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1 Review

2 **Involvement of aquaglyceroporins in energy metabolism in health and** 3 **disease**

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13 disorders, drug targets.

14 **Abstract**

15 Aquaglyceroporins are a group of the aquaporin (AQP) family of transmembrane water channels.
16 While AQPs facilitate the passage of water, small solutes, and gases across biological membranes,
17 aquaglyceroporins allow passage of water, glycerol, urea and some other solutes. Thanks to their
18 glycerol permeability, aquaglyceroporins are involved in energy homeostasis. This review provides an
19 overview of what is currently known concerning the functional implication and control of
20 aquaglyceroporins in tissues involved in energy metabolism, i.e. liver, adipose tissue and endocrine
21 pancreas. The expression, role and (dys)regulation of aquaglyceroporins in disorders affecting energy
22 metabolism, and the potential relevance of aquaglyceroporins as drug targets to treat the alterations of
23 the energy balance is also addressed.

24 **1. Introduction**

25 AQPs are a family of transmembrane protein channels featuring six transmembrane helices
26 surrounding a narrow water-permeable channel in which water flows in a single file [1]. In biological
27 membranes, AQPs assemble in tetramers, creating a fifth narrow channel through the center that was
28 suggested to be permeable to CO₂ [2]. Thirteen mammalian AQPs have been identified and named
29 AQP0 to AQP12 [3,4]. AQPs structure feature an internal tandem repeat of three transmembrane
30 segments and a pair of highly conserved signature asparagine-proline-aspartate (NPA) motifs forming
31 a pore and defining the permeability selectivity [3,5,6].

32 According to their sequences homologies and permeability, mammalian aquaporins are grossly
33 subdivided into three main groups: (i) *orthodox aquaporins* (AQP0, AQP1, AQP2, AQP4, AQP6, and
34 AQP8), homologues that were initially thought to only allow the passage of water, (ii)
35 *aquaglyceroporins* (AQP3, AQP7, AQP9, and AQP10), homologues allowing the passage of water,
36 glycerol and some small solutes, and (iii) *unorthodox aquaporins* (or *superaquaporins*; AQP11 and
37 AQP12), two AQPs characterized by their divergent evolutionary pathway, and whose channel
38 selectivity is still matter of debate. However, this classification is not exhaustive since additional
39 solutes can pass through, sometimes even more efficiently than the molecules that were initially

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4 40 considered to be the only substrates. AQP3, AQP6, AQP8, and AQP9 are also permeated by ammonia
5 41 and are also termed *ammoniaporins* [7]. AQP1, AQP3, AQP5, AQP8, AQP9, and AQP11 also allow
6 42 the passage of hydrogen peroxide, and are therefore called *peroxiporins* [8–11]. Moreover, AQP7 and
7 43 AQP9 have been reported to move arsenite [12], AQP6 to be permeable to nitrate and halide ions
8 44 [13,14], AQP1 to be a gated ion channel [15], and AQP9 to facilitate monocarboxylate passage [16].
9 45 Some AQP channels have also been reported to facilitate transmembrane movement of gases of
10 46 biological relevance such as NO, CO₂ or O₂ [17–19]. The biological significance of the passage of
11 47 these other molecules remains largely unknown and object of debate.

12 48 In light of their expression in many mammalian tissues and organs, AQPs ensure diverse roles in
13 49 healthy and pathological conditions [3,4,20]. Among the functions ascribed to aquaglyceroporins in
14 50 the various organs in which they are expressed, important roles are played in liver, adipose tissue and
15 51 endocrine pancreas where they participate to energy metabolism. By facilitating the uptake/release of
16 52 glycerol, substrate used for the synthesis of triacylglycerols (TAG) and for gluconeogenesis depending
17 53 on the states of energy balance, aquaglyceroporins have important implications in the control of fat
18 54 accumulation in adipose tissue and liver, in glucose homeostasis and oxidative stress [21–25]. In
19 55 addition, in endocrine pancreas, AQP7-mediated glycerol uptake plays a role in β -cell insulin secretion
20 56 [26–28].

21 57 This review provides the current state of knowledge concerning the involvement of
22 58 aquaglyceroporins in energy metabolism by focusing on their expression, role and regulation in liver,
23 59 adipose tissue and endocrine pancreas. In addition, the review provides an overview of the role of
24 60 aquaglyceroporins in disorders affecting energy metabolism, the consequences of disorders on
25 61 aquaglyceroporins expression and function, and the main pharmacological tools available to target
26 62 aquaglyceroporins.
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29 64 **2. Structure and characteristics of human aquaglyceroporins**

30 65 The overall AQP structure revealed by multiple studies confirmed the existence of the initially
31 66 suggested “hourglass model” [29]. In accordance with this model, each AQP monomer forms a pore
32 67 composed of six transmembrane helices connected by five loops (A to E loops; two intracellular and
33 68 three extracellular). At the vicinity of the extracellular channel mouth, the so-called aromatic/arginine
34 69 selectivity filter (ar/R SF) featuring the most constricted part of the pore controls the passage of
35 70 molecules based on their size [30]. A arginine residue, highly conserved among AQPs and charged
36 71 positively, is located in a typically aromatic sidechain environment excluding the passage of protons
37 72 while accommodating the passage of glycerol and urea [31,32]. Underneath, two highly conserved
38 73 NPA motifs present in the B and E loops and half-helices contribute to the formation of the pore center
39 74 and ensure the exclusion of charged solutes [5,33–37]. Aquaglyceroporins contain two additional
40 75 peptide spans located within the second and third extracellular loops as compared to orthodox AQPs,
41 76 and two relatively conserved NPA motifs in which the presence of an aspartic acid (D) in the second
42 77 NPA motif expands the pore to allow the passage of larger solutes such as glycerol [38,39].
43 78 Aquaglyceroporins features an ar/R SF region wider than other AQPs, allowing the passage of larger
44 79 solutes [40]. Recent studies have confirmed that AQPs pore size determines exclusion properties but
45 80 not solute selectivity [41]. Despite groundbreaking studies on AQPs structure and better structural
46 81 insights into AQPs selectivity [1], structural information concerning aquaglyceroporins remains quite
47 82 limited mainly in reason of to the absence of crystallized proteins.
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49 83 **2.1. AQP3**

50 84 To our knowledge, the human AQP3 protein crystal structure has not been obtained. However,
51 85 structural studies have been conducted using a homology model of tetrameric AQP3 built based on the
52 86 crystal structure of the glycerol uptake facilitator protein (GlpF) from *Escherichia coli* [38,42–44].
53 87 *Xenopus laevis* oocytes and cells expressing AQP3 and atomistic modelling and large-scale simulations
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4 88 have established the permeability of AQP3 to water, glycerol, urea and H₂O₂ [45–52]. AQP3
5 89 permeability to urea has been the subject of some controversies [53], but recent data indicate the lack
6 90 of urea permeability of human AQP3 expressed in HEK293 cells [41]. A more recent study revealed
7 91 the mechanisms of AQP3 water and glycerol permeation at the atomistic level using metadynamic
8 92 approaches [35]. Both single-file water and glycerol permeate human AQP3 in a bidirectional way in
9 93 the absence of osmotic gradient, with free energy for permeation smaller for water than for glycerol
10 94 [35]. The bidirectional water flux is disrupted consequently to glycerol molecules permeating the pore
11 95 [35]. Although ar/R SF being the narrowest part of the pore, the NPA represents an energy barrier for
12 96 both water and glycerol permeation [35]. Water and glycerol make hydrogen bonds with amino acids
13 97 located within the ar/R SF (Y212, A213 and R218), the NPA (N83, N215) and the cytoplasmic pocket
14 98 (H81, E191) [35]. Human AQP3 point mutation Y212A affected glycerol but not urea permeability,
15 99 while combined point mutations G203H/Y212A abolished both glycerol and urea permeabilities, and
16 100 point mutation G203H reduced protein expression [41].

2.2. AQP7

21 101 The AQP7 sequence has the particularity to contain conserved NPA motifs modified to NAA (in
22 102 positions 94-96) and NPS (in positions 226-228) for the first and second motifs, respectively [54,55].
23 103 *Xenopus laevis* oocytes or cells expressing rat, mouse or human AQP7 permeate water, as well as
24 104 glycerol, urea and H₂O₂ [50,56,57]. Compared to orthodox AQPs, AQP7 displays lower water
25 105 permeability in cell-based assays [58,59] but similar water permeability in proteopolymersome assay
26 106 performed using a glycerol-free set up [60], or reduced water permeability in the presence of glycerol
27 107 using molecular dynamics simulations [61]. The AQP7 structure has been elucidated in recent works
28 108 using X-ray crystallography and molecular-dynamics stimulations [57,61]. Human AQP7 displays a
29 109 pore of around 3 Å of diameter at the selectivity filter [61] comprising amino acid residues F74, G222,
30 110 Y223 and R229 [57]. AQP7 tetramers contain two monomers with three molecules of glycerol and two
31 111 monomers with two molecules of glycerol within the pore [57]. The AQP7 structure is quite flexible
32 112 as its F74 residue undergoes rapid conformational changes from a closed to an opens state, allowing
33 113 the passage of glycerol without strong orientation restriction [57], with a partly rotating movement that
34 114 may serve to break hydrogen bonds and allow its movement along the channel pore [61]. Water
35 115 molecules pass along glycerol molecules to allow glycerol permeation as both molecules compete for
36 116 hydrogen-bonding sites in the pore [62]. This competition mechanism along with the rotation of
37 117 glycerol might facilitate glycerol release [61]. Studies of heterologous expression of human AQP7 in
38 118 yeast displayed similar glycerol permeability for this aquaglyceroporins at pH values ranging between
39 119 6 and 8 [58,59]. However, at acidic pH (pH 5), both glycerol [58,63] and water permeabilities were
40 120 reduced with no change in pore size [63]. The reduction of glycerol permeability of AQP7 at acidic pH
41 121 was suggested to be due to the protonation of Y135 and H165 rather than a true gating mechanism.
42 122 Furthermore, the presence of some pH-sensitive amino acids at the monomer-monomer interface of
43 123 the tetramer suggests that the decreased permeability of AQP7 results from the cooperativity between
44 124 AQP7's monomers [63].

2.3. AQP9

50 125 Human AQP9 has been shown to be permeable to water as well as to glycerol, urea and H₂O₂
51 126 [12,41,50,56,64,65]. Despite the lack of structure for human AQP9, two-dimensional crystallization of
52 127 rat AQP9 indicates that monomers form a tetrameric structure [39]. An homology model of tetrameric
53 128 AQP9 built based on the crystal structure of the bacterial glycerol uptake facilitator protein (GlpF)
54 129 reveals the conserved pore residues (G80, H82, N84, N216, R219 in rat AQP9) located at the faces
55 130 presenting groups involved in formation of hydrogen bonds, predominantly in the center of the
56 131 channel, while the residues conserved only among all aquaglyceroporins (I60, V68, L172, I196, I200,
57 132 G208, G212, A214, M215) located within helices 2, 4 and 5 and loop E all sit in the center of the
58 133 channel on its hydrophobic portion [39]. In addition, residues forming portion of the channel and
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conserved among all AQP9 homologs (F64, G81, M91, V176, F180, L209, C213) fall into the hydrophobic face of the pore and are located within helices 2 and 4, loops B and E, and pore helix HB [39]. Proton-exclusion mechanism likely regulates solute permeation in AQP9 similarly to other AQPs as the constricted region of the pore in all AQP9 is conserved similarly to AQPs [39]. Large solute permeation across AQP9 may require solute-induced change in the pore region [39].

2.4. AQP10

Human AQP10 expressed in HEK293 cells exhibit some permeability to glycerol and urea [41], while permeability to H₂O₂ remains to be determined. The relatively recent crystal structure determination of AQP10 unveiled its unique characteristic to be stimulated by pH reduction as it displays a pH-dependent glycerol-specific gating at the intracellular interface, in contrast to other AQPs [60].

In-depth unveiling of the aquaglyceroporins structure contributes to better understanding of diseases and provides additional perspectives for the design of new therapeutic tools.

3. Hepatic aquaglyceroporins

Once imported into hepatocytes glycerol is phosphorylated leading to glycerol-3-phosphate (G3P), an essential metabolite in fat accumulation being required for TAG synthesis and gluconeogenesis occurring during fasting or exercise thereby being of critical importance for glucose homeostasis [66]. Glycerol derived from the diet, lipolysis or reabsorbed at the proximal renal tubules contributes to blood free glycerol levels. Furthermore, cell glycerol content results from the conversion of G3P arising from glycolysis, or the conversion of pyruvate, lactate and alanine through a pathway called *glyceroneogenesis* that occurs in the white and brown adipose tissues, and in the liver to sustain TAG synthesis, especially when TAG metabolism is augmented [66].

Liver exerts a central function in glycerol metabolism, considering its predominant (70–90%) contribution to the whole-body glycerol metabolism [66]. The use of hepatic glycerol implies sexual dimorphism. Indeed, glucose metabolism and lipid metabolism predominate in men and women, respectively. Under physiological conditions, glycerol metabolism is gender-dependent, especially during hypoglycemia, fasting or exercise [67] (see section 3.1). The liver express all four aquaglyceroporins with difference in term of levels and cellular and subcellular localization. Among these aquaglyceroporins, AQP9 is the most abundantly expressed. Table 1 summarizes some characteristics of liver aquaglyceroporins.

Table 1. Localization and suggested roles of hepatic aquaglyceroporins.

Aquaglyceroporin	Permeability	Cell type	Subcellular location	Suggested role	References
AQP3	Water, glycerol, hydrogen peroxide, ammonia, urea	Kupffer cells (h)	Undefined	Repopulation of Kupffer cells in liver regeneration	[68]
		Hepatocytes (h)	Undefined	Uptake of glycerol (?)	[69]
		Stellate cells (h)	Undefined	Cell activation	[70]

AQP7	Water, glycerol	Hepatocytes (h, m)	IC; others?	TAG synthesis; uptake of glycerol (?)	[69]
		Cholangiocytes (h)	APM	Unknown	[68]
		Endothelial cells (h)	APM	Unknown	[68]
AQP9	Water, glycerol and other polyols, hydrogen peroxide, lactate, ammonia, arsenite, urea and some other uncharged solutes	Hepatocytes (h, m, r)	BLPM	Uptake of portal glycerol for gluconeogenesis and TAG synthesis; import of water from portal blood (bile formation); urea extrusion; arsenic detoxification; liver regeneration	[71–77],
		Cholangiocytes (h)	Undefined	Unknown	[68]
AQP10	Water, glycerol, urea (?)	Hepatocytes (h)	Undefined	Unknown	[69]

APM, apical plasma membrane; BLPM, basolateral plasma membrane; h, human; IC, intracellular; m, mouse.

3.1. Expression in human and rodents

AQP9 is located at the sinusoidal plasma membrane of rodent and human hepatocytes [71,78]. In rat liver, the AQP9 expression is stronger in the pericentrolobular region of the hepatic lobule than in the periportal area [73,79]. Males exhibit higher hepatic expression of AQP9 than females both in rodents and human [22,80,81].

At a lesser extent compared to AQP9, human hepatocytes also express the other three aquaglyceroporins, AQP3, AQP7, and AQP10 [68,69]. Hepatic transcript levels of AQP3 are higher in women than men whereas no sexual dimorphism in the liver transcriptional levels have been observed regarding AQP7 and AQP10 [69]. AQP3 is also reported in human hepatic stellate cells (HSC) [82] and in human Kupffer cells [68].

3.2. Function

3.2.1 AQP9

AQP9 is the main pathway through which glycerol is imported from portal blood [72,83], a not insignificant aspect given that the glycerol passage through the membrane limits the rate of glycerol use by the liver [84]. Once imported into the cell, glycerol is promptly converted into G3P by the glycerol kinase. G3P is of particular importance as substrate for *de novo* synthesis of glucose (gluconeogenesis) during short term starvation [85] and for TAG synthesis during feeding [75]. As expected, liver glycerol permeability is reduced in *Aqp9*-depleted (*Aqp9*^{-/-}) mice, while plasma glycerol and TAG levels are increased [73,83]. Likely due to its broad selectivity, additional roles for AQP9 in the liver have been suggested in primary bile formation [74,86] and hepatic extrusion of catabolic urea [76]. In liver, AQP9 also mediates excretion of arsenic [77]. AQP9 has also been immunodetected in

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4¹⁹³ the cholangiocytes lining mouse intrahepatic bile ducts [68], but the related physiological meaning is
5¹⁹⁴ still elusive.

6¹⁹⁵ 3.2.2 AQP3, AQP7 and AQP10

7¹⁹⁶ The functional role of AQP3, AQP7, and AQP10 in human hepatocytes, if any, remains to be proven.
8¹⁹⁷ A role for AQP3 in lipogenesis of quiescent HSC was suggested after finding that its expression
9¹⁹⁸ decreases during cell activation [70,82]. In human Kupffer cells AQP3 has been reported to be involved
10¹⁹⁹ processes occurring during liver regeneration liver diseases (such as cell repopulation, migration,
11²⁰⁰ cytokine secretion) [68]. Overall, the roles of AQP3, AQP7, and AQP10 in liver remain to be assessed
12²⁰¹ by additional studies.

13²⁰² 3.3. Regulation

14²⁰³ In rodents, hepatocyte *Aqp9* is transcriptionally downregulated by insulin [87], explaining why
15²⁰⁴ rodent models of type 1 diabetes [88] and subjects with insulin resistance [69] show increased hepatic
16²⁰⁵ levels of AQP9 as well as the diminished glycemia (-10–40%) of obese diabetic leptin receptor-
17²⁰⁶ deficient db/db mice lacking *Aqp9* [73]. The hepatic glucose metabolism has been modeled as
18²⁰⁷ multiple steps integrating the hepatocyte uptake and handling of glycerol in the glucose-insulin axis as
19²⁰⁸ a function of the glycogen stores, plasma glycerol and glycerol permeability to hepatic AQP9 protein
20²⁰⁹ levels [89]. The model can assess the contribution of AQP9 to murine liver glycerol permeability over
21²¹⁰ time during the course metabolic status [89]. Hepatocyte AQP9 is also regulated transcriptionally by
22²¹¹ leptin [22,75]. Unexpectedly, leptin and insulin regulate AQP9 transcription differently in rodents and
23²¹² humans [22,90]. In the human hepatocellular carcinoma cell line HepG2, insulin and leptin were seen
24²¹³ to increase and decrease the expression of AQP9. This was reported to occur through the activation of
25²¹⁴ the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR)
26²¹⁵ signaling cascade [75] and AMP protein kinase (AMPK), via forkhead box a2 (Fox a2) [91,92].
27²¹⁶ However, the question regarding the insulin and leptin regulation of hepatocyte AQP9 in human
28²¹⁷ remains open and needs further investigation since no AQP9 expression was found in a recent work
29²¹⁸ using HepG2 cells [78]. Fasting-induced increase of AQP9 in rat liver was found to be 2.6 times higher
30²¹⁹ in males than females, in line with the concurrent observation that during fasting, male rats showed
31²²⁰ unmodified plasma glycerol level whereas the plasma glycerol levels were increased in the female
32²²¹ counterpart [93], and with the liver glycerol permeability that was higher in males. Ovariectomized
33²²² rats behave similarly to male rats. These *in vivo* findings and the *in vitro* data obtained on hepatocytes
34²²³ treated with estrogen infer that sexual dimorphism account for differential AQP9 regulation during
35²²⁴ fasting and concomitant higher plasma glycerol levels in female [93]. The peroxisome proliferator-
36²²⁵ activated receptor α (PPAR α) downregulated AQP9 in rat male periportal hepatocytes [94].
37²²⁶ Scarce is the knowledge about the regulation of AQP3, AQP7 and AQP10 in the healthy liver. In
38²²⁷ HepG2 hepatocytes, AQP3 and AQP7 were found to be upregulated and downregulated, respectively,
39²²⁸ by leptin [75].

40²²⁹ 3.4. Metabolic disorders

41²³⁰ Due to their role in mediating the membrane passage of glycerol, liver aquaglyceroporins,
42²³¹ especially AQP9, are of pathophysiological relevance in liver metabolic disorders (Table 2). Seen their
43²³² peroxiporin activity, roles for AQP9 and AQP3 cannot be ruled out in disorders of the redox balance
44²³³ and inflammatory diseases.

45²³⁴ **Table 2.** Suggested pathophysiological roles of liver aquaglyceroporins in energy balance disorders.
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Aquaglyceroporin	Cell type	Pathophysiological involvement	References
AQP3	Kupffer cells (h)	Cell migration and secretion of pro-inflammatory cytokines	[68]
	Hepatocytes (h)	Insulin resistance (?)	[75]
	Stellate cells (h)	NAFLD, NASH, fibrosis and cancer (PNPLA3 I148M variant)	[70]
AQP7	Hepatocytes (h, m)	Insulin resistance	[75]
	Cholangiocytes (h)	Unknown	
	Endothelial cells (h)	Unknown	
AQP9	Hepatocytes (h, m, r)	NAFLD; NASH; obesity; T2D; hepatocellular carcinoma	[22,69,95–99]
	Cholangiocytes (h)	Unknown	
AQP10	Hepatocytes (h)	Unknown	

h, human; m, mouse; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing 3; r, rat; TAG, triacylglycerols; T2D, type 2 diabetes mellitus.

Non-alcoholic fatty liver disease (NAFLD) is a complex disease characterized by ectopic accumulation of TAG in hepatocytes which varies from simple fatty liver to non-alcoholic steatohepatitis (NASH) and to cirrhosis [100]. NAFLD is frequently associated with the metabolic syndrome often accompanied by obesity and diabetes mellitus with insulin resistance. Involvement of hepatocyte AQP9 has been reported in human NAFLD as well as in studies using rodent or cell models of NAFLD [69,95–97,99]. The decreased AQP9 expression seen in obesity and/or diabetes could contribute to decreasing the substrate availability for *de novo* TAG synthesis and limiting fat overaccumulation in hepatocytes and to reducing gluconeogenesis to hamper the aggravation of hyperglycemia [22,69,97]. Despite comparable levels of liver AQP9 expression, obese women diverged from obese men by their lower liver glycerol permeability [69]. This may reflect the lower incidence of insulin resistance and NAFLD in women than men. However, no relationship was found between hepatic AQP9 expression and extent of liver steatosis in patients with morbid obesity [101]. Also, *Aqp9* knockdown in db/db mice did not change the hepatic fat content [102]. These results are not consistent with a study using a cell model of NAFLD where silencing of AQP9 prevented or alleviated the degree of intracellular steatosis [103]. A work with male and female mice fed for 24 weeks with a high-fat diet showed that only males displayed liver steatosis without significant modification in AQP9 expression [81]. The originating factors and onset of the fatty liver disease, pathogenic pattern and gender of the studies subjects and animals should be taken into account to appreciate the involvement of AQP9 in the disease. Further work is needed to precisely determine the etiopathological implication and modulation of AQP9 in fatty liver disease. Liver AQP9 represents an

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4²⁶¹ interesting potential drug target for therapeutic intervention in NAFLD and NASH [24,104]. Although
5²⁶² AQP9 did not appear very relevant in hepatic steatosis of alcoholic origin, modification in AQP9-
6²⁶³ induced glycerol permeability in response to the binding of acetaldehyde, produced by the oxidation
8²⁶⁴ of ethanol by alcohol dehydrogenase, to the hepatocyte plasma membrane [105].

9²⁶⁵ Estrogen treatment was found to protect mice against ovariectomy-induced hepatic steatosis by
10²⁶⁶ increasing the expression of hepatocyte AQP7. Therefore, AQP7 could become a therapeutic
11²⁶⁷ HSCtarget for the prevention or treatment of liver steatosis in postmenopausal women [106].

12²⁶⁸ PNPLA3 (adiponutrin) I148M variant has been associated with NAFLD, NASH, fibrosis and
14²⁶⁹ cancer [70]. AQP3 was found to be strongly reduced in human HSC carrying the PNPLA3 I148M
15²⁷⁰ polymorphism paralleling the decrease in PPAR γ activation, which could be rescued by the PPAR γ
16²⁷¹ agonist rosiglitazone and the inhibition of JNK. This observation leads to the idea that targeting AQP3
18²⁷² within HSCs activation may be a strategy to treat liver fibrosis in PNPLA3 I148M patients.

20²⁷⁴ 4. Adipocyte aquaglyceroporins

21²⁷⁵ Aquaglyceroporins play important roles in the physiology of energy metabolism in adipose tissue.
22²⁷⁶ Indeed, upon feeding, glycerol enters the adipocytes and participate to the synthesis of TAG, while
23²⁷⁷ upon fasting glycerol exits to the bloodstream upon lipolysis activation. It is therefore no surprise that
24²⁷⁸ aquaglyceroporins have been the center of special attention in adipocytes. This review provides a
25²⁷⁹ concise and explicit overview of the various facets of aquaglyceroporins in adipocytic glycerol
26²⁸⁰ metabolism. Additional information on the subject can be found in previous reviews on the subject (see
27²⁸¹ [23,24,107–112].

30²⁸² 4.1. Expression

31²⁸³ 4.1.1. Human expression

32²⁸⁴ Human adipocytes express AQP7 [55,113] and both *Aqp7* mRNA and protein levels are
33²⁸⁵ upregulated upon differentiation of human preadipocytes into adipocytes [114]. Some controversy
34²⁸⁶ exists concerning the location of AQP7 to endothelial cells surrounding adipocytes only [93], or also
35²⁸⁷ to adipocytes [115]. The expression of AQP3 [75], AQP9 [75] and AQP10 [115] in human adipocytes
36²⁸⁸ has not reached a shared consensus. Indeed, some studies could not detect the expression of both AQP3
37²⁸⁹ and AQP9 [114] or only AQP9 [78]. Worth notifying that AQP11, an unorthodox homologue allowing
38²⁹⁰ the passage of glycerol, is also expressed in human fat cells [116,117].

41²⁹¹ 4.1.2. Mouse expression

42²⁹² Mouse adipocytes express AQP7 [55,118] but appear to lack AQP3 and AQP9 expression
43²⁹³ [119,120]. As in human, the expression of AQP7 has been located in endothelial cells surrounding
44²⁹⁴ adipocytes only [121], or also in adipocytes [122]. Upon differentiation of 3T3-L1 cells into
45²⁹⁵ adipocytes, both *Aqp7* mRNA and protein levels are upregulated [122–126], while only AQP3 and
46²⁹⁶ AQP9 protein levels are upregulated [124–126]. In some cases, AQP3 and AQP9 expression could not
47²⁹⁷ be detected in adipocytes differentiated from 3T3-L1 cells [119].

48²⁹⁸ In addition, AQP11 is expressed in mouse adipocytes [116,125]. AQP10 expression is irrelevant in
49²⁹⁹ mouse as it is a nonfunctional pseudogene in that species [127].

50³⁰⁰ Beige and white adipocytes differentiated from 3T3-L1 cells display *Aqp7*, *Aqp3* and *Aqp9* mRNA
51³⁰¹ levels, while only AQP9 protein levels were detected [128]. Time-course analysis of aquaglyceroporins
52³⁰² mRNA levels during the differentiation process revealed significant increase in *Aqp7* and *AQP9*
53³⁰³ mRNA levels during the differentiation into white adipocyte[128].

54³⁰⁴ 4.1.3. Rat expression

55³⁰⁵ Rat adipocytes express AQP3, AQP7, and AQP9, while the expression of AQP10 has not been
56³⁰⁶ reported [69,129].

60³⁰⁷ 4.2. Regulation

4.2.1. Trafficking mechanism

Protein trafficking plays a key role in the regulation of aquaglyceroporins function. Indeed, aquaglyceroporins localized in subcellular vesicles translocated to the plasma membrane (to ensure transcellular glycerol passage) upon stimulation with insulin and hormones inducing the activation of cAMP signaling pathway [75,115,122,130,131].

cAMP-inducing hormones or agents induced translocation of AQP3, AQP7 and AQP10 from intracellular vesicles to the plasma membrane, but had no effect on AQP9 localized at the membrane [75,115]. Upon insulin stimulation of 3T3-L1 adipocytes, AQP3 and AQP7 localization was ntmostly prese surrounding the lipid droplets and AQP9 localization increased at the plasma membrane [75].

4.2.2. Transcriptional mechanism

The transcription of aquaglyceroporins is regulated by hormones (i.e, insulin, catecholamines, steroids) and inflammatory mediators (i.e, TNF α and lipopolysaccharides) [75,113,118,125,132]. Both human and mouse *Aqp7* transcription is upregulated by peroxisome proliferator-activated receptor gamma (PPAR γ) [118,133] and modulated by the Küppel-like factor 15 (KLF-15), a transcription factor playing a role in lipogenesis [134].

4.2.3. Epigenetic mechanism

DNA methylation at GpG dinucleotides, one of the epigenetic mechanisms by which cells modulate gene expression, can regulate *Aqp7* expression [135]. The presence of high proportion of methylated GpG-sites in *Aqp7* gene promoter correlated with a downregulation of the gene expression [136]. While some miRNAs can interact with some aquaglyceroporins, their role in aquaglyceroporins regulation in adipose tissue remains to be investigated [137,138]. Further analyses will be required to shed more light on the role of epigenetic mechanisms in aquaglyceroporins regulation in adipose tissue.

4.2.4. pH-induced mechanism

So far, all aquaglyceroporins, with the exception of AQP9, have been reported to be regulated by a pH-dependent gating mechanism.

Indeed, rat AQP3 glycerol passage was significantly lower at pH 6.0 and completely abolished at pH below 5.7 [47,139], while another study showed it was unmodified at pH in the range 6-7 [140]. Extracellular pH reduction also decreased AQP3 water permeability, involving H53, Y124 and H154 residues [141]. The investigation of the molecular mechanisms responsible for pH-dependent AQP3 gating disclosed important structural features [139].

Human AQP7 displayed similar glycerol permeability at both high and low pH (pH range 6-8) [58,59]. In contrast, at pH 5, that may not be quite meaningful physiologically, glycerol [58,63] and water permeabilities are reduced, but not the pore size [63]. The reduction in glycerol permeability of AQP7 may be consecutive to the protonation of Y135 and H165 rather than a true gating mechanism. In addition, the protonated residues may be responsible for a cooperativity change between the AQP7 monomers within the tetramer that alters permeability [63].

Human AQP10 displays a pH-dependent glycerol-specific gating at the intracellular interface, in contrast to other AQPs [60]. Upon double protonation induced by low pH exposure, the amino acid H80 played a critical role in the regulation as it acted as the pH-sensor propagating conformational changes leading to the opening of AQP10 [60]. Indeed, upon H80 protonation, H80 reorients and stabilizes with E27 prior to leading to a structural shift of F85 and the rearrangement of the loop (containing V76-S77) in conjunction with R94 to allow the passage of glycerol [60].

4.2.5. Phosphorylation mechanism

Current knowledge about phosphorylation of aquaglyceroporins is very limited. Indeed, only human AQP3 was shown to be phosphorylated upon cAMP-induced hormone on T514 and this phosphorylation paralleled the increase in AQP3 trafficking to the plasma membrane in an intestinal

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4354 epithelial cell line [142]. It remains to be determined whether other aquaglyceroporins can undergo
5355 phosphorylation upon hormone stimulation in parallel with their trafficking.

6356 **4.3. Function**

8357 Adipose tissue ensures the synthesis and storage of TAG during feeding, and their degradation and
9358 release during fasting [66,143]. These processes require transmembrane passage of glycerol through
10359 aquaglyceroporins. As such, aquaglyceroporins participate to energy metabolism. The function of
11360 aquaglyceroporins has been deciphered using aquaglyceroporin knockout animals, adipocyte
12361 differentiation models, and adipose tissue samples. The role played by AQP7 in adipocytic glycerol
1362 release has been unveiled using differentiated adipocytes [119] and *Aqp7* knockout mice
14362 [120,144,145]. In addition, AQP3 could contribute to cell glycerol exit during lipolysis, and AQP9
15363 could allow cell glycerol entrance during TAG synthesis [75]. The involvement of AQP9 in adipocyte
16364 glycerol uptake relies on various facts. AQP9 expression located at the plasma membrane of mouse
17365 3T3-L1 adipocytes, and was higher upon insulin stimulation [75]. Insulin increased AQP9, in addition
18366 to AQP3 and AQP7, in human omental adipocytes, while leptin decreased AQP9, as well as AQP7,
19366 expression though the PI3K/Akt/mTOR pathway [75]. Due to the low activity of the adipose glycerol
20367 kinase during lipogenesis, glycerol uptake is likely limited. Using selective AQPs inhibitors, the
21368 relative contribution of aquaglyceroporins to the glycerol permeability of 3T3L1 adipocytes were
22369 estimated to 68% for AQP3, 3% for AQP7 and 12% for AQP9 [126]. However, the contribution of
23370 AQP7 to glycerol permeability was estimated to 45% using RNA interference [144]. Plausible
24370 explanations may account for the observed variability in AQP7 contribution to adipocytic glycerol
25371 permeability. Indeed, incomplete effects of the inhibitors and possible off target effects of RNA
26372 interference could respectively underestimate or overestimate AQP7 contribution to adipocytic
27373 glycerol permeability [126].

31376 Lower expression and lack of regulation of aquaglyceroporins in beige adipocytes versus white
32377 adipocytes differentiated from 3T3-L1 cells are in agreement with the commitment of beige adipocytes
33378 to metabolize fat to produce heat instead of performing TAG synthesis and degradation that involves
34379 glycerol passage [128].

37381 Adipocytes undergo rapid intracellular acidification or alkalization in response to lipolytic agents
38382 or insulin, respectively [146–150]. Therefore, adipocyte intracellular pH modifications are likely
39383 involved in pH-induced gating mechanisms of aquaglyceroporins. Under fasting, lipolysis is known to
40384 induce intracellular pH acidification that does not drop below pH 6.5 [60,146]. Therefore, under these
41384 conditions, AQP3 and AQP7 will likely retain a glycerol permeability superior to 50% [63], while
42385 AQP10 will likely display high glycerol permeability [60], both contributing to glycerol efflux. In
43386 contrast, under feeding, insulin-induced intracellular pH alkalization will likely trigger high AQP3-
44387 and AQP7-mediated glycerol permeability contributing to glycerol influx, but low AQP10-mediated
45388 glycerol permeability. While it remains to be determined whether AQP9 also undergoes a pH-
46388 dependent gating mechanism, AQP9 may be still involved in glycerol import upon feeding (see above).

48390 AQP11 is expressed in the endoplasmic reticulum and its expression is upregulated during human
49391 adipocyte differentiation and lipolysis [117]. Physiologically, AQP11 allows glycerol passage for TAG
50392 synthesis in nascent lipid droplets in the endoplasmic reticulum [117].

51392 Based on current understanding of the role of aquaglyceroporins in adipocyte energy metabolism,
52393 we proposed an updated working model whereby mainly AQP3, AQP7 and AQP10 would be involved
53394 in glycerol exit during lipolysis, while mainly AQP3, AQP7 and AQP9 would be involved in glycerol
54395 entrance during lipogenesis [126]. However, the contribution of each aquaglyceroporins to glycerol
55396 metabolism in adipose tissue remains to be fully deciphered, possibly with the use of mice carrying the
56397 conditional knockout of one or more aquaglyceroporin gene(s) or using isoform-specific and potent
57397 inhibitors.

60400 **4.4. Metabolic disorders**

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A brief overview on the role of adipocytic aquaglyceroporins in metabolic disorders will be provided, due to available extensive review on the subjects [24,90,107–111]. Table 3 summarizes the involvement of aquaglyceroporins in metabolic disorders.

Table 3. Suggested pathophysiological roles of adipose tissue aquaglyceroporins.

Aquaglyceroporin	Cell type	Pathophysiological involvement	References
AQP7	Adipocytes (h, m, r) Endothelial cells (h, m)	Obesity Metabolic syndrome Insulin resistance T2D	[75,101,114,135,144,145,151–157] [158,159] [136] [133,160]
AQP3	Adipocytes (h, m, r)	Unknown	
AQP9	Adipocytes (h, m, r)	Unknown	
AQP10	Adipocytes (h)	Unknown	
AQP11	Adipocytes (h, m)	Obesity	[117]

h, human ; m, mouse ; r, rat; T2D, type 2 diabetes mellitus.

The capacity of *Aqp7* knockout mice to develop or not an obesity phenotype may arise from their genetic background [144,145,161]. The site of AQP7 expression, exclusively in endothelial cells [93,121] or in endothelial cells and adipocytes [115,122], could account for the susceptibility to develop obesity [162]. In an animal model of metabolic syndrome (i.e. rats depleted in n3-polyunsaturated fatty acids (PUFA), the level of AQP7 protein, but not mRNA, was decreased in adipose tissue (PUFA depletion is likely responsible for the absence of PPAR γ activation that is then incapable to transactivate AQP7 [118] and thereby glycerol passage [158]. Lipopolysaccharide, known to play an important role in obesity [163], decreased glycerol permeability and increased TAG content in 3T3-L1 adipocytes, without modification of AQP7 protein expression [126].

Animals fed an obesogenic high fat diet displayed various modulations of *Aqp3*, *Aqp7* and *Aqp9* mRNA levels [81,129,164–166]. Data variability may arise from differences in animal species (rat, mouse), adipose tissue investigated, duration of obesogenic diet, animal genetic background and assay conditions.

DNA methylation of the human *AQP7* gene has been associated with adiposity. Indeed, *Aqp7* gene methylation was significantly increased in four adiposity phenotypes including android fat mass, android:gynoid fat ratio and trunk:limb fat ratio [135]. Furthermore, high proportion of methylated GpG-sites detected in *Aqp7* gene promoter correlated with a downregulation of the gene expression in human hypertrophic white adipose tissue that is generally associated with insulin resistance and type 2 diabetes [136]. However, *Aqp7* gene methylation could not be detected in rats fed with high fat diet to create an obesogenic environment [167]. Nevertheless, *Aqp7* downregulation, achieved using interfering RNA knockdown of *Aqp7*, stimulated lipid storage in mesenchymal stem cells subsequently differentiated into adipocytes [136], consistently with the role of AQP7 to ensure the passage of glycerol, used as a substrate and product of lipid metabolism [119].

The human *AQP7* gene is located in a chromosomal region linked to type 2 diabetes (T2D) [133,160] and metabolic syndrome [159]. Therefore, several studies have assessed AQP7 expression in patients with obesity, T2D and metabolic syndrome. However, no correlation has been observed between *AQP7* gene mutations and obesity or T2D [113]. Adipose tissue from obese subjects presents either higher or lower AQP7 expression, according to the studies [75,101,151–157]. Clinical features

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441 of obese subjects, as well as their gender, may account for these apparent contradictory data [22].
5442 Lower KLF-15 expression that could reduce *AQP7* expression may account for insulin resistance in
6443 adipose tissue [134].

8444 *AQP11* has recently been shown to act an endoplasmic reticulum-resident protein involved in
9445 endoplasmic reticulum stress induced by lipotoxicity and inflammation in human obesity [117]. The
10446 peroxiporin activity of *AQP11* appears to lessen the endoplasmic reticulum stress caused by
11447 lipotoxicity and inflammation [117].

12448 Despite numerous studies aiming at evaluating the role of aquaglyceroporins in energy metabolism
13449 disorders, additional studies will be required to deepen our knowledge on the subject.

16451 **5. Pancreatic endocrine aquaglyceroporins**

18452 **5.1.Expression**

19453 Endocrine cell clustered into islets of Langerhans and single endocrine cells scattered among the
20454 pancreas constitutes the endocrine pancreas. The islets of Langerhans are clusters of few to several
21455 thousand cells of five main well-characterized endocrine cell types: β -cells producing insulin; α -cells
22456 producing glucagon; δ -cells producing growth hormone-inhibiting hormone (GHIH - also termed
23457 somatostatin); γ -cells producing pancreatic polypeptide (PP); and ϵ -cells producing ghrelin [168,169].
24458 Among human and rodent islets of Langerhans, the endocrine cells types vary in their proportions
25459 [170–172] and localization (core or rim of islets) [170,171]. From a physiological point of view, both
26460 insulin and glucagon play major roles in the control of energy metabolism in post-prandial and in pre-
27461 prandial states, respectively. Indeed, it is well known that insulin, secreted in response to increased
28462 glycaemia, and glucagon, secreted in response to decreased glycaemia, are anabolic and catabolic
29463 hormones, respectively, controlling the mobilization and storage of carbohydrates, lipids and amino
30464 acids.

31465 Current state of knowledge reports the expression of *AQP7* in rodent (rat and mouse) β -cells [26–
32466 28,128,161]. In mouse and rat β -cells, *AQP3* mRNA expression has not been detected [161,173]. While
33467 *AQP9* mRNA expression has not been detected in mouse β -cells, its expression was detectable in 10%
34468 of rat pancreatic samples analyzed [173].

35469 The possible presence of additional aquaglyceroporins in β -cells, as well as aquaglyceroporins
36470 expression in other islet endocrine cell types require further investigations.

41471 **5.2. Regulation**

42472 In response to triggered lipolysis in adipocytes, increased blood glycerol levels will induce local β -
43473 cells intracellular glycerol concentration increase and subsequent β -cell activation. In addition, insulin
44474 or other agents downregulating *AQP7* expression will likely increase the intracellular β -cell glycerol
45475 concentration and induce cell activation [113,118]. Recent data partly corroborated this hypothesis as
46476 *Aqp7* overexpression decreased insulin release in rat RIN-m5F β -cells [128]. However, reduced *Aqp7*
47477 expression had no impact on insulin release [128]. In addition, tumor necrosis factor-alpha ($\text{TNF}\alpha$)
48478 and LPS, respectively decreasing or increasing *Aqp7* mRNA expression, both decreased insulin release
49479 in rat RIN-m5F β -cells [128]. This apparent contradiction might be related to the measurement of
50480 *Aqp7* mRNA levels rather than *AQP7* protein expression levels or cell glycerol permeability under
51481 $\text{TNF}\alpha$ and LPS treatment, to the condition of insulin secretion (in the absence or presence of glycerol),
52482 or to the effects of cytokines released upon $\text{TNF}\alpha$ and LPS stimulation. As such, additional
53483 experiments will help shedding light on this issue.

57484 **5.3. Function**

58485 The role of *AQP7* in β -cell intracellular glycerol content and both insulin production and secretion
59486 has been deciphered using *Aqp7*-knockout (KO) mice [27,121,144,161], *ex vivo* β -cells [26] and
60487 BRIND-DB11 β -cell line [28]. A reduction in β -cell number, size and insulin content was documented
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in *Aqp7*-KO mice as compared to wild type (WT) mice [161]. In addition, *Aqp7*-KO mice displayed higher intra-islet levels of both glycerol and TAG, higher glycerol kinase activity, but no glycerol efflux in response to forskolin [161]. Depending on the genetic background of *Aqp7*-KO mice models used, the animals displayed various phenotypes characterized by either normal glycaemia with underdetermined insulinemia [121], normal glycaemia with hyperinsulinemia [161], or hyperglycaemia and hyperinsulinemia [144]. The various phenotypes of *Aqp7*-KO mice models could result from combined interactions between AQP7 and other gene products. This hypothesis warrants additional experiments to decipher the AQP7 interactome. Figure 1 resumes the phenotype of *Aqp7*-KO mice.

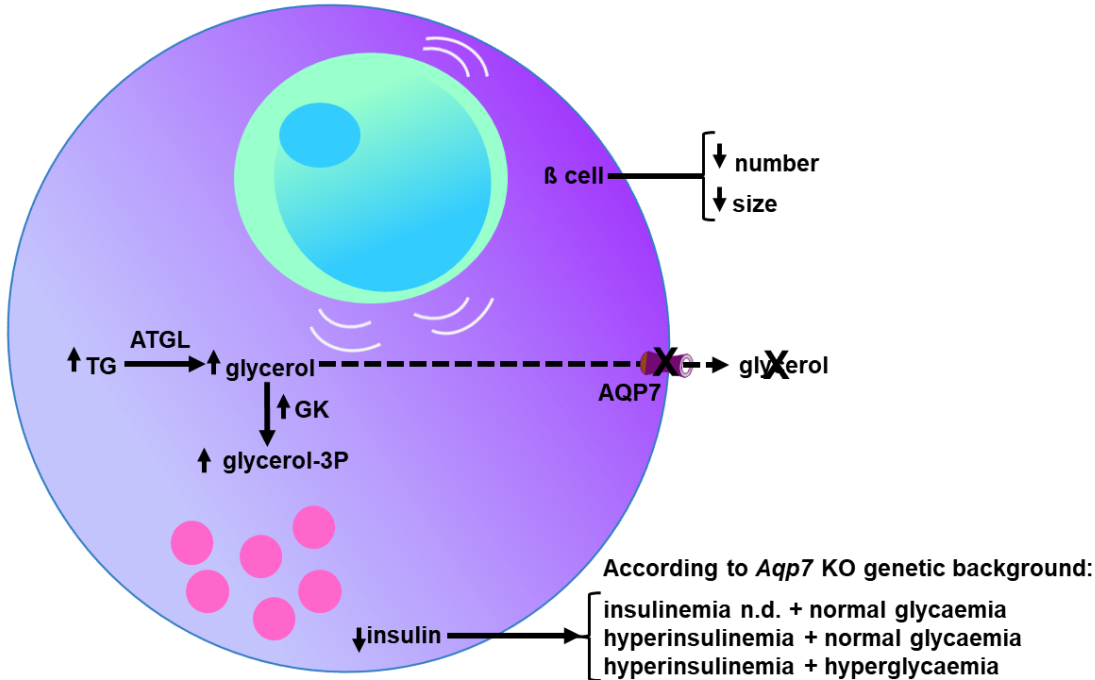


Figure 1. Characteristics of *Aqp7*-KO mice. ATGL: adipose triglyceride lipase; GK: glycerol kinase; TAG, triacylglycerols.

The current model of insulin secretion is based on the existence of sequential mechanisms including: glucose entry through glucose transporter type 2 (GLUT2), glucose metabolism, intracellular ATP level increase, ATP-sensitive potassium channels inhibition, membrane depolarization, voltage-dependent calcium channels opening, intracellular calcium concentration increase, and insulin-containing granules release [174]. Glucose entry in β -cell leads to cell swelling [175], and subsequent activation of volume-regulated anion (VRAC) channel inducing regulatory volume decrease, cell depolarization, voltage-dependent calcium channel activation, intracellular calcium level increase, and insulin secretion [176,177]. Upon exposure of rat β -cells or rat BRIN-DB 11 cells to extracellular isosmotic addition of glycerol, cells encounter cell swelling and subsequent here above-mentioned mechanisms leading to insulin release (Fig. 2) [26,28,178]. Both glycerol entry and metabolism are likely accounting for the observed β -cells activation [26]. Extracellular isosmotic addition of glycerol, extracellular hypotonicity or increased D-glucose level induced increased insulin release in β -cells from *Aqp7*-KO mice as compared to WT mice [27]. The role played by glycerol and AQP7 in insulin secretion corroborate the correlation between glucose-induced insulin secretion and β -cell lipolysis [179–181]. Consequently, AQP7 allows glycerol passage, and modulate the insulin secretion pathway at a distal downstream site [27].

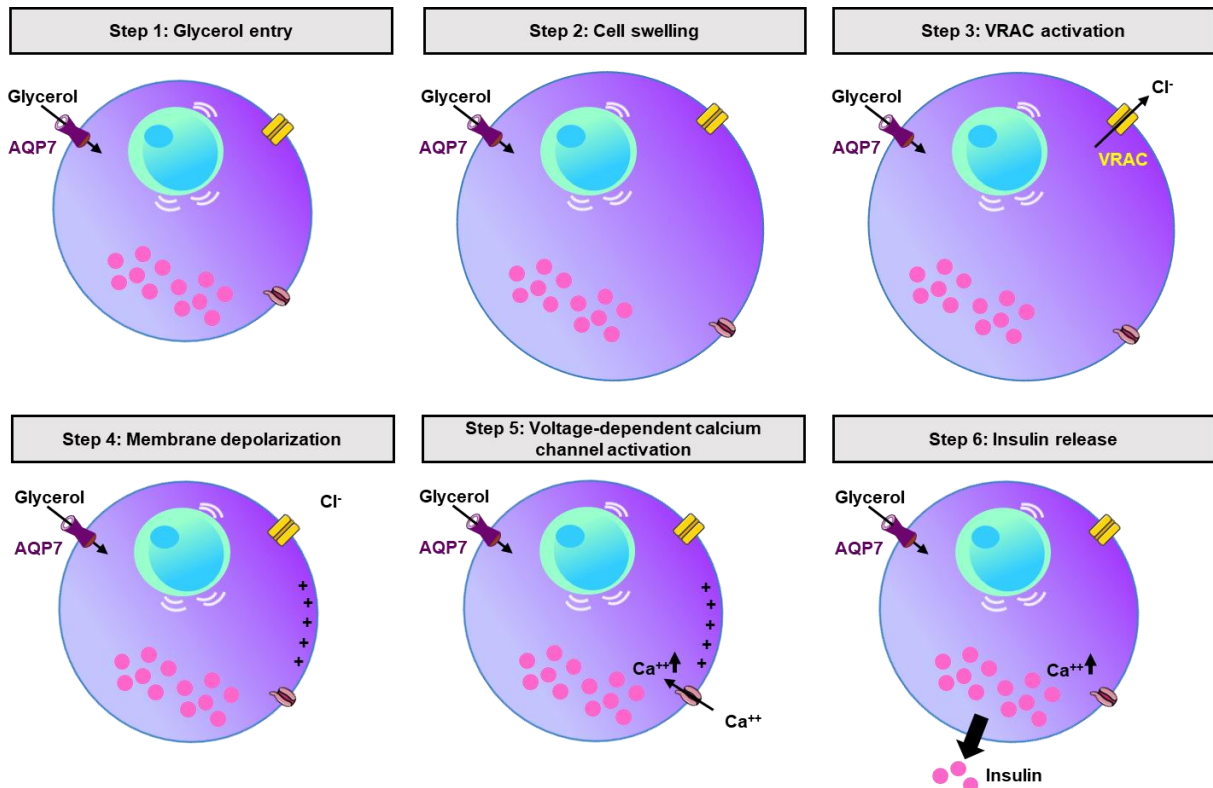


Figure 2. AQP7 function in β -cell insulin secretion. Following glycerol entry through AQP7 in β -cells, multiple steps lead to insulin secretion.

5.4. Metabolic disorders

Aquaglyceroporins are described to be of pathophysiological relevance in metabolic disorders affecting the pancreas (Table 4). Indeed, in the context of type 2 diabetes, both insulin resistance and insulinemia may also induce an intracellular glycerol concentration increase in β -cells [178]. In obesity, β -cell activation of TNF α receptor and TLR4 may play a role in type 2 diabetes by modulating *Aqp7* expression. Indeed, TNF α and LPS, inducing cytokine production/release, respectively decreased or increased *Aqp7* mRNA expression in rat RIN-m5F β -cells [128]. Opposite to lean rats, pancreas from obese rats have by higher AQP7 mRNA and protein levels. In addition, *Aqp7* mRNA levels are negatively correlated with total ghrelin and glucagon-like peptide 1 (GLP-1) concentrations [173]. Restored pancreatic AQP7 expression and improved pancreatic β -cell function have been observed in obese rats with sleeve gastrectomy [173]. AQP7 could become a the target for therapeutic intervention in metabolic diseases, and in particular obesity and diabetes [112].

Table 4. Suggested pathophysiological roles of pancreas aquaglyceroporins.

Aquaglyceroporin	Cell type	Pathophysiological involvement	References
AQP7	β -cells (m,r)	Obesity Diabetes	[112,128,173] [161]
AQP9	β -cells (r*)	unknown	[173]

m: mouse: r: rat; * present in about 10% of samples

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4539 Overall, additional studies are necessary to further deciphering the function of AQP7 in β -cells and
5540 metabolic diseases, assess the possible presence and role of other aquaglyceroporins, as well as
6541 determine the expression of aquaglyceroporins in other islet endocrine cell types.
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9543 **6. Main pharmacological tools targeting aquaglyceroporins**

10544 Reflecting the strong interest in the subject, the full characterization of what it is already known
11545 and search for new and more selective and potent inhibitors of aquaglyceroporins and, more generally,
12546 of all aquaporin groups is currently very intense [24,182,183]. For the purpose of this review, only
13546 aquaglyceroporin inhibitors are considered.
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15548 The Cu^{2+} and Ni^{2+} ions were reported to bind to extracellular loop sites of human AQP3 [141,184].
16549 In the related studies, inhibitor potency was not fully clarified as it resulted to vary depending on the
17550 employed buffer. Indeed, Cu^{2+} inhibited AQP3 water and glycerol permeability with an IC_{50} ranging
18551 from $\sim 5 \mu\text{M}$ in bicarbonate buffer to the millimolar range in Tricine or HEPES buffers, and had no
19552 effect of AQP7 permeabilities in bicarbonate buffer [141,184]. It is worth mentioning that the normal
20552 human plasma Cu^{2+} levels range from 1 to $15 \mu\text{M}$. Nickel chloride (1 mM) reduced AQP3 water
21553 permeability by about 60% in human bronchial epithelium cells overexpressing the protein, compared
22554 to sham-transfected cells. The inhibitory effect exerted by Ni^{2+} ions likely occurs on the extracellular
23555 loop residues Trp-128, Ser-152, and His-241 [141].
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25556 The Hg^{2+} ion binds to a pore-lining cysteine residue found on most AQPs including AQP3, AQP9
26557 and AQP10 (but not AQP7) and blocks the channel. However, mercury and its compounds are toxic
27558 due to their non-specificity causing off-target effects the reason why it is used only in cell-based
28559 studies.
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30560 Some gold^{III} compounds have been reported to block the molecular passage operated by
31561 aquaglyceroporin channels. The Au^{III} -based compound $[\text{Au}(\text{phen})\text{Cl}_2]\text{Cl}$ (phen=1,10-phenanthroline;
32562 Auphen) was proved to inhibit AQP3 [185]. Biophysical and computational analyses suggested binding
33563 of Auphen to the side chain of a cysteine residue (Cys40) located nearby of the AQP3 pore constriction
34564 [43]. Auphen was also reported to inhibit mouse and human AQP7 through direct binding to a
35564 methionine (Met47) at the pore mouth facing the intracellular compartment [186]. AQP3 has been
36565 shown to be blocked by Aubipy ($[\text{Au}(\text{bipy})\text{Cl}_2]^+$ (bipy=2,2'-bipyridine), another gold coordination
37566 compounds [187–189].
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39568 Phloretin, a bioactive chalcone with various health benefits [184], is a proven blocker of the facilitated
40568 passage of glycerol and urea across membranes [49,190]. Although it does not allow to distinguish
41569 between the various aquaglyceroporins phloretin is largely employed in the biophysical
42570 characterization of the aquaglyceroporin-mediated passage of glycerol and urea in basic research
43571 [76,83]. Small molecule inhibitors of AQP9 were identified after screening a commercially available
44572 library of drug-like molecules [72,191]. One of the identified inhibitors, the heterocyclic compound
45573 HTS13286, inhibited the AQP9-mediated glycerol permeation with an IC_{50} of $\sim 2 \mu\text{M}$. The limited
46574 solubility in water ($\sim 25 \mu\text{M}$ in 1% DMSO-containing aqueous solution) makes HTS13286 not suitable
47574 for *in vivo* applications. However, HTS13286 is revealing a good blocker of AQP9 for characterizing
48575 the biophysical properties and function *in vitro* not to mention the possibility of identifying structural
49576 derivatives with a more potent inhibitory action on AQP9 and/or with higher water solubility for
50577 potential drug development.
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52578 Novel inhibitors of AQP3 and AQP7 have recently been identified [50]. The heterocyclic
53579 compounds DFP00173 and Z433927330 blocked the glycerol permeability of human erythrocytes with
54580 an IC_{50} of $\sim 0.2 \mu\text{M}$ and $\sim 0.6 \mu\text{M}$, respectively. In CHO cells, compound DFP00173 specifically
55581 blocked mouse AQP3 with an IC_{50} of $\sim 0.1 \mu\text{M}$ but not AQP7 and AQP9, while Z433927330 inhibited
56582 mouse AQP7 with an IC_{50} of $\sim 0.2 \mu\text{M}$ but not AQP3 and AQP9. The IC_{50} values for Z433927330 for
57583 mouse AQP3 and AQP9 were $\sim 0.7 \mu\text{M}$ and $\sim 1.1 \mu\text{M}$, respectively. In the same study by Sonntag and
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4 coworkers, the previously identified AQP9 inhibitor RF03176 [72] was found to block
5 aquaglyceroporin H₂O₂ permeability. Molecular docking suggested binding of Z433927330,
6 DFP00173 and RF03176 to amino acids residues at the cytoplasmic entrance of the AQP9 pore.
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9 7. Conclusions and outlook

10 Adipose, liver and endocrine pancreas aquaglyceroporins play a significant role in the function
11 these organs exert in metabolic and energy homeostasis. In the light of their physiological functions,
12 aquaglyceroporins play relevant roles in diseases accompanied by metabolic and energy imbalances.
13 Modulation of aquaglyceroporins by some bioactive phytochemicals is found to prevent or improve
14 some chronic metabolic disease. New and more sustainable aquaglyceroporins inhibitors are being
15 discovered offering promise for novel therapeutic strategies where targeting aquaglyceroporins may
16 help managing obesity, diabetes and NAFLD/NASH. Additional and important achievements on the
17 role and regulation of aquaglyceroporins in health and disease as well as their translational value are
18 anticipated.
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21 Conflict of Interest

22 The authors declare no conflict of interest.
23

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