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REVIEW

Aquaglyceroporins: implications in adipose biology and obesity

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Abstract Aquaporins (AQPs) are membrane water/glycerol channels that are involved in many physiological processes. Their primary function is to facilitate the bidirectional transfer of water and small solutes across biological membranes in response to osmotic gradients. Aquaglyceroporins, a subset of the AQP family, are the only mammalian proteins with the ability to permeate glycerol. For a long time, AQP7 has been the only aquaglyceroporin associated with the adipose tissue, which is the major source of circulating glycerol in response to the energy demand. AQP7 dysregulation was positively correlated with obesity onset and adipocyte glycerol permeation through AQP7 was appointed as a novel regulator of adipocyte metabolism and whole-body fat mass. Recently, AQP3, AQP9, AQP10 and AQP11 were additionally identified in human adipocytes and proposed as additional glycerol pathways in these cells. This review contextualizes the importance of aquaglyceroporins in adipose tissue biology and highlights aquaglyceroporins' unique structural features which are relevant for the design of effective therapeutic compounds. We also refer to the latest advances in the identification and characterization of novel aquaporin isoforms in adipose tissue. Finally, considerations on the actual progress of aquaporin research and its implications on obesity therapy are suggested.

Keywords Aquaporin · Adipocyte · Obesity · Diabetes · Membrane permeability

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Introduction

Obesity is considered one of the pathological features of metabolic syndrome, which also includes insulin resistance, hypertension, and dyslipidemia [1]. The specific mechanisms that may lead from obesity towards its metabolic complications such as insulin resistance and type 2 diabetes remain poorly understood. The "adipose tissue expandability" hypothesis is an attractive concept for explaining adipose tissue dysfunction that has been supported by the growing evidence from clinical as well as experimental model studies [2]. When adipose tissue ceases to store lipids efficiently as a consequence of reduced adipose tissue expandability (reduced fatty acid uptake or increased fatty acid spillover from adipose tissue), metabolic alterations may arise from ectopic accumulation of lipids in non-adipose organs, such as liver, muscle and pancreas [3–5]. In contrast to adipocytes, the poor adaptation of these tissues to store lipids triggers lipotoxic disruption of cellular function. According to this model, adipose lipid stores, especially peripheral subcutaneous compartments, are viewed as lipid-buffering tissues that help maintaining the homeostasis of daily lipid fluxes [2, 3].

In the last decade, adipose aquaporins (AQPs) emerged as key players in adipose tissue biology [6]. Glycerol permeation in adipocyte membranes carried out by these proteins seems to be intrinsically related to the mechanisms

of obesity and type 2 diabetes. More specifically, impaired glycerol transport through plasma membrane aquaporin-7 (AQP7) has been correlated with triglyceride accumulation and obesity onset. Recently, other AQPs, namely AQP3, AQP9, AQP10 and AQP11, were reported in adipose tissue, although their involvement in the obesity mechanisms is still unclear.

This review gives an overview on recent data implicating aquaglyceroporins in adipose tissue biology. Special attention is given to aquaglyceroporins' structural features, bridging basic aspects of AQP research and clinical data indicating adipose AQPs as potential obesity/type 2 diabetes targets.

Adipose tissue metabolism

Adipose tissue plays an important role in glucose and lipid metabolism in the mammalian body. Adipocytes continuously synthesize and hydrolyze triacylglycerols (TAG) in response to energy balance (Fig. 1a, b) [7].

In conditions of energy expenditure, TAG stored in adipocytes is hydrolyzed to glycerol and free fatty acids (FFA) (Fig. 1b). Glycerol, as a precursor of glycerol-3-phosphate (G3P), represents an important metabolite in the control of both fat and glucose homeostasis for building the backbone of TAG and for as a substrate for gluconeogenesis [8, 9]. In addition, glycerol is an energy substrate via the G3P shuttle, a mechanism that allows the generation of ATP in the mitochondria through the oxidation of G3P [9].

There are three major sources for G3P: (1) glucose, via glycolysis; (2) glycerol, after phosphorylation by glycerol kinase and (3) pyruvate, lactate, alanine and intermediaries of the citric acid cycle via glyceroneogenesis ([10, 11] reviewed in [12]).

Adipose tissue is the major source of plasma glycerol [8]. Serum glycerol concentrations at rest are approximately between 0.05 and 0.1 mmol/L [8, 13–18]. Prolonged fasting [19, 20], exercise [21] and metabolic dysfunctions such as obesity [19, 22], hyperthyroidism [8] or diabetes [15, 16] elevate glycerol levels; whereas insulin, in contrast, has the opposite effect [8, 23]. All these changes are associated with the state of fat mobilization or deposition, the condition of which is reflected also by serum levels of FFA [8].

Several membrane proteins have been related to FFA transport [24], whereas only one protein, AQP7, remains the major transporter for glycerol in adipocytes [25]. Thus, the regulation of glycerol transport by AQP7 contributes to the control of fat accumulation and glucose homeostasis [9]. It is presumed that the glycerol channel(s) in adipocyte prevent(s) acute rise in intracellular osmotic pressure when glycerol is rapidly produced during lipolysis. However, the complete picture of the underlying mechanisms responsible for glycerol release from adipocytes remains incomplete [7].

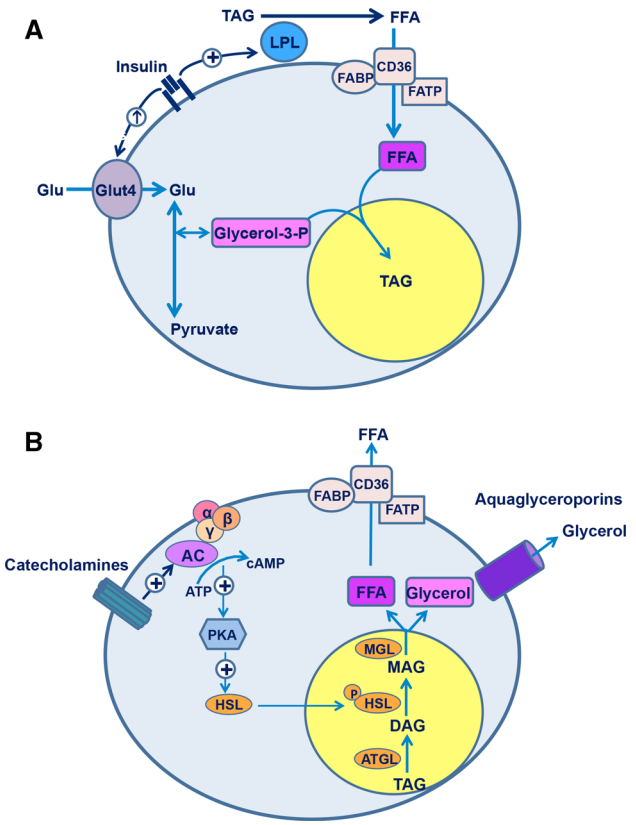

Fig. 1 Lipogenesis and lipolysis in adipocytes. **a** Under lipogenic conditions, adipocytes synthesize TAG from the esterification of free fatty acids (FFA) with G3P. Pancreatic cells release insulin in response to an increase in blood glucose level during feeding state. On adipocytes, insulin binds to receptors inducing GLUT4 translocation to the plasma membrane, enhancing the entry of glucose. In addition, insulin activates lipoprotein lipase (LPL) located on the cell surface of the vascular endothelium, which removes FFA from chylomicrons and very low-density lipoproteins (VLDLs). Several membrane proteins, including fatty acid-binding protein (FABP), fatty acid translocase (FAT, CD36) or fatty acid transporter protein (FATP), facilitate the FFA transport across the adipocyte membrane. **b** In circumstances of negative energy balance, such as fasting or exercise, TAG stored in adipocytes are hydrolyzed to glycerol and FFA. Increased catecholamine levels activate adrenergic receptors located on adipocyte cell surface, subsequently stimulating adenylyl cyclase and cyclic AMP production (cAMP) that stimulate protein kinase A (PKA), which in turn phosphorylates and activates hormone-sensitive lipase (HSL). During lipolysis, TAG are hydrolyzed into glycerol and FFAs by adipocyte triglyceride lipase (ATGL), HSL and monoacylglycerol lipase (MAG), and both products are released into the bloodstream. Glycerol permeates through aquaglyceroporins, being AQP7 the main efflux channel

Aquaporins

Water homeostasis is central to the physiology of all living cells. All biological membranes have an intrinsic water permeability that depends upon their lipid composition. In

addition, the presence of water channels enables the fine-tuning of water permeability in biological membranes, and hence controlling the flux of water into and out of the cells [26].

In the last 20 years, water transport across biomembranes became a very hot area of research in biochemistry and molecular cell biology, and one of the greatest achievements was the discovery of water channels, also known as AQPs [27].

AQPs represent an ancient family of small integral membrane proteins ($\approx$30 kDa) that facilitate the bidirectional transfer of water and small solutes across biological membranes in response to osmotic gradients [27–29]. Compared with other biological processes, the translocation of water molecules by AQPs is extremely fast, on a nanosecond timescale. These selective channels belong to the large superfamily of major intrinsic proteins (MIP) transmembrane channels. They have been found in virtually all living organisms, including eubacteria, archaea, fungi, protozoa, plants and animals [30], which indicates their importance in maintaining fluid homeostasis [31].

In microorganisms, AQPs are believed to aid survival by providing protection against osmotic shock and rapid freezing [32, 33]. *Escherichia coli* has two MIP family proteins: one is a glycerol facilitator (GlpF) [34] and the other is a specific channel for water (AQPZ) [35]. More AQP genes are present in the genomes of multicellular organisms: *Arabidopsis thaliana* contains 35 putative *AQP* genes [36], while mammalians express 13 AQPs with specific organ, tissue, and cellular localization [31].

Based on their primary sequences, mammalian AQPs have been divided into three subfamilies: (a) the orthodox AQPs, which are considered to be water selective (AQPs 0, 1, 2, 4, 5, 6 and 8); (b) the aquaglyceroporins (AQP3, 7, 9, 10), which are permeable to glycerol and other small solutes in addition to water; and (c) the unorthodox superaquaporins (AQP11, 12), the most recently identified AQPs, which seem to be mostly intracellular, with unique (Asn-Pro-Ala) NPA boxes and unusual pore structures and functions [37, 38].

Mammalian AQPs are expressed in a wide range of tissues and are involved in many biological functions, including transepithelial fluid transport, cell migration, brain edema, neuroexcitation cell proliferation, epidermal water retention and adipocyte metabolism [39].

Aquaporin structure

The three-dimensional structures of several AQPs have enabled the conceptualization of a general fold, revealing the structural determinants that are essential for AQPs extraordinary permeation rate and selectivity. The atomic model of AQP1 derived from a 3.8 Å resolution potential map obtained by electron crystallography was the first atomic structure of a human membrane protein to be solved and gave the first insight into AQPs' specific structural features that are essential for water specificity [40]. Medium- and high-resolution structures of several AQPs belonging to different subfamilies have been ever since determined, namely, from archaea [41], bacteria [42, 43], yeast [44], protozoa [45], plants [46] and mammals [47–50]. More recently, molecular dynamics (MD) simulations have complemented the experimental data by providing the progression of the biomolecular system at atomic resolution [51].

The reported structures have revealed that AQPs are grouped as homotetramers embedded in the lipid bilayer [52], consisting of four independent monomers, each behaving as an independent channel [53] and sharing a conserved overall typical hourglass fold [40, 54] (Fig. 2). The monomers interact with two of its neighbors, forming the tetramer with a central pore (Fig. 2b). It has been suggested that this pore, which is not involved in water conductance [40], may permeate gases [55–57] and function as a gated cation channel [58, 59]. Each AQP monomer is a small protein with usually fewer than 300 amino acids and comprised of six transmembrane helices (H1-6) arranged in a right-handed helical bundle and connected by five loops (A–E), with both amino and carboxyl termini located in the cytoplasm (Fig. 3a, b) [60, 61].

Despite the diversity of primary sequences, most AQPs have two highly conserved asparagine–proline–alanine (NPA) signature motifs that are thought to be critical to water and substrate permeation [54]. The NPA repeats are located at the end of the two short α-helices (loop B and E) that fold back into the membrane, with loop B entering from the exoplasmic side and the loop E entering from the cytoplasmic side [54, 62] (Fig. 3a, b).

To achieve the water specificity, the pore region contains selective filters with size constrictions and/or charge characteristics that enable water molecules to pass through, while preventing permeation of protons or any solutes above 2.8 Å [47]. Two main constriction sites that act as selectivity filters were identified in AQP channels: one is positioned on the extracellular face of the channel in the aromatic/arginine (ar/R) constriction region, whereas the other is located in the central part of the channel at the NPA region [33, 51, 63] (Fig. 3b).

Closer to the extracellular exit of the channel, the highly conserved ar/R region forms the narrowest part of the pore [47, 51]. In water-specific AQPs, this region is approximately 2.8 Å in diameter, being identical to that of a water molecule [64].

Despite their extreme water permeability, AQPs strictly prevent the conduction of protons [64], an important feature that prevents the dissipation of the electrochemical potential gradient.

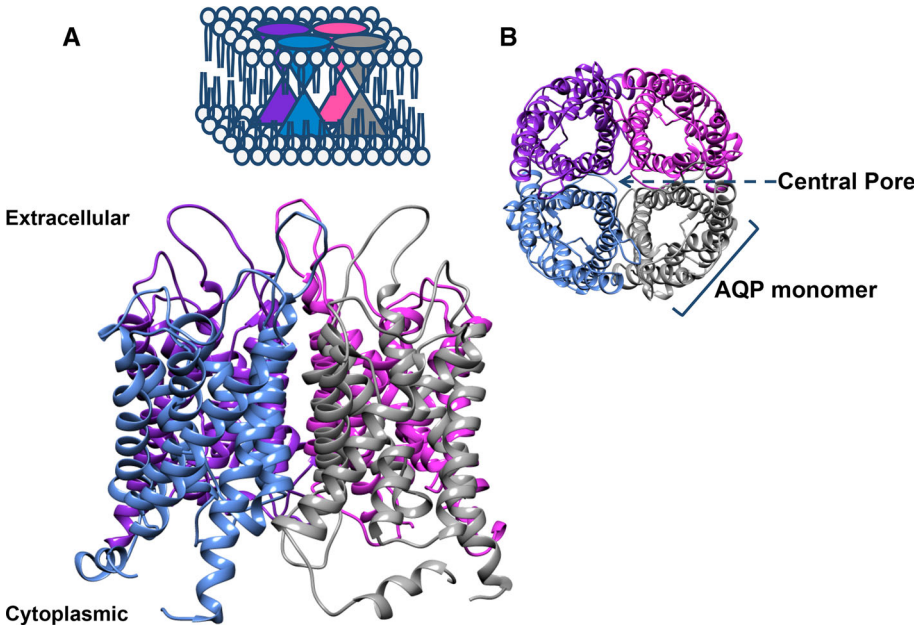

Fig. 2 Tetrameric structure of aquaporins **a** side and **b** top views of bovine AQP1 homotetramer, based on its X-ray structure (Protein Data Bank (PDB) code: 1J4N). Figures were generated with Chimera (http://www.cgl.ucsf.edu/chimera)

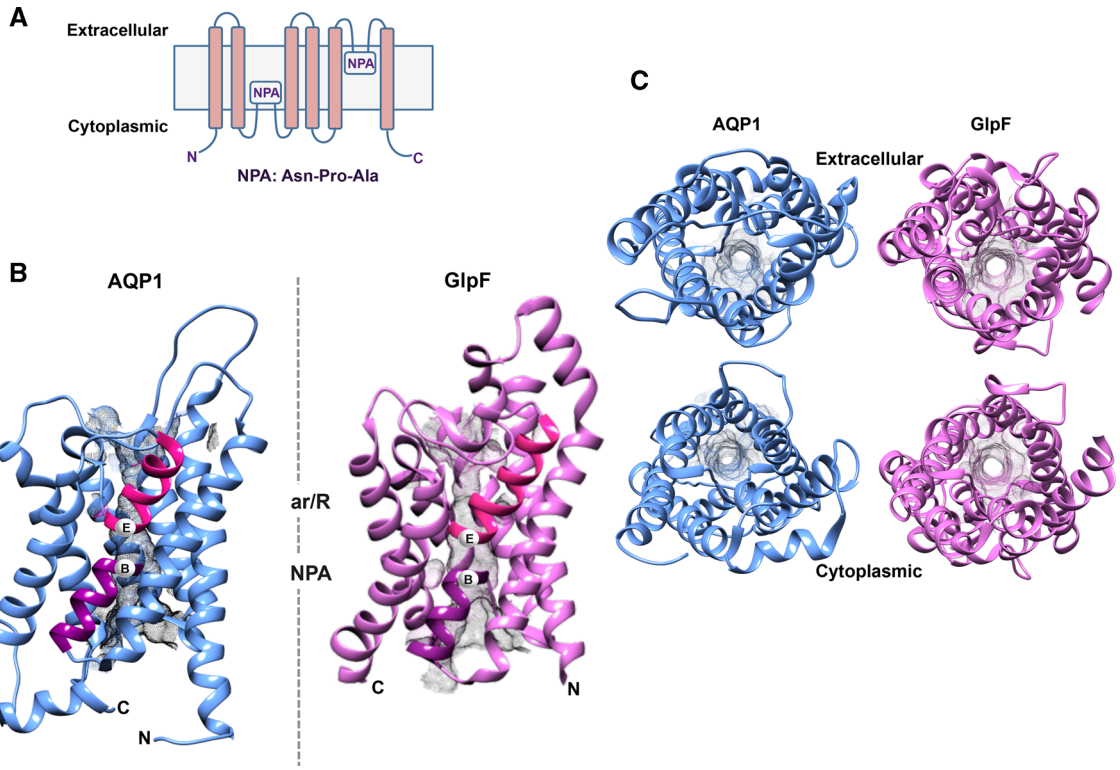

Fig. 3 Detailed view of AQP1 and Glpf monomers. **a** Topology map showing the basic aquaporin fold. **b** Overall structure of bovine AQP1 (bAQP1) and GlpF, highlighting the two half-helices entering from loops B and E to form a pseudo seventh transmembrane helix and the selectivity filters regions ar/R and NPA. **c** View into the extracellular and intracellular vestibules of bovine AQP1 and Glpf. The *gray mesh* represents the residues lining each pore. **b** and **c** were generated with Chimera (http://www.cgl.ucsf.edu/chimera), based on X-ray structures (PDB codes 1J4N and 1FQY)

Up to date, there are no X-ray structures solved for mammalian aquaglyceroporins. But the existing structure of *E. coli* glycerol facilitator GlpF suggests that the AQP general fold topology previously described is also conserved among aquaglyceroporins [42, 65] (Fig. 3b). However, GlpF permeability to water is substantially less

than that measured for its bacterial water-specific counterpart AqpZ [34, 66], which might result from more subtle structural divergences among orthodox and glycerol facilitator subfamilies. In fact, specific amino acid replacements in GlpF have critical effects on the characteristics of the constriction region by increasing both its size (Fig. 3c) and hydrophobicity [47]. Since the constriction region of GlpF is wider than that of AQP1 (3.4 and 2.8 Å, respectively), it seems that the more hydrophobic nature of GlpF is the primary factor responsible for its relatively reduced ability to transport water [47, 67].

The GlpF channel lining is strongly amphipathic, with oxygen and nitrogen lined up on one side and carbon on the opposite side of the lumen surface. This amphipathic channel uniquely matches the chemical structure of glycerol, which is a composite of the polar hydroxyl group arranged on a non-polar alkyl backbone [42].

The absence of crystal structures for mammalian aquaglyceroporins has been partially overcome by the conception of homology models. The assembly of three-dimensional models, having GlpF channel as a template, allowed accessing the structural uniqueness of human AQP3 (hAQP3) [68] and AQP7 (hAQP7) [69] aquaglyceroporins. These models confirmed both the existence of two constriction sites (ar/R and NPA selective filters) and the amphipathic nature of these channels, which is predicted to be a common feature of all aquaglyceroporins. However, key differences were also detected between these two isoforms. The most relevant was the identification of several methionine residues lining hAQP7 pore (not present in hAQP3), which are predicted to directly interact with glycerol [69]. Not only differences on glycerol permeation are expected between hAQP3 and hAQP7, but also different mechanisms of regulation by modulatory compounds. In fact, when investigating the effects of gold (III) complexes in the activity of both hAQP3 and hAQP7, Cys40 in hAQP3 extracellular pocket is the likely candidate for binding to gold (III) complexes [68]; whereas in hAQP7, Met47 located in the channel entrance facing the cytoplasm was appointed as the interacting residue [69].

Aquaporin-7 in fat accumulation

Aquaporin-7 (AQP7) is an aquaglyceroporin that can facilitate the transport of water and small uncharged solutes such as glycerol across lipid bilayers [70]. The physiological roles of the aquaglyceroporins have been more difficult to ascertain, in part due to incomplete understanding of the metabolic role of glycerol in mammals [29].

AQP7 is expressed in human, rat and mouse white adipose tissue [71, 72] and is a functional water and glycerol channel in adipocytes [73]. In adipose tissue, controversy still exists regarding AQP7 subcellular localization. Some studies have localized AQP7 simultaneously in adipocytes and in the capillaries of adipose tissue [73–76]. However, the undetectable AQP7 labeling in adipocyte membranes in other works [77, 78] has not supported the appointed role for AQP7 in glycerol transport in these cells.

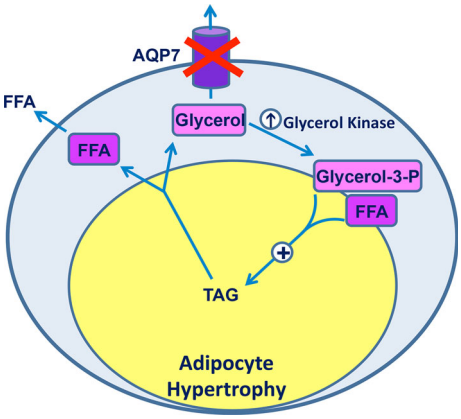

Fig. 4 Proposed mechanism for mice adipocyte hypertrophy with AQP7 deficiency knockdown of AQP7 in mice has been associated with increased intracellular glycerol content and induction of glycerol kinase in adipocytes. Glycerol kinase promotes re-esterification of glycerol and accelerates triglyceride accumulation in adipocytes. AQP7-null mice develop obesity and diabetes accompanied by adipocyte hypertrophy after 12 weeks of age (adapted from [7])

In obese *db*/*db* mice [72, 79] and in obese Otsuka Long Evans Tokushima fatty (OLETF) rats, *Aqp7* mRNA levels were found elevated [80]. From these data, it was speculated that AQP7 dysregulation might lead to an increased supply of glycerol for hepatic gluconeogenesis and to increased glucose levels in type 2 diabetes [25]. The progress in the understanding of the role of AQP7 was further driven by the independent creation of mouse lines devoid of AQP7. Two studies have related the depletion of AQP7 in mice to the development of obesity and adipocyte hypertrophy [81, 82]. Besides increased body weight, also derangement of whole-body metabolism was observed in aged AQP7-depleted mice [82]. A mechanism for progressive TAG accumulation in adipocytes of AQP7 deficient mice has been proposed: reduced plasma membrane glycerol permeability would result in an increased intracellular glycerol concentration that would activate glycerol kinase, increase G3P and hence TAG biosynthesis (Fig. 4) [82]. These findings suggest adipocyte glycerol permeability as a regulator of adipocyte metabolism and whole-body fat mass, and the modulation of adipocyte AQP7 expression should be regarded as a target in obesity therapy [83, 84]. However, other research groups have also generated AQP7-depleted mice [77, 85] that presented different phenotypic traits, not confirming the susceptibility to developing obesity in the absence of AQP7. In all,

despite the marked differences between the AQP7 mouse models, they all point towards AQP7 being involved in glycerol metabolism.

In line with the potential role of AQP7 in regulating adipocyte metabolism in mice, several studies have been attempting to disclose the possible connection between AQP7 and obesity/diabetes in humans [7]. However, the pathophysiologic role of AQP7 is not obvious, and its involvement in human obesity is far from clearly defined.

In support for being involved in increased susceptibility for obesity onset, the *AQP7* gene is localized in a chromosomal region (9 p13.3–p21.1) with reported linkage to type 2 diabetes [86] and to the metabolic syndrome [87]. Also, an association between a specific polymorphism in the *AQP7* gene promoter (A-953G SNP) and obesity was reported [88]. Regarding AQP7 functionality, a missense mutation G264V in the coding region of *AQP7* gene that disrupts glycerol and water transport in the *Xenopus oocyte* expression system was discovered [89]. This missense mutation has also been related to hyperglyceroluria and a mild platelet secretion defect [90] as well as to an impaired exercise-induced plasma glycerol increase [89]. The G264V AQP7 heterozygous variant was also found to be 8 % of a Caucasian population (in contrast to 3.75 % in the Japanese population [89]), but no association was found with obesity nor diabetes [91]. The authors of these works adverted, however, for the need of more adequately sample-sized studies for the accurate determination of genetic associations. In addition to the allelic variant G264V in the human *AQP7* gene, more single nucleotide polymorphisms for genes encoding AQP7 and other human aquaglyceroporins have been reported (reviewed in [92]).

White adipose tissue is distributed throughout the body mainly in subcutaneous regions (subcutaneous depot) and surrounding visceral organs (visceral depot) [93]. Morphological differences have been observed between subcutaneous and visceral tissue compartments, as well as different responses to lipolytic and lipogenic stimuli [94]. In regard to the implication of AQP7 in human obesity, Rodriguez and co-workers [76] highlighted the fat depot-specific differences in *AQP7* gene expression. On one hand, the upregulated *AQP7* expression in visceral fat depots suggests an increase in overall lipolytic capacity; whereas, on the other, the downregulated *AQP7* expression in subcutaneous fat mass could indicate the intracellular glycerol accumulation and progressive adipocyte hypertrophy.

The possible correlation between AQP7 expression and type 2 diabetes also requires further clarification. The polymorphism (SNP) (A-953G) in the promoter of *AQP7*, other than obesity, has also been associated with secondary development of type 2 diabetes [88]. Furthermore, some studies show an increased AQP7 abundance in visceral adipose tissue from type 2 diabetes subjects as compared with controls [75, 76]. On the contrary, other reports show no association between *AQP7* abundance and the genetic predisposition to type 2 diabetes, neither in visceral [95, 96] nor in subcutaneous adipose tissue [75, 76, 78, 96].

Regulation of AQP7 in adipocytes

Studies on the regulation of *AQP7* gene expression in adipocytes have mostly been conducted to investigate the role of AQP7 in glycerol metabolism.

In mice, the dependence of AQP7 expression on the nutritional status is well documented. *Aqp7* mRNA expression is enhanced by fasting, a condition favoring increased glycerol production from stored TAG, and decreased by refeeding [72]. These nutrition-related changes in *Aqp7* and plasma glycerol are the opposites of those for plasma insulin levels [72]. In fact, it was also shown that insulin suppresses *Aqp7* transcription via an insulin negative response element (IRE) located on the promoter region of the *Aqp7* gene, and that the phosphatidylinositol 3-kinase pathway was triggered for this inhibition [97]. Under lipolytic conditions, catecholamine stimulation induces AQP7 translocation to the plasma membrane [72, 76], whereas the reduced insulin signal increases *Aqp7* mRNA level, ultimately resulting in augmentation of glycerol release from adipocytes [72].

In humans, *AQP7* gene was also shown to be negatively regulated by insulin through an insulin response element (IRE) found in its promoter [89], but this was not sustained by the elevated AQP7 protein expression elicited by insulin in human visceral adipocytes [76]. Furthermore, a recent study described lower AQP7 protein abundance in men in parallel with a trend for lower insulin sensitivity, supporting the positive regulation of AQP7 by insulin [78].

Interestingly, many studies have reported the upregulation of AQP7 by thiazolidinediones [80, 89, 98], which are synthetic peroxisome proliferator-activated receptor gamma (PPARγ) and insulin sensitizers, and the downregulation of AQP7 by insulin resistance inducers, such as leptin [76], adrenergic agonists, TNFα and steroids [99]. In addition, ghrelin which is an oxygenic and lipogenic hormone, showed to stimulate TAG accumulation in human adipocytes in parallel to a decrease in the AQP7 protein levels [100]. Overall these data suggest that the fine-tuning of cytosolic glycerol content might be one mechanism regulating fat cell enlargement.

PPARγ is a regulator of adipocyte differentiation and regulates the transcription of several adipose-specific genes [7]. It is reported that in both murine 3T3-L1 [72, 99] and human Simpson–Golabi–Behmel syndrome cells [75], *AQP7* mRNA expression increases in parallel with adipocyte differentiation, which is consistent with the

upregulation of AQP7 by PPARγ. In addition, glycerol release into the media also increases in parallel with AQP7 mRNA levels in differentiating 3T3-L1 adipocytes [72]. In human adipocytes, *Aqp7* expression was also positively correlated with PPARγ, and with several PPARγ target genes, emphasizing the common regulatory pathways of these genes [75].

It was recently reported that AQP7 is regulated in response to physical training in a gender-dependent manner in subcutaneous adipose tissue. Thus, women responded by increasing the abundance of AQP7, whereas in men a reduced expression was observed, resulting in a more than twofold higher abundance of AQP7 in women as compared with men [78]. Along the same line, one report shows that obese women have higher *AQP7* mRNA levels in adipose tissue in both subcutaneous and visceral depots as compared with obese men [101]. Furthermore, Kondo and co-workers [89] have shown that a male subject homozygous for the G264V substitution in AQP7 (a loss of function mutation), although clinically normal, had impaired plasma glycerol increase in response to exercise, thus supporting that AQP7 is important for facilitating the release of glycerol from adipose tissue under these conditions.

Other aquaporins in adipose tissue

Several authors have speculated the existence of glycerol channels in adipose tissue in alternative to AQP7 [89, 91, 102]. In fact, glycerol secretion was not completely abolished in AQP7-depleted adipocytes [82, 102], suggesting that other mechanism(s) must be involved in glycerol transport. Recently, AQP3, AQP9 [76] and AQP10 [103] aquaglyceroporins and the superaquaporin AQP11 [69] were identified in human adipocytes. Although the presence of aquaglyceroporins in adipocytes other than AQP7 was not corroborated by other studies [72, 75], given the ubiquity that characterizes the AQP family, the presence of novel adipose AQPs is not utterly unexpected.

AQP3 has a wide tissue distribution and is physiologically relevant for diuresis, skin hydration, wound healing and tumor angiogenesis/spread. AQP3-deficient mice have impaired urine-concentration function and reduced skin elasticity and wound healing capacity. Yet these mice possess the advantage of higher resistance in the formation of skin tumors [39], which is consistent with AQP3 activity being positively correlated with cell proliferation [104]. Within the adipose tissue, AQP3 was identified in both visceral and subcutaneous human fat depots [76]. But unlike AQP7, a higher AQP3 expression was observed at the stromal vascular fraction (SVF) in comparison with the adipose fraction [73, 75, 76]. This is consistent with AQP3 expression in immune cells [39], which are important components of the SVF. At the adipocyte subcellular level, AQP3 seems to locate simultaneously at the plasma membrane and at the cytoplasm of 3T3-L1 cells [76]. AQP3 is translocated from the cytosolic fraction to the plasma membrane in response to the lipolytic stimulation of beta-adrenergic receptors in murine [105] and human [76] adipocytes, highlighting the relevance of this glycerol channel in glycerol efflux after lipolysis.

AQP9 was also detected in adipocytes, yet it seems to be constitutively expressed at the plasma membrane [76]. Besides the recent association with adipose tissue, AQP9 is abundantly expressed in the liver, epididymis and skin [25]. Although mostly associated with the hepatic function, AQP9 has been long described in straight association with adipose tissue metabolism. A coordinated regulation of adipose tissue AQP7 and hepatic AQP9 seems to be of the utmost relevance for the control of fat accumulation and glucose homeostasis [79]. First, gene expression of *Aqp9* in liver and *Aqp7* in adipose tissue was increased in insulin-resistant *db*/*db* mice [79]. Second, mice fasting and re-feeding, respectively, increased and decreased hepatic *Aqp9* mRNA expression, mirroring the pattern for AQP7 expression in adipose tissue [79]. Recently, it has been reported that obesity-associated non-alcoholic fatty liver disease (NAFLD) is associated with a reduction in hepatic AQP9 and glycerol permeability in experimental animals [106] and patients [107], suggesting a compensatory mechanism whereby the liver counteracts further TAG accumulation within its parenchyma as well as reduces hepatic gluconeogenesis in insulin-resistant states.

AQP10, which is expressed almost exclusively in human small intestine, was recently proposed to be an alternative pathway for glycerol efflux in human adipocytes [103]. This protein was both detected at the plasma membrane and in the cytoplasm of human adipocytes, and its ability to transport both water and glycerol in adipocytes was demonstrated. Similar to AQP7, AQP10 translocation from the lipid droplets to the plasma membrane in response to the beta-adrenergic agonist isoproterenol was reported.

AQP11 was the latest aquaporin to be associated with human adipose tissue. In rodents AQP11 is expressed in multiple tissues, including kidney and liver [38] where it locates intracellularly [108, 109] mostly associated with the endoplasmic reticulum (ER). Interestingly there seems to be a correlation between AQP11 functionality and ER stability, pointing to AQP11 involvement in maintaining an environment suitable for protein folding [110]. AQP11 knockout mice die prematurely due to advanced renal failure resulting from cysts originated from the ER [109]. Moreover, selective AQP11 deletion in the liver resulted in disrupted rough endoplasmic reticulum (RER) homeostasis and increased sensitivity to RER injury upon metabolic challenge with amino acids [111].

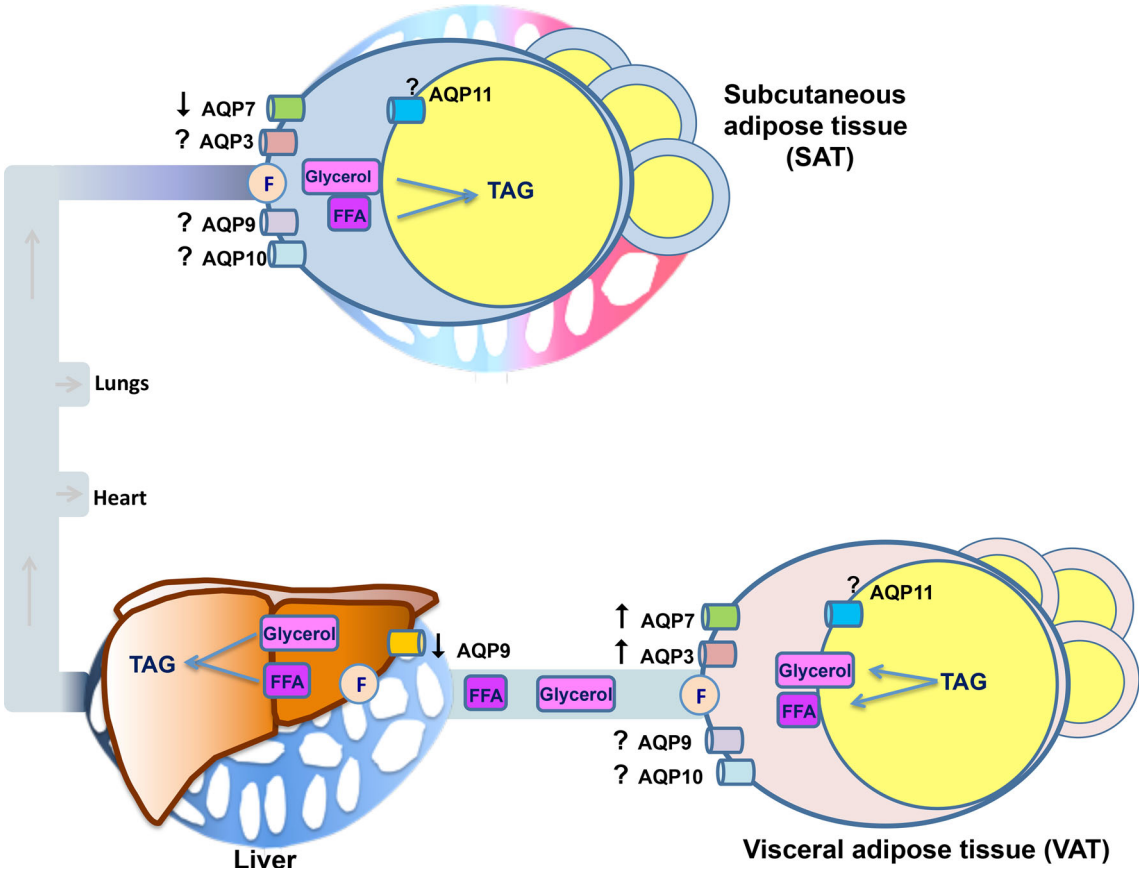

Fig. 5 Model for aquaglyceroporins implication in human obesity-associated type 2 diabetes. Obesity/type 2 diabetes (T2D) is associated with dysregulation of aquaglyceroporins in adipose tissue and liver. Obese subjects exhibit elevated expression of AQP3 and AQP7 in visceral adipocytes and low AQP7 levels in subcutaneous adipose cells, a condition reflecting increased lipolysis and adipose tissue hypertrophy, respectively. FFA released from visceral adipose tissue (VAT) though FFA transporters (F) reach the liver at high concentrations directly through the portal vein, a step leading to excessive hepatic TAG deposition and, ultimately, hepatotoxicity. Thus, liver AQP9 downregulation in obese/T2D patients suggests a compensatory mechanism whereby liver prevents further TAG accumulation and reduces hepatic gluconeogenesis. Subcutaneous adipose tissue (SAT) venous drainage is conducted through systemic veins being less important for liver metabolism. Being more insulin-sensitive than VAT and, therefore, more avid in the absorption of circulating FFA and TAG, SAT is functioning as a buffer of circulating lipids protecting other tissues from lipotoxic effects. AQP7 downregulation may represent a feedback regulatory mechanism to prevent depletion of these adipose stores. The role of AQP9, 10 and 11 in adipose tissue and their interplay need further investigation

In humans, AQP11 was recently detected intracellularly in both subcutaneous and omental adipocytes presumably in association with the ER [112]. Immunofluorescence labeling indicates that AQP11 colocalizes with perilipin in the vicinity of lipid droplets, yet the physiological relevance of this still remains unclear [112]. Due to its intracellular location, the disclosure of AQP11 transport features has proven to be challenging and its ability to transport water and/or other solutes is still controversial [109, 113]. However, recent permeability assays, with AQP11 either reconstituted in liposomes or expressed in CHO cells and in adipocytes, revealed that this protein is a functional water [112, 114] and glycerol [112] channel and may represent an intracellular glycerol gateway from the lipid stores.

Future perspectives

The ubiquity of AQPs expression in mammalians has been recognized for long, but only recently demonstrated in adipose tissue. There is evidence supporting the implication of the novel AQPs in adipose tissue glycerol metabolism, but further work is required to solidify this notion.

AQP7 has been appointed a potential drug target in obesity therapy [83] and metabolic syndrome [7]. However, it is still difficult to predict the outcome of an AQP7 modulator in these pathological settings. Given the overlapped expression of AQPs in adipose tissue, it is possible that the depletion of one isoform is compensated by another (Fig. 5). This could account for the

large heterogeneity of phenotypes associated with dysregulated AQP7 expression, making adipose AQPs elusive obesity targets [39]. Another relevant question raised by this ubiquitous expression is related with the establishment of specific interactions amongst adipose AQPs, which might be important to adipose tissue homeostasis. A proposed model for the possible involvement of aquaglyceroporins in obesity/type 2 diabetes is depicted in Fig. 5.

Adipose tissue is now recognized as the predominant contributor to systemic inflammation that characterizes the obese state. Overproduction of pro-inflammatory adipokines and reduced production of anti-inflammatory and insulin sensitizing adipokines constitute another important element of adipose tissue dysfunction in obesity [115]. In fact, metabolic dysregulation is among the factors that trigger the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome [116]. This is a multiprotein complex that activates caspase-1, enabling the processing and secretion of the pro-inflammatory cytokine IL-1β by macrophages [116]. The exact mechanisms underlying NLRP3 activation are still unclear, but recently AQP1-mediated water fluxes were implicated in the IL-1β release by macrophages through the NLRP3 inflammasome axis [117]. Interestingly, AQP3 was also implicated in macrophage immune response, specifically regarding the phagocytic and migration activities of these cells [118]. Altogether these data might hint for a deeper involvement of adipose tissue aquaporins in the systemic inflammatory processes associated with obesity.

Finally, the scarcity of AQPs structural information has limited the progress of their characterization. Good quality protein three-dimensional information is important for more accurate structure-based drug design and also for a better understanding of AQPs interaction partners. The overall gain of mechanistic insight into adipose AQPs function and regulation will be essential for a better understanding of adipose tissue physiology.

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