8
Aquaporins and Adipose Tissue: Lesson from Discovery to Physiopathology and to the Clinic of Aquaporin Adipose (AQP7)

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8.1
Introduction

The metabolic syndrome comprises a cluster of insulin resistance, elevated blood pressure, and atherogenic dyslipidemia, and is a major cause of cardiovascular disease in industrial countries [1]. Accumulation of body fat, especially intra-abdominal fat deposits, stands upstream of the metabolic syndrome [1]. In the early 1990s, our group pioneered research on the molecular mechanism of the metabolic syndrome [2–4]. We analyzed the gene expression profile of human adipose tissue in collaboration with the Human Body Map Project team, and found that adipose tissue, especially visceral fat, expressed a variety of genes for secretory proteins, including growth factors and cytokines [5]. Traditionally, adipose tissue has been considered as a simple energy storage organ in which lipogenesis and lipolysis are observed in response to whole-body energy balance, but it is also currently understood as an endocrine organ that secretes a variety of bioactive molecules [5]. We named these adipocyte-derived molecules "adipocytokines" [1]. We and others have provided evidence for the potential role of dysregulation of adipocytokines in the development of the metabolic syndrome [1]. To find a novel therapy for metabolic syndrome, it is necessary to focus on the biology and science of adipocyte that the authors have named "adiposcience."

Adipocytes hydrolyze triglyceride and rapidly liberate free fatty acids (FFAs) and glycerol into the circulation [6]. The underlying mechanism responsible for glycerol release from adipocytes remains unclear. Our team also discovered a highly expressed gene from the human adipose tissue cDNA library in 1997 [5]. This gene, encoded by a protein belonging to the aquaporin (AQP) family, was named AQP adipose (aquaporin adipose AQPap) [21, 22], which also had glycerol permeability and was later found to be a human homolog of AQP7 [9]. In this chapter, we review a series of studies on AQP7 to define the function and significance of the glycerol gateway molecule, and discuss the relation between AQPs and glycerol metabolism.

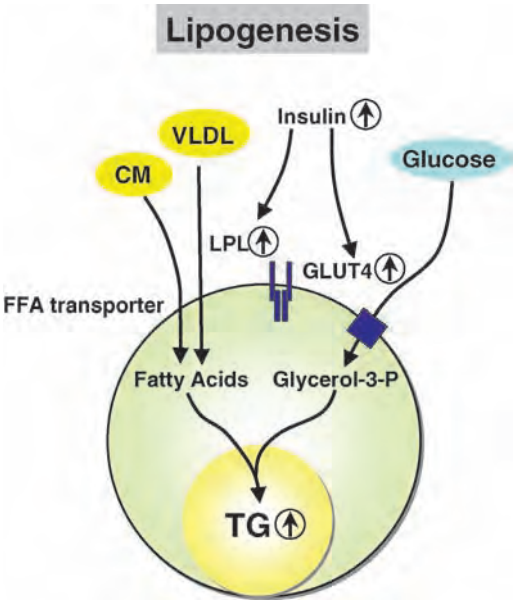

Figure 8.1 Schematic presentation of lipogenesis in adipocytes. Under lipogenic conditions, β-cells in the pancreas secret insulin in response to an increase in plasma glucose concentration. Insulin binds to the insulin receptor located on the surface of adipocytes, transfers GLUT4 to the plasma membrane, and takes glucose into the cell. Intracellular glucose is converted to glycerol-3-phosphate (glycerol-3-P). Insulin also activates LPL located on the cell surface of the vascular endothelium. Activated-LPL removes fatty acids from intestine-derived CM and liver-derived VLDL, and then fatty acids are taken into adipocytes. Fatty acids and glycerol-3-phosphate are esterified into triglyceride (TG).

CM= chylomicron

8.2 Characteristics of Adipocytes and Gycerol Metabolism in the Mammalian Body

Adipocytes continuously synthesize (lipogenesis) and hydrolyze (lipolysis) triglyceride in response to whole-body energy balance (Figures 8.1 and 8.2) [6]. 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 triglyceride accumulation. The β-cells in the pancreas secret insulin in response to an increase in plasma glucose concentration during feeding. Insulin acts on adipose tissues as well as skeletal muscles, transfers glucose transporter 4 (GLUT4) to plasma membrane, and takes glucose into the cell [11]. 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 [12]. In adipocytes, glycerol-3-phosphates converted from glucose and fatty acids are esterified into triglyceride.

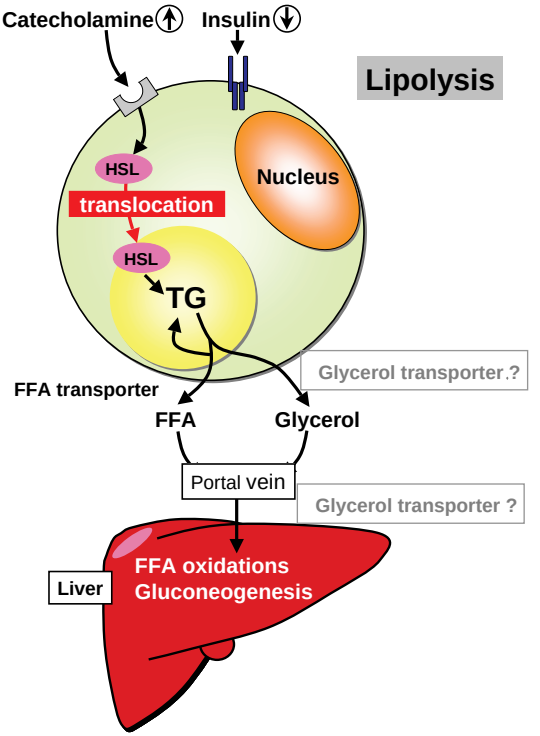

Figure 8.2 Schematic presentation of lipolysis in adipocytes, and pathway of glycerol and FFA from adipocytes to liver. Under lipolysis or starvation, sympathetic nerves are activated and catecholamines are increased. Catecholamines stimulate adrenergic receptors located on the surface of adipocytes. Activation of adrenergic receptors results in activation of HSL. HSL hydrolyzes triglyceride (TG) to FFA and glycerol, and both are released into the bloodstream. FFAs are partly re-esterified to triglyceride and incorporated to lipoprotein, and glycerol is used as a substrate of gluconeogenesis in liver. We hypothesized that AQP7 may function as a glycerol transporter in adipocytes [52].

HSL=Hormone Sensitive Lipase

Fatty acid-binding protein (FABP; aP2) [13], fatty acid translocase (FAT) [14], and fatty acid transport protein (FATP) [8, 15] are recognized as fatty acid transporters in adipocytes (Figure 8.1).

In contrast to the feeding state, exercise and/or fasting induce lipolysis in adipocytes [16]. Fasting stimulates sympathetic nerves and elevates catecholamines, such as adrenaline and noradrenalin, which in turn stimulate adrenergic receptors located on the surface of adipocytes. Activation of adrenergic receptors results in converting ATP to cAMP. Elevation of intracellular cAMP activates hormone-sensitive lipase (HSL) by phosphorylation. Phosphorylated HSL hydrolyzes triglyceride to FFA and glycerol, and both are released into the bloodstream. FFA and glycerol are utilized for thermogenesis and gluconeogenesis, respectively (Figure 8.2). During the long history of mammals and humans, starvation has been a matter of life or death. Adipocytes play a crucial role in energy supply under starvation to maintain

energy homeostasis and have contributed to the survival of animals during long fasting periods.

During lipolysis, adipocytes liberate FFA and glycerol rapidly outside the cells. It had been considered that FFA and glycerol are transported outside the cell by simple diffusion. Interestingly, recent studies revealed the participation of several molecules in the transport of FFA, such as FATP [8, 15], FABP [13], and FAT [14]. However, the molecular mechanism involved in the transport of glycerol was poorly understood. The rapid increase in glycerol production during lipolysis results in an acute rise in intracellular osmotic pressure, which could damage the cell. We hypothesized that adipocytes may have a molecule for glycerol (Figure 8.2)

8.3
Adipose Glycerol Channel: AQP7

8.3.1
AQP7: A Putative Adipose-Specific Glycerol Channel

The AQPs are a group of channel-forming integral membrane proteins that selectively transport water and, in some cases, small neutral solutes, such as glycerol and urea [16–18]. The discovery of AQPs led to the finding that rapid movement of water molecules across the cell membrane is required in specialized cell types or tissues [16–18]. The AQPs are a family of homologous water channels widely distributed in plants, unicellular organisms, invertebrates, and vertebrates [16–19]. There are two subfamilies – the aquaporins, which transport only water, and the aquaglyceroporins, which transport glycerol in addition to water (Figure 8.3a) [19, 20]. 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. For example, the studies of AQP2, as the gene responsible for nephrogenic diabetes insipidus, highlighted the significance of AQP in human diseases [7].

We analyzed the gene expression profile of human visceral and subcutaneous fat to clarify the molecular mechanism of obesity-related diseases [5]. We identified a novel cDNA belonging to the AQP family during this analysis, and named it AQPap because its mRNA is expressed abundantly in adipose tissues and adipocytes [10, 21, 22]. AQPap is a human counterpart of AQP7 that was independently cloned from rat testes by another group at the same time [9]. In Figure 8.4, each AQP is specifically expressed in specialized tissues. The adipose tissue expresses two aquaporins, AQP1 and AQP7. AQP7 is highly expressed in white adipose tissue (WAT), brown adipose tissue (BAT), and testes [22]. The testes express a shorter size of AQP7 mRNA because of a different polyadenylation site [10]. A weak expression of AQP7 is also observed in the heart, skeletal muscles, and kidneys [22].

We analyzed the function of AQP7 using in *Xenopus* oocytes. AQP7-expressing *Xenopus* oocytes showed water and glycerol permeability [10, 21] (Figure 8.3b). This gain of function was inhibited by $HgCl_2$ and recovered by 2-mercaptoethanol, similar

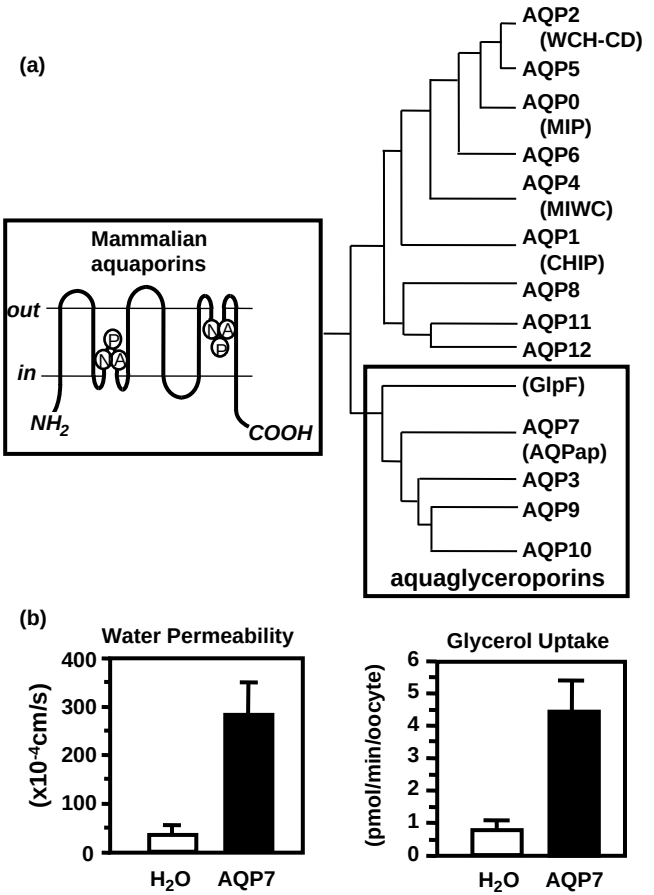

Figure 8.3 Phylogenic analysis of the mammalian AQPs and functional analysis of AQP7 in *Xenopus* oocytes. The phylogenic tree of the mammalian AQP gene family is shown (a). Water-permeable AQPs are AQP0, 1, 2, 4, 5, 6 and 8. Glycerol-permeable aquaglyceroporins are boxed (AQP3, 7, 9, 10 and GlpF). GlpF is the *Escherichia coli* homolog. AQP11 and AQP12 belong to the unclassified subfamily. Osmotic water permeability (P_f) and [^{14}C]glycerol uptake of oocytes injected with water (open bar) or 50 ng of human AQPap cRNA (closed bar) are shown (b). The oocytes injected with AQP7 cRNA showed 4.5-fold stimulation of glycerol uptake compared with those of water-injected oocytes [10].

to the other AQPs [21, 22]. These results indicated that AQP7 could be subcategorized as an aquaglyceroporin. In mammals, AQP3, 7, 9, and 10 are considered to belong to the aquaglyceroporin subfamily at present (Figure 8.3a) [19, 20]. AQP1 is a simple water channel (Figure 8.3a). Therefore, AQP7 is the sole aquaglyceroporin in the adipose tissue (Figure 8.4).

During the differentiation of 3T3-L1 adipocytes, the amount of glycerol released into the media increases and such an increase correlates with augmentation of the AQP7 mRNA expression level [22]. Many adipose-specific genes are controlled by the

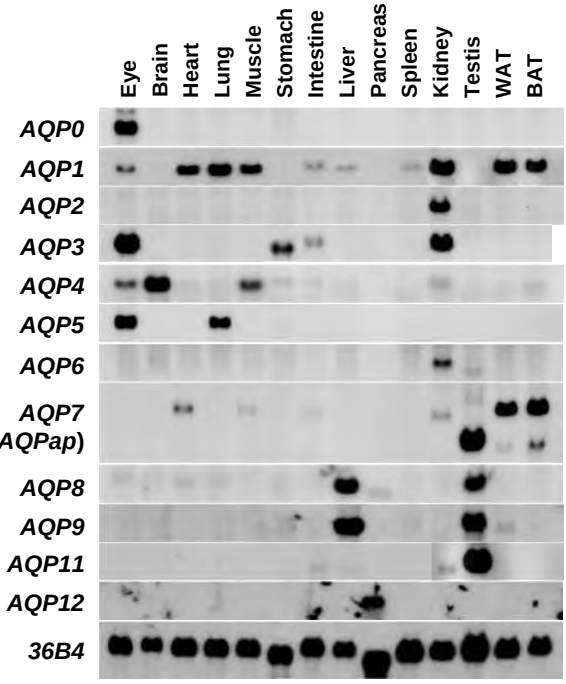

Figure 8.4 Tissue distribution of AQPs in mice. C57Black6 mice (12 weeks old) were sacrificed in the fed state and various tissues were removed. RNAs were extracted and subjected to Northern blot analyses. Shown is Northern blotting analysis of AQPs in the various tissues. From [22, 23].

nuclear factor peroxisome proliferator-activated receptor (PPAR)-γ, which is a master regulator of adipocytes differentiation and regulates several adipose-specific genes at the transcriptional level [24]. PPAR-γ forms a heterodimer with retinoic acid X receptor (RXR)-α and binds to peroxisome proliferator response element (PPRE) [24]. We identified the PPRE sequence in the AQP7 promoter [25]. The heterodimer of PPAR-γ and RXR-α binds to the PPRE site of the AQP7 promoter and upregulates AQP7 mRNA expression in adipocytes (Figure 8.5a). Furthermore, transfection of PPAR/RXR and exogenous PPAR-γ ligands, thiazolidinediones, stimulated the promoter activity of AQP7 [25]. AQP7 PPRE actually bound to PPAR/RXR complex (Figure 8.5) [25]. AQP7 is the adipose-specific novel PPAR-γ target gene.

8.3.2
Function and Regulation of AQP7 in Adipocytes

Catecholamine activates lipolysis and may accelerate glycerol release from adipocytes through AQP7. Epinephrine does not affect AQP7 gene expression (Figure 8.6a). In 3T3-L1 adipocytes, AQP7 is localized at the periphery of the nucleus in the steady state [22]. However, AQP7 translocates to the plasma membrane following epinephrine stimulation, which induces lipolysis (Figure 8.6b) [22]. Epinephrine elevates

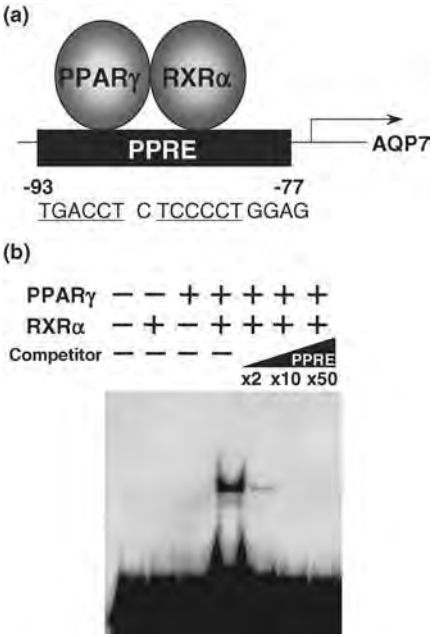

Figure 8.5 Specific binding of the PPAR-γ/RXR-α complex to the PPRE in the AQP7 promoter. The putative PPRE in the AQP7 promoter is underlined (a). The PPAR-γ/RXR-α complex directs and specifically binds to the PPRE in the AQP7 promoter as shown by electrophoretic mobility shift assay (b) [25].

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 [26]. Briefly, AQP2 exists in the principal cells of the renal collecting duct. Immunogold electron microscopy studies showed that very little AQP2 protein is found in the apical membrane of collecting duct principal cells, but most 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 antidiuretic 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 adenylyl cyclase 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. It is necessary to determine the phosphorylation site of AQP7 in the future.

Moreover, fasting activates lipolysis. We investigated the regulation of AQP7 expression during fasting and refeeding in animals. In mice, fasting was associated with increased AQP7 mRNA level, whereas refeeding reduced the level in parallel with plasma glycerol levels [10]. These nutrition-related changes in AQP7 and plasma glycerol were in clear contrast to insulin levels (Figure 8.6c) [10]. The expression of

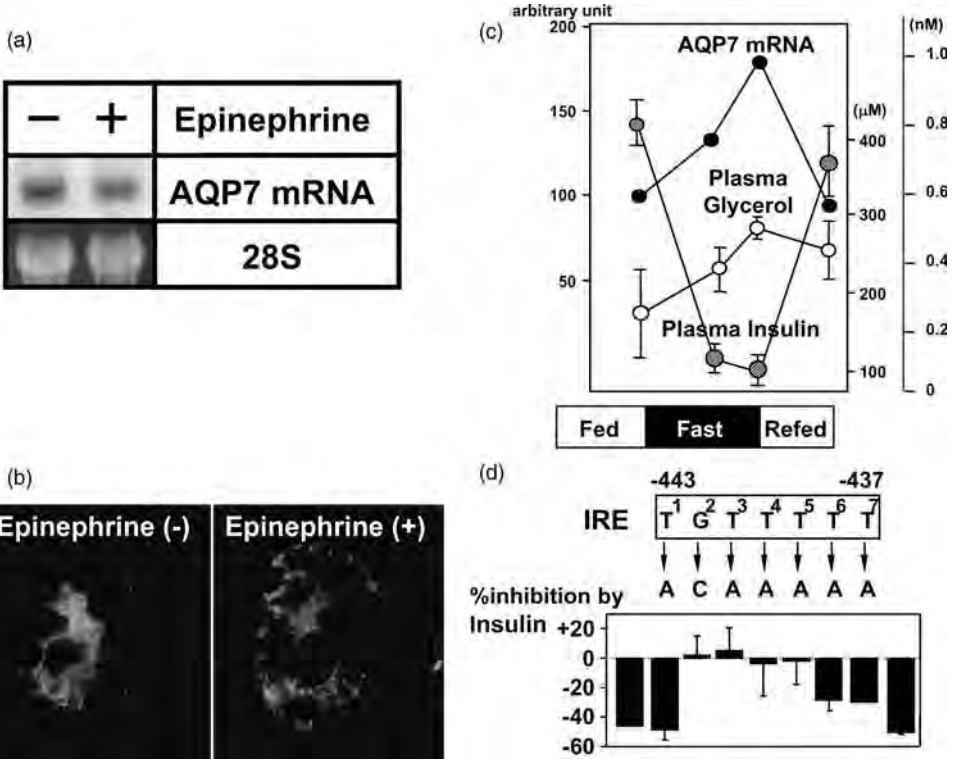

Figure 8.6 Catecholamine-induced translocation and insulin-mediated repression of the AQP7 through negative IRE. We examined the effect of lipolytic hormone on AQP7 mRNA levels. Administration of epinephrine did not change AQP7 mRNA levels in 3T3-L1 adipocytes (a). When the 3T3-L1 adipocytes were stimulated by epinephrine for 20 min, the signal in the plasma membrane became robust in comparison to the intracellular regions, suggesting that AQP7 was translocated from intracellular regions to the plasma membrane (b) [22]. Next, we examined the effect of fasting on AQP7 mRNA expression in adipose tissues. Adipose AQP7 mRNA levels were increased by fasting and suppressed by refeeding, similar to the change of glycerol. Plasma insulin levels were decreased in the fasted mice and restored in the refed mice (c). We found negative IRE in the human and mouse AQP7 promoter. Single point mutation analysis of the IRE sequence in the AQPap promoter using the promoter assay in 3T3-L1 adipocytes (d) [10]. The bases in the heptanucleotide IRE are numbered 1–7. The value for the wild-type construct without insulin was arbitrarily set at 1.0. In the lower panel, the percent inhibition of AQPap-mediated luciferase activity in mutant constructs by insulin is plotted below the corresponding mutated base pair.

AQP7 was upregulated in the adipose tissue of streptozotocin (STZ)-induced insulin deficient mice [10]. In fact, in 3T3-L1 adipocytes, insulin suppresses AQP7 mRNA levels in a dose- and time-dependent manners. Many genes negatively controlled by insulin such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK), which are key enzymes of gluconeogenesis, have a negative insulin

response element (IRE) in their promoter. We found IRE in the promoter of AQP7 [10]. Single nucleotide substitution analysis revealed that four core nucleotides at the center of AQP7 IRE, GTTT, are necessary for the negative regulation by insulin (Figure 8.6d) [10]. These results indicated that AQP7 mRNA expression is closely regulated by insulin at the transcriptional level. Taken together, plasma adipocyte-derived glycerol levels through AQP7 are partly determined by insulin and catecholamine (Figure 8.7).

8.3.3 Human AQP7 Genetic Mutation

Next, we studied the mutations in the human AQP7 gene for further clarification of the significance of AQP7. We screened human AQP7 gene mutation in 160 subjects including controls, diabetics, and obese individuals, and three types of polymorphism, which predict 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),

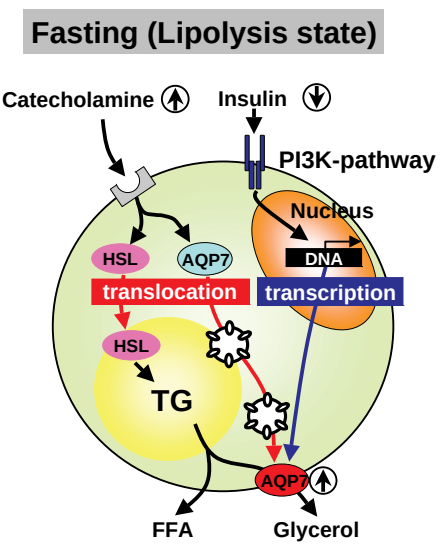

Figure 8.7 Schematic presentation of the regulatory mechanisms that control adipose AQP7 under the fasting state. Catecholamine translocates HSL to the lipid droplets, while related stimuli move AQP7 to the plasma membrane. HSL hydrolyzes triglyceride (TG) to fatty acids and glycerol. Moreover, AQP7 mRNA levels are elevated by the decrease of the insulin signaling cascade. Thus, long-term regulation of AQP7 is under the control of insulin, whereas short-term regulation is under the control of catecholamines. These two different regulatory pathways of AQP7 ensure the efficient release of glycerol from adipocytes under fasting conditions. PI3K, phosphoinositide-3-kinase.

HSL = Hormone Sensitive Lipase

one 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 first bilayer-spanning domain), 13 subjects; and 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 sixth bilayer-spanning domain), six subjects (Figure 8.8a) [28]. When we injected cRNA carrying each mutation, oocytes normally expressed AQP7 protein [28]. V59L and R12C were functionally normal, but the G264V mutation lacked both water and glycerol permeability, while those expressing R12C or V59L mutant proteins retained water and glycerol permeability (Figure 8.8b) [28]. G264V mutation is located in the sixth bilayer-spanning domain. Structural analysis of AQP1 shows that the conserved GxxxG motif in the third and sixth bilayer-spanning domain is important for functional pore conformation of AQP family protein; glycine can be sometimes replaced by alanine in the motif (Figure 8.8c) [29]. In the sixth bilayer-spanning domain of human AQP7, A260, G264, and G268 form this motif. The functional defect in the G264V mutant might be caused by a disturbance of this motif (Figure 8.8c) [28]. We found a homozygote carrying the G264V mutation. When

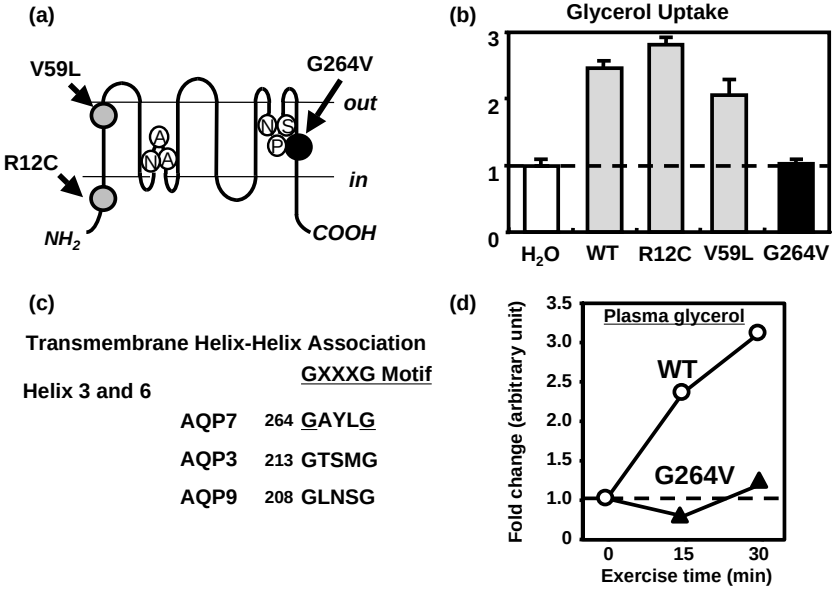

Figure 8.8 Genetic mutations of the human AQP7 gene, and functional analysis of the AQP7 mutant proteins in both *Xenopus* oocytes and human. Three identified missense mutations, AQP7-R12C, V59L and G264V, in the topology of AQP7. NAA and NPS are amino acid residues composed of NPA motifs highly conserved among AQP family proteins (a). We note the functions of these mutant AQP7 proteins using glycerol permeability of oocytes expressing the G264V mutant is lower (WT, wild-type) (b). G264V is located in the GXXXG motif in the α- helix structures present in the third and sixth transmembrane region (c). This motif is well conserved in aquaglyceroporins (c). The plasma glycerol level is not increased in the subject homozygous for the G264V mutation during exercise (d) [28].

subjected to physical exercise after prolonged (20 h) fasting, 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 (Figure 8.8d) [28]. This result indicates that AQP7 may be a crucial molecule for maintaining plasma glycerol levels in human. However, obesity and diabetes were not observed in subjects with homozygous mutation of G264V. Furthermore, it has been reported that single nucleotide polymorphisms (SNPs) in the AQP7 gene may modulate the risk of obesity and dysregulated glycerol release [30]. Further analysis of the human AQP7 gene and/or frequency of the AQP7 mutation or SNP in subjects with the metabolic syndrome should be performed in the future.

8.3.4
Adipose-Derived Glycerol and Gluconeogenesis through AQP7 – Lessons from AQP7-Deficient Mice and Cells

We identified only one subject with a loss-of-function mutation of AQP7 [28]. To further clarify the physiological function of AQP7 *in vivo*, we generated AQP7 knockout mice and analyzed their phenotype [31]. We removed exons 1, 2, and 3, and replaced them with a *neo* cassette. No difference was found in plasma FFA levels between wild-type and knockout mice, but AQP7 knockout mice exhibit lower portal glycerol concentrations under fasting state than wild-type mice under the same condition [31]. Administration of β_3-adrenergic agonist, which specifically affects adipocytes and enhances lipolysis, results in impaired plasma glycerol elevation in AQP7 knockout mice, but does not modulate the normal increase of plasma FFA in both wild-type and AQP7 knockout mice [31]. Similar results are obtained in *in vitro* 3T3-L1 adipocytes introduced by RNA interference (RNAi) [31]. Briefly, epinephrine-mediated glycerol release is significantly disturbed in AQP7 knockdown 3T3-L1 adipocytes, while epinephrine-mediated FFA release from AQP7 knockdown adipocytes is similar to that of 3T3-L1 adipocytes transfected with control RNAi. A longer starvation test demonstrated that AQP7 knockout mice exhibit impaired plasma glycerol elevation associated with severe hypoglycemia in comparison with wild-type mice [31]. 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 (Figure 8.9a).

During the experiments with AQP7 knockout mice, we noticed an unexpected phenotype. There is no difference in body weight of wild-type and AQP7 knockout mice at a young age, but we found that AQP7 knockout mice developed mild obesity after 12 weeks of age [11]. Adipose tissue weights of AQP7 knockout mice are significantly heavier than wild-type mice at 20 weeks of age. The mass of the WAT was larger in knockout mice compared to wild-type mice (Figure 8.9b) [32]. Histological analysis shows an increase in hypertrophic adipocytes in AQP7 knockout mice [32]. Recently, Hara-Chikuma *et al.* [33] also reported an increased number of hypertrophic adipocytes in AQP7 knockout mice, although the body weights of their AQP7 knockout mice were similar to those of wild-type mice. The difference of phenotypes

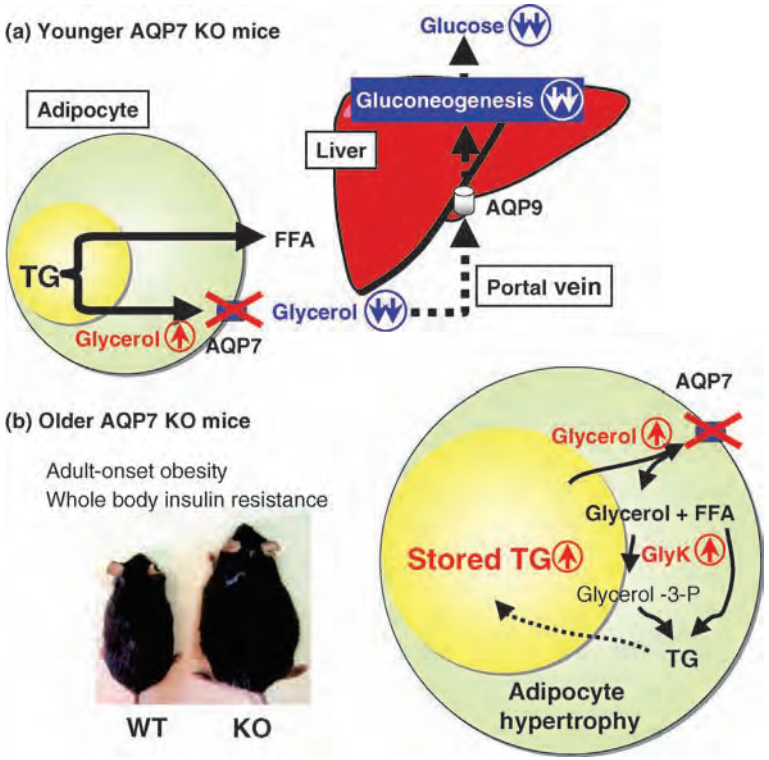

Figure 8.9 Schematic presentation of the physiological role of AQP7 based on the analysis of AQP7 knockout mice. (a) Summary of younger mice. Under starvation, AQP7 knockout (AQP7-KO) mice exhibit impairment of plasma glycerol elevation that results in severe hypoglycemia in comparison with wild-type (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 [31]. (b) Summary and photo of older mice. AQP7 knockout mice develop obesity accompanied by adipocyte hypertrophy after 12 weeks of age. The high intracellular glycerol contents induce the enzymatic activity of glycerol kinase in adipose tissues of AQP7 knockout mice at a young age. Glycerol kinase promotes re-esterification of glycerol and accelerates triglyceride (TG) accumulation in adipocytes. Moreover, AQP7 knockout mice exhibit whole-body insulin resistance associated with obesity [32]. Glycerol-3-P, glycerol-3-phosphate.

may be accounted for by the genetic background of mice. Why was AQP7 deficiency associated with the development of obesity? Intracellular glycerol contents of AQP7 knockout mice are significantly higher than those of wild-type mice at a young age [32]. Under normal conditions, the adipose tissue exhibits only low glycerol kinase activity and it is believed that glycerol is not reused for lipogenesis in adipocytes. 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 [34–36]. Actually, the activity of adipose glycerol kinase of AQP7 knockout mice is elevated before development of obesity, relative to

wild-type mice. A previous study indicated that overexpression of glycerol kinase promotes re-esterification of glycerol and accelerates triglyceride accumulation in adipocytes [36]. Knockdown of AQP7 in 3T3-L1 adipocytes using RNAi increases intracellular glycerol content, elevates glycerol kinase activity, enhances oleic acid uptake, and finally results in triglyceride accumulation (Figure 8.9b) [32]. Thus, AQP7 knockout mice exhibit whole-body insulin resistance associated with obesity. In summary, a deficiency of adipose AQP7 influences not only glycerol metabolism, but also glucose metabolism *in vivo*.

8.4
Hepatic Glycerol Channel: AQP9

8.4.1
AQP9: A Putative Hepatic-Specific Glycerol Channel

AQP9 was independently identified in human leukocytes and liver [37], and rat liver [38, 39]. AQP9 is also highly expressed in mouse liver and testes (Figure 8.4). In hepatocytes, immunohistochemistry showed that AQP9 is localized at the sinusoidal plasma membrane [40]. Rat AQP9-expressing *Xenopus* oocytes exhibit water and glycerol permeability [37, 39]. A series of studies on *Xenopus* oocytes also demonstrated that rat AQP9 permeates urea, mannitol, sorbitol, and uracil. Consistent with this finding, another group found that rat AQP9 is permeable to water, glycerol, and urea [39]. These results indicate a broad selectivity of rat AQP9. However, another group found AQP9-induced permeability to be restricted to water and urea in humans [37]. Thus, there are conflicting results regarding AQP9-induced permeability between rats and humans.

Intra-abdominal visceral fat accumulates mainly in the mesentery [2–4]. 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 [2–4]. FFA derived from visceral fat during lipolysis elevates liver acyl-CoA synthetase and microsomal triglyceride transfer protein mRNA levels, and reduces degradation of apolipoprotein B. These changes induce the release of apolipoprotein B from the liver and increase plasma triglyceride concentrations. Hypertriglyceridemia, which is often observed in subjects with visceral fat accumulation, is partly accounted for by the increase in FFA derived from adipose tissues [41]. Glycerol, which is another product from adipose triglyceride during lipolysis, flows directly into the liver via the portal vein and becomes a substrate for gluconeogenesis. The liver has glycerol kinase activity and can activate glycerol to use it for gluconeogenesis [34, 36]. Expression of AQP1, 8 and 9 was identified in the liver (Figure 8.4). The only aquaglyceroporin among these AQPs is AQP9 (Figure 8.3a). AQP9 is considered as the sole glycerol channel in liver and is localized at the sinusoidal plasma membrane facing the portal vein [40]. Taken together, AQP9 may act as a channel of glycerol uptake in the liver (Figure 8.2). AQP9 mRNA levels increase by fasting and decrease by feeding [42]. 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 a time- and dose-dependent manner in H4IIE rat hepatocytes. Administration of STZ results in increased AQP9 mRNA [42] and protein [43] levels in insulin-insufficient mice. Promoter analysis demonstrates that insulin closely reduces AQP9 mRNA at the transcriptional level through IRE on AQP9 promoter, as in the regulation of AQP7 by insulin.

8.4.2
Gluconeogenesis through AQP9 – Lessons from AQP9-Deficient Mice

Another group generated AQP9 knockout mice and analyzed their phenotype [44]. In the absence of physiological stress, knockout mice did not display any visible behavioral or severe physical abnormalities. Compared with control mice, plasma levels of glycerol and triglycerides were markedly increased in AQP9 knockout mice, whereas glucose, urea, FFAs, and cholesterol were not significantly different. Oral administration of glycerol to fasted mice resulted in an acute rise in blood glucose levels in AQP9 knockout mice, revealing no defect in utilization of exogenous glycerol as a gluconeogenic substrate and indicating a high gluconeogenic capacity in nonhepatic organs. In summary, AQP9 is important for hepatic glycerol metabolism, and may play a role in glycerol and glucose metabolism in diabetes mellitus.

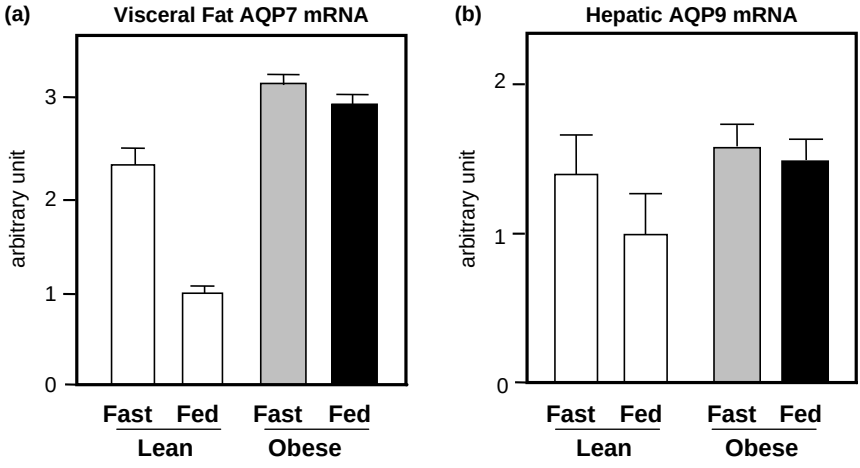

Figure 8.10 Overexpression of adipose AQP7 and hepatic AQP9 in obesity. Total RNA (10 μg) of visceral fat and liver in obese db^+/db^+ ($n=8$) and lean db^+/db^+ ($n=8$) mice were analyzed by Northern blotting. Columns and bars represent the mean ± standard error of the results. Two aquaglyceroporins, adipose AQP7 (a) and hepatic AQP9 (b), are coordinately regulated. Both AQPs are upregulated under starvation and downregulated after feeding. However, downregulation of both AQP7 and AQP9 expression is blunted and remains high in obesity [42].

8.5
Coordination of Adipose Glycerol Channel, AQP7, and Hepatic Glycerol Channel, AQP9

In the feeding state, an increase in plasma insulin concentration results in suppression of lipolysis and the mRNA expression of adipose AQP7, and in reduced glycerol release from adipocytes (Figure 8.10) [10, 22, 42]. Feeding also reduces hepatic AQP9 mRNA and glycerol-based gluconeogenesis (Figure 8.11) [42]. In the physiological feeding state, high plasma insulin coordinately suppresses the AQP7 mRNA for glycerol release from adipocytes and AQP9 in mRNA for glycerol uptake into liver, through the IRE in the AQP7 and AQP9 gene promoter, respectively [10, 42].

8.6
Dysregulation of AQP7 and AQP9 in Obesity with Insulin Resistance

We also studied the regulation of AQP7 and AQP9 in obesity. Both adipose AQP7 and hepatic AQP9 were suppressed in the fed state of lean mice through insulin

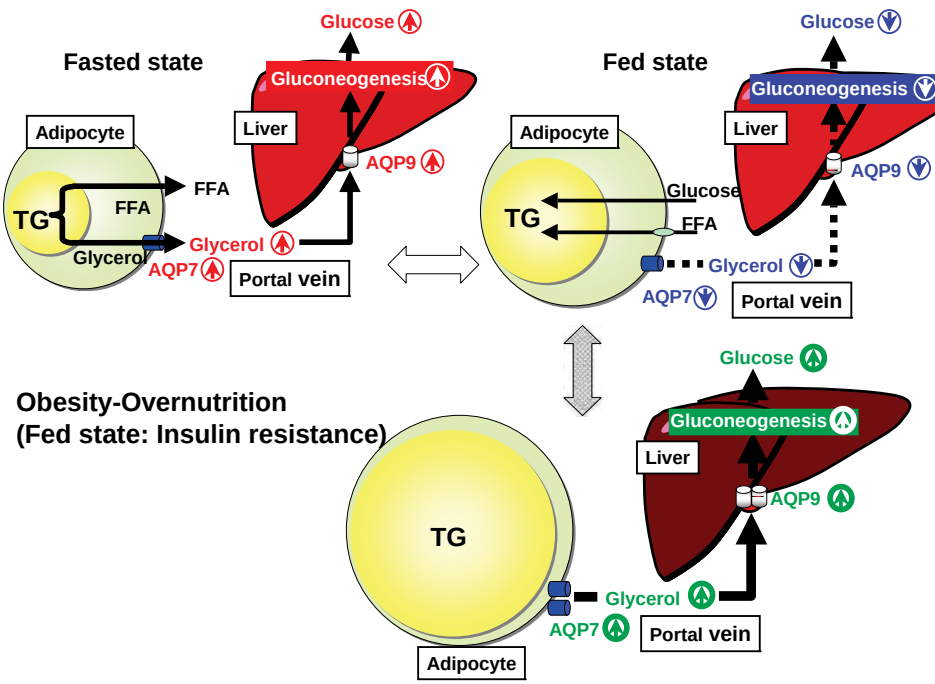

Figure 8.11 Schematic presentation of physiological and pathophysiological involvement of adipose AQP7 and hepatic AQP9. The coordinated regulation of AQP7 and AQP9 in the physiological fasted and fed states may lead to systemic glucose metabolism via adipose-derived glycerol. In contrast, in the pathological fed state of insulin resistance, the coordinated augmentation of AQP7 and AQP9, in spite of hyperinsulinemia, augments the utilization of glycerol for hepatic glucose production [42]. TG, triglyceride.

(Figure 8.10) [10, 42]. However, the suppression of the two AQPs is impaired in obese and insulin-resistant animals in spite of hyperinsulinemia, resulting in portal hyperglycerolemia, which at least partly participates in the systemic hyperglycemia (Figures 8.10 and 8.11) due to more utilization of adipose-derived glycerol for hepatic glucose production and release [42]. Considered collectively, physiological and pathological coordinated regulation of organ-specific glycerol channels, adipose AQP7 and hepatic AQP9, may contribute to glycerol and glucose metabolism (Figure 8.11) [42].

8.7 Conclusions

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. Glycerol release is enhanced with increased expression of adipose AQP7 to maintain glucose levels during starvation. However, in AQP7 knockout mice glycerol release is disturbed and causes hypoglycemia [23, 31, 32, 45–47]. Take together, we demonstrated that AQP7 serves as a glycerol channel in adipocytes [23]. Investigation of AQP-dependent glycerol metabolism should provide a pivotal insight for the design of novel therapeutic strategies to combat metabolic syndrome [48–51].

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