (ACS) and microsomal triglyceride transfer protein (MTP) mRNA levels, and reduces degradation of apolipoprotein B (ApoB). These changes induce the release of ApoB from the liver and increase plasma triglyceride concentrations. Hypertriglyceridemia, which is often observed in subjects with visceral fat accumulation, is partly accounted for the increase in FFA derived from adipose tissues [46]. Glycerol, which is another product from adipose TG during lipolysis, directly flows into the liver via portal vein and become substrate for gluconeogenesis. AQP9 is considered as the sole glycerol channel in liver and is localized at the sinusoidal plasma membrane facing the portal vein [44]. Taken together, AQP9 may act as a channel of glycerol uptake in the liver (Fig. 3A). AQP9 mRNA levels increase by fasting and decrease by feeding [47]. These changes in AQP9 mRNA are similar to those of glycerol kinase, which is a key enzyme involved in the conversion of glycerol to glycerol-3-phosphate, and PEPCK, which is a key enzyme for gluconeogenesis. Insulin suppresses AQP9 mRNA levels in time- and dose-dependent manners in H4IIE hepatocytes. Promoter analysis demonstrates that insulin reduces AQP9 mRNA via IRE locating at −496/−502 promoter region. Administration of streptozotocin (STZ) results in increased AQP9 mRNA [47] and protein [48] levels in insulin-insufficient mice.

4.3. Coordination of adipose and liver glycerol channels

In feeding state, a rise in plasma insulin concentration results in suppression of lipolysis and the mRNA expression of adipose AQP7, and results in reduced glycerol release from adipocytes. Feeding also reduces liver AQP9 mRNA and glycerol-based gluconeogenesis (Fig. 3B). However, high

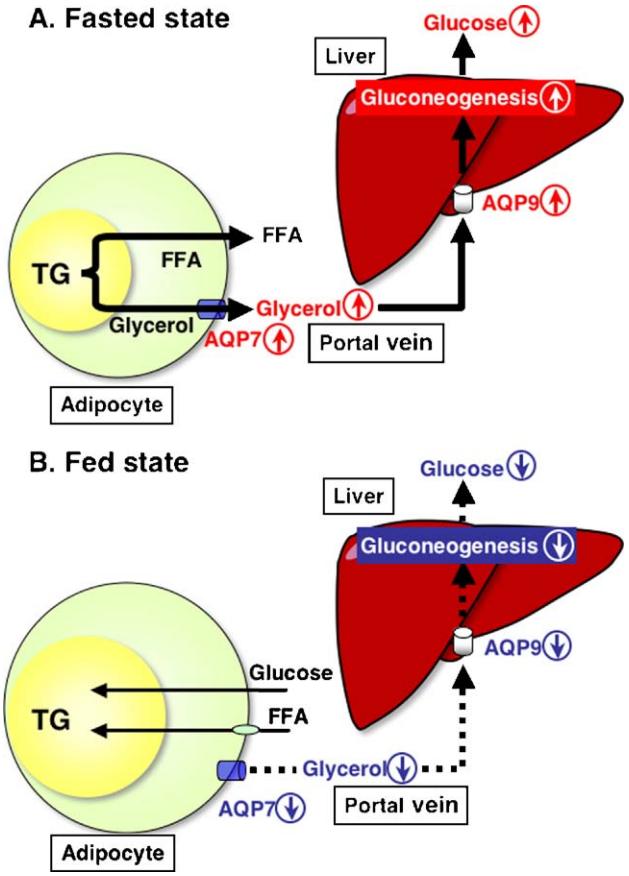

Fig. 3. Coordinated regulation of adipose AQP7 and liver AQP9 during fasting and feeding states. (A) A model illustrating the regulation of AQPs under fasted state. Fasting induces lipolysis in adipocytes and gluconeogenesis in liver. As shown in Fig. 1, AQP7 mRNA levels are elevated, AQP7 protein translocates to the plasma membrane, and AQP7 serves as an efficient release of glycerol in adipocytes under fasting conditions. Fasting also increases AQP9 mRNA levels in liver, the increased portal glycerol directly flows into the liver, and AQP9 may contribute to the entry of glycerol into hepatocytes. In the liver, glycerol is one of substrates for gluconeogenesis. The glycerol cascade from adipose tissue to liver is maintained by the coordinated regulation of AQP7 and AQP9 under fasting state. (B) A model illustrating the regulation of AQPs under fed state. After feeding, the increased plasma insulin makes a metabolic switch from lipolysis to lipogenesis in adipocytes, and suppresses glucose production from liver. Adipose AQP7 mRNA levels are reduced under feeding state, while glucose transporter 4 (GLUT4) translocates to the plasma membrane and takes glucose into adipocytes. Adipocytes store triglyceride (TG) by esterificating glucose and fatty acids. Portal glycerol levels decrease in parallel with the reduction of adipose AQP7 and liver AQP9 mRNAs.

adipose AQP7 and liver AQP9 mRNA levels are observed in obese and insulin-resistant animals in spite of hyperinsulinemia. These animals show increased glycerol release from adipose tissues in parallel with the increase of AQP7 mRNA, and also increased glycerol levels in portal vein. Finally, the high glycerol levels in portal vein causes gluconeogenesis and results in hyperglycemia through the pathological induction of liver AQP9 [47]. Considered collectively, physiological and pathological coordinated regulation of organ-specific glycerol channels, adipose AQP7 and liver AQP9, may contribute to glycerol and glucose metabolism in vivo (Fig. 3).

5. Analysis of AQP7-deficient mice

5.1. Adipose-derived glycerol and gluconeogenesis through AQP7

Recently, we generated and analyzed AQP7 knockout (AQP7-KO) mice [49]. AQP7-KO mice exhibit lower plasma and portal glycerol concentrations under fasting state than wild-type (WT) mice under the same condition. Administration of β3-adrenergic agonist, which specifically effects on adipocytes and enhances lipolysis, results in impaired plasma glycerol elevation in AQP7-KO mice but does not modulate the normal increase of plasma FFA in both WT and AQP7-KO mice. Similar results are obtained in in vitro 3T3-L1 adipocytes introduced by RNAi. Briefly, adrenaline-mediated glycerol release is significantly disturbed in AQP7-knockdown 3T3-L1 adipocytes, while adrenaline-mediated FFA release from AQP7-knockdown adipocytes is similar to that of 3T3-L1 adipocytes transfected with control-RNAi. Longer starvation test demonstrated that AQP7-KO mice exhibit impaired plasma glycerol elevation associated with severe hypoglycemia in comparison with WT mice. The results of a series of studies indicate that AQP7 acts as an adipose glycerol channel in vivo and that adipose-derived glycerol is a significant substrate for gluconeogenesis (Fig. 4A).

5.2. Adipocytes dysfunction in AQP7-deficient cells

There is no difference in body weight of WT and AQP7-KO mice at young age, but AQP7-KO mice become obese after 12 weeks of age [50]. Adipose tissue weights of AQP7-KO mice are significantly heavier than WT mice at 20 weeks of age. Histological analysis shows an increase in hypertrophic adipocytes in epididymal WAT of AQP7-KO mice. Moreover, AQP7-KO mice exhibit whole body insulin resistance associated with obesity (Fig. 4B). Recently, Hara-Chikuma et al. [51] also reported increased number of hypertrophic adipocytes in AQP7-KO mice, although the body weights of their AQP7-KO mice were similar to those of WT mice. The difference of phenotypes may be accounted for the genetic background of mice.

Food intake, rectal temperature, and oxygen consumption of AQP7-KO mice are similar to WT mice at young age. At young age, there are no apparent differences in adipose mRNA levels, which are related to adipogenesis, lipogenesis, lipolysis, and thermogenesis, between AQP7-KO and WT mice. However, intracellular glycerol contents of AQP7-KO mice are significantly higher than those of WT mice at young age. A recent study reported that glycerol induces conformational changes and enzymatic activity of glycerol kinase, which is a key enzyme in the conversion of glycerol to glycerol-3-phosphate [52]. Actually, the activity of adipose glycerol kinase of AQP7-KO mice is elevated before development of obesity. Previous study indicated that over-expression of glycerol kinase promotes re-esterification of glycerol and accelerates TG accumulation in adipocytes [53]. Knockdown of AQP7 in 3T3-L1 adipocytes increases intracellular glycerol contents, elevates glycerol kinase activities, enhances oleic acid uptake, and finally results in TG accumulation [50].

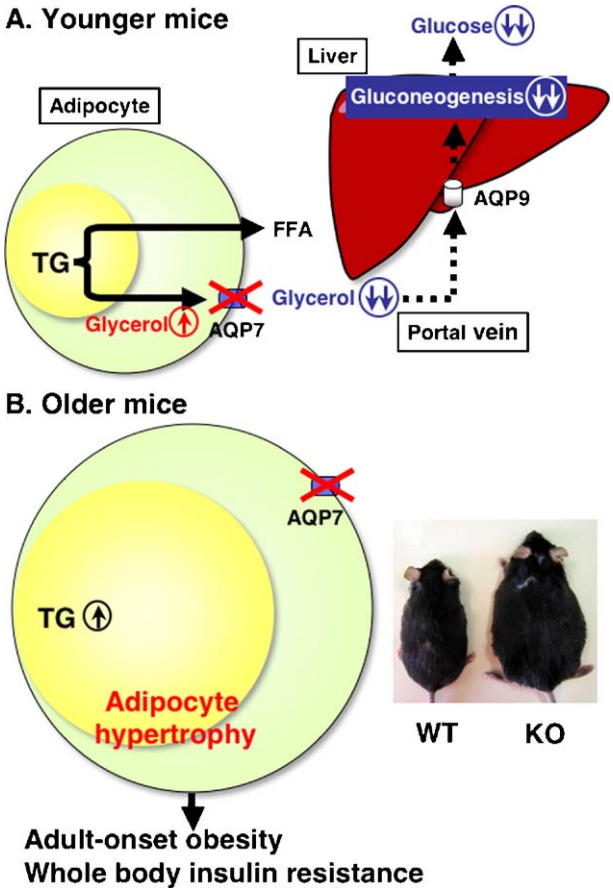

Fig. 4. Schematic presentation of the physiological role of AQP7 based on the analysis of AQP7 knockout mice. (A) The summary of younger mice. Under starvation, AQP7 knockout (AQP7-KO) mice exhibit impairment of plasma glycerol elevation and result in severe hypoglycemia in comparison with wild-type (WT) mice. There is evidence that AQP7 acts as an adipose glycerol channel in vivo and that adipose-derived glycerol is a significant substrate for gluconeogenesis. (B) The summary and photo of older mice. AQP7-KO mice develop obesity accompanied by adipocyte hypertrophy after 12 weeks of age. Photo in the right panel shows WT and KO mice at 40 weeks of age. The high intracellular glycerol contents induce the enzymatic activity of glycerol kinase in adipose tissues of AQP7-KO mice at young age. Glycerol kinase promotes re-esterification of glycerol and accelerates triglyceride (TG) accumulation in adipocytes. Moreover, AQP7-KO mice exhibit whole body insulin resistance associated with obesity.

In summary, deficiency of adipose AQP7 influences on not only glycerol metabolism but also glucose metabolism in vivo.

6. Conclusions and outlook

The discovery of AQP has made a great impact on life sciences. Structural and functional analyses of AQPs indicate that AQPs do not only permeate water. Novel metabolic mechanisms have been clarified by the demonstration that some AQPs act as glycerol channels. Investigation of AQPs-dependent glycerol metabolism should help the design of novel therapeutic strategies for metabolic syndrome. Future studies

should further refine our understanding of the association between glycerol metabolism and AQPs.

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