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Aquaglyceroporins serve as metabolic gateways in adiposity and insulin resistance control

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Aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) encompass a subfamily of aquaporins that allow the movement of water and other small solutes, especially glycerol, through cell membranes. Adipose tissue constitutes a major source of glycerol via AQP7. We have recently reported that, in addition to the well-known expression of AQP7 in adipose tissue, AQP3 and AQP9 are also expressed in omental and subcutaneous fat depots. Moreover, insulin and leptin act as regulators of aquaglyceroporins through the PI3K/Akt/mTOR pathway. AQP3 and AQP7 appear to facilitate glycerol efflux from adipose tissue while reducing the glycerol influx into hepatocytes via AQP9 to prevent the excessive lipid accumulation and the subsequent aggravation of hyperglycemia in human obesity. This Extra View focuses on the control of glycerol release by aquaglyceroporins in the adipose tissue and briefly discusses the importance of glycerol as a substrate for hepatic gluconeogenesis, pancreatic insulin secretion and cardiac ATP production.

Key words: glycerol, aquaporin, fat accumulation, glucose homeostasis, insulin secretion, ATP production

Abbreviations: Akt; protein kinase B; AQP, aquaporin; CD36, fatty acid translocase; DHAP, dihydroxyacetone phosphate; FABP, fatty acid binding protein; FATP, fatty acid transporter protein; FFA, free fatty acids; GK, glycerol kinase; GLUT, glucose transporter; GPD, glycerol-3-phosphate dehydrogenase; IRE, insulin response elements; LPL, lipoprotein lipase; mTOR, mammalian target of rapamycin; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; PI3K, phosphatidylinositol-3-kinase; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; TAG, triacylglycerols; TZD, thiazolidinediones

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Aquaglyceroporins and Glycerol as Metabolic Substrate

Over the past four decades, evidence for the existence of physiological systems aimed at body weight and energy homeostasis has been provided.[1] The adipose tissue has been extensively investigated and has been revealed to act as an endocrine organ that secretes a large number of hormones, growth factors, enzymes, cytokines, complement factors and matrix proteins, collectively termed as adipokines.[2] Upper body excess adiposity is associated with increased incidence of metabolic disturbances, elevated risk of cardiovascular disease and premature death.[3-5] Additionally, selective reduction of visceral adiposity with diet, exercise or bariatric surgery is accompanied by improvements in intermediary metabolism.[6,7] In spite of the growing understanding of the fundamental biology of energy homeostasis, it is evident that crucial pathways have yet to be identified. Aquaglyceporins, integral plasma membrane proteins with proven transport and signaling capabilities, have emerged as key players in shifting the focus of obesity and insulin resistance development.[8,9]

Aquaporins are channel-forming integral membrane proteins that allow the movement of water across the cell membranes.[10,11] These selective channels belong to the large superfamily of major intrinsic proteins (MIP) transmembrane channels and have been found in virtually every living organism, including eubacteria, archaea, fungi, protozoa, plants and animals. Both site-directed mutagenesis and high-resolution electron microscopy studies have revealed that the secondary structure of aquaporins contains six transmembrane α-helices with two highly conserved non-bilayer-spanning loops with the motif asparagine-proline-alanine.[12] The three-dimensional structure of aquaporins resembles an hourglass;[13,14] within the lipid bilayer, aquaporins form a tetrameric quaternary structure, with each monomer defining a single pore.[14] To date, 13 mammalian aquaporins (AQP0-12)

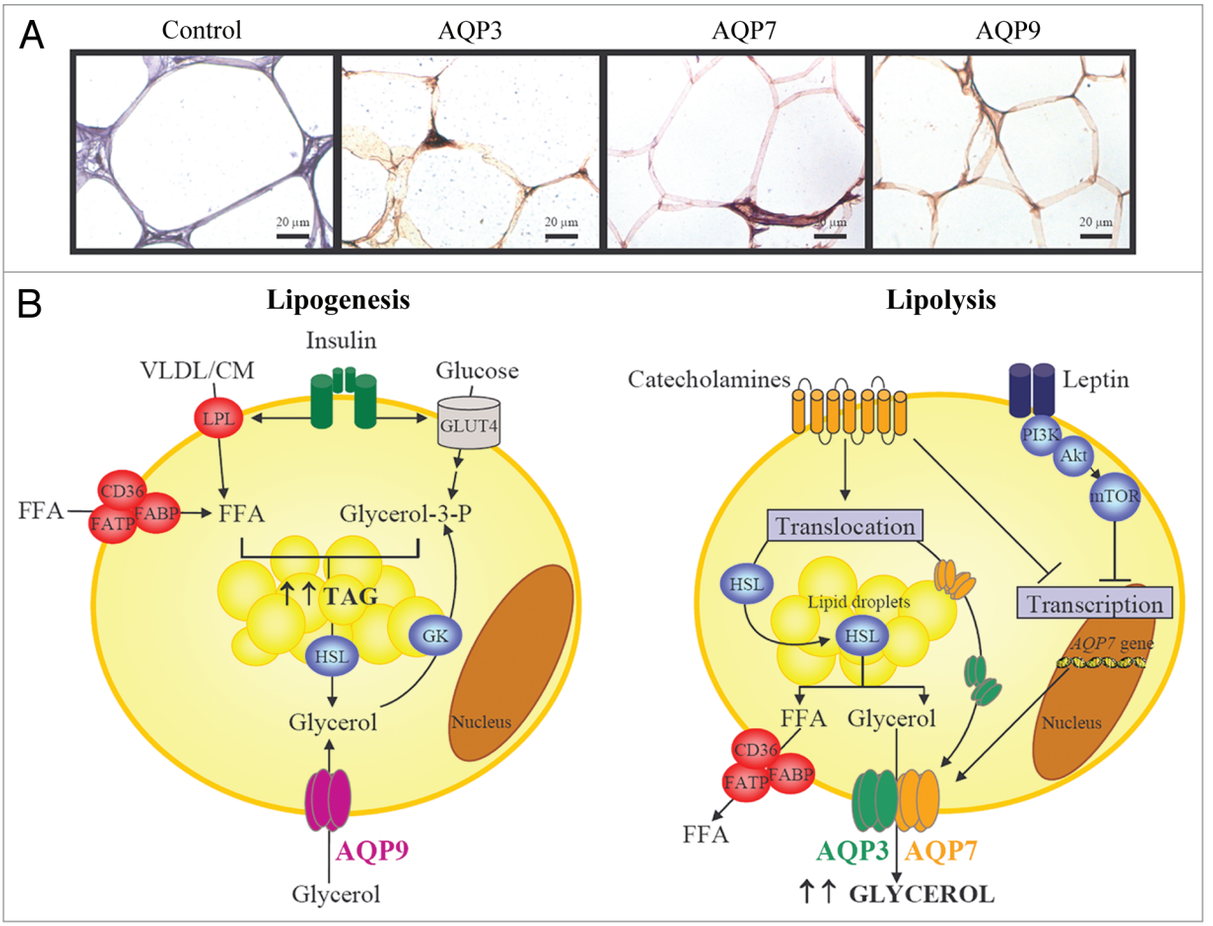

Figure 1. Proposed role of aquaglyceroporins in lipolysis and lipogenesis. (A) Immunohistochemical detection of AQP3, AQP7 and AQP9 in omental adipose tissue obtained from obese patients (magnification x400). (B) In response to lipogenic conditions, adipocytes synthesize triacylglycerols from FFA and glycerol-3-phosphate. LPL and fatty acid transporters (FABP, FATP and CD36) facilitate the FFA transport across the membrane. Glycerol-3-phosphate proceeds from: (1) glucose, (2) glycerol from HSL-dependent lipolysis which is phosphorylated by GK or (3) AQP9-mediated-glycerol uptake. Lipolytic stimuli are characteristically embodied by stimulation of adrenergic receptors by catecholamines, leading to the translocation of HSL to the lipid droplets and its activation as well as to a parallel translocation of AQP3 and AQP7 to the plasma membrane to facilitate the glycerol release. Interestingly, leptin and catecholamines downregulate AQP7 expression, suggesting a negative feedback regulation in lipolytic states to restrict glycerol release from adipocytes. CM, chylomicron; CD, fatty acid translocase; FFA, free fatty acids; FABP, fatty acid binding protein; FATP, fatty acid transporter protein; GK, glycerol kinase; GLUT4, glucose transporter 4; HSL, hormone sensitive lipase; LPL, lipoprotein lipase; TAG, triacylglycerols; VLDL, very low density lipoprotein.

have been identified. Functional studies have defined a subgroup of aquaporins (AQP3, AQP7, AQP9 and AQP10) as aquaglyceroporins, given their permeability, not only to water, but also to small solutes, such as glycerol and urea.[8,10]

Circulating glycerol results from fat lipolysis, diet-derived glycerol or glycerol reabsorbed in the proximal tubules.[15-17] Glycerol represents an important metabolite for the control of fat accumulation, as the carbon backbone of triacylglycerols (TAG) and for glucose homeostasis, given that glycerol constitutes the major substrate for hepatic gluconeogenesis during fasting.[16,18] In addition, glycerol also serves as an energy substrate for pancreatic cells and cardiomyocytes via the glycerol-3-phosphate shuttle, a reaction that allows the NADH synthesized in the cytosol by glycolysis to contribute to the oxidative pathway in the mitochondria to generate ATP.[19] Thus, the regulation of glycerol transport by aquaglyceroporins contributes to the control of fat accumulation, glucose homeostasis, cardiac energy production and pancreatic insulin secretion, among other functions. The present Extra View focuses on advances in the metabolic and cardiac effects of aquaglyceroporins in rodents and humans.

Lipogenesis and Lipolysis (AQP3 and AQP7)

Adipose tissue constitutes the most important source of plasma glycerol.[15] To date, AQP7 was considered the unique glycerol channel in the adipose tissue, but it has been recently shown that AQP3 and AQP9 represent novel additional pathways for the transport of glycerol in human adipocytes (**Fig. 1A**).[20,21] Furthermore, the existence of other yet-undiscovered glycerol facilitators cannot be discarded.[21,22] Teleological reasons for the presence of several glycerol channels in adipocytes include the complex and redundant signaling network

involved in the regulation of lipid and glucose metabolism. The redundancy of proteins exerting similar biological functions is usual in physiology, in particular with biologically critical processes, e.g., the main glucose transporter in insulin-sensitive tissues is GLUT4, but other glucose transporters GLUT1-12 are also expressed and active in liver, adipose tissue or skeletal muscle.[23] Aquaglyceroporins display different subcellular localization in murine 3T3-L1 adipocytes; while AQP3 is present in the plasma membrane and cytoplasm, AQP7 resides predominantly in the cytoplasm upon the lipid droplets, whereas AQP9 is constitutively expressed in the plasma membranes.[21] The main function of aquaglyceroporins in adipocytes is the control of glycerol uptake and release, two key steps for TAG synthesis (lipogenesis) and hydrolysis (lipolysis), respectively.[8] In this regard, aquaglyceroporins are regulated by lipogenic (mainly insulin) and lipolytic (exemplified by leptin and catecholamines) hormones (**Fig. 1B**).[21]

Under lipogenic conditions, such as overnutrition or lack of exercise, adipocytes synthesize TAG from the esterification of free fatty acids (FFA) with glycerol-3-phosphate. 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 membrane.[24] In addition, lipoprotein lipase (LPL) (located on the surface of adipocytes) removes FFA from chylomicrons and very low-density lipoproteins.[25] The other metabolite required for TAG biosynthesis, glycerol-3-phosphate, derives from three metabolic sources: (1) glucose, since glycerol-3-phosphate constitutes a secondary metabolite of glycolysis;[8,18] (2) lipolysis-derived glycerol, which is phosphorylated by glycerol kinase (GK)[26] and (3) aquaglyceroporin-mediated glycerol uptake (**Fig. 1B**).[21] AQP9 appears to be the channel responsible for glycerol influx into adipocytes, since it is a constitutively expressed integral membrane protein not acutely regulated by vesicular trafficking.[21,27] Insulin stimulates lipogenesis through the stimulation of glucose uptake after the translocation of glucose transporter 4 (GLUT4) to the plasma membrane as well as through the activation of LPL and, hence, through FFA uptake after binding to its receptor in adipocytes.[8] Under physiological conditions, insulin regulates the expression of aquaglyceroporins to either increase or decrease glycerol uptake and/or release from adipocytes.[17,18] Insulin reportedly represses the expression of *Aqp7* gene in murine adipocytes through negative insulin response elements (IRE) in its gene promoter.[24,28] Interestingly, insulin increases the protein expression of AQP3, AQP7 and AQP9 in human adipocytes through the phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway,[21] suggesting divergent effects of insulin on the regulation of aquaglyceroporins in humans compared to rodents. Plausible explanations for these diverse effects of insulin in humans compared to rodents might be related to species-specific differences in the physiological relevance of aquaglyceroporins (mouse vs. human) based on the following facts: (1) glycerol becomes the major substrate for hepatic gluconeogenesis in rodents during fasting and post-absorptive states, while pyruvate constitutes the main substrate of the gluconeogenic pathway in humans;[15,18] (2) the rare cases of human AQP3 and AQP7 deficiency identified so far do not resemble the same evident phenotypic characteristics observed in mice[29,30] and (3) the expression of aquaglyceroporins is elevated in insulin-resistant states,[28,31] resulting in increased plasma glycerol and hepatic glucose production in obese, insulin-resistant subjects.[21,29]

In circumstances of negative energy balance, such as fasting or exercise, TAG are hydrolyzed to glycerol and FFA by the adipose triglyceride lipase (ATGL) as well as the hormone-sensitive lipase (HSL) and released into the bloodstream. Catecholamines (noradrenaline and adrenaline) regulate lipolysis through lipolytic β-adrenoceptors (β_1, β_2 and β_3) and anti-lipolytic α_2-adrenoceptors.[32] The β-adrenoceptors couple to G_s proteins that activate adenylate cyclase, leading to an increase in cAMP production, which is followed by activation of protein kinase A (PKA) that induces HSL phosphorylation and translocation from the cytosol to the lipid droplets, thereby increasing lipolysis (**Fig. 1B**). AQP3 and AQP7 facilitate glycerol efflux from murine 3T3-L1 adipocytes in response to the β-adrenergic agonist isoproterenol-induced lipolytic stimulus via its translocation from the cytosolic fraction (AQP3) or lipid droplets (AQP7) to the plasma membrane.[21,33-35] Short-term treatment with isoproterenol induces AQP3 and AQP7 translocation without changes in their expression, whereas long-term stimulation with isoproterenol reduces the expression of *Aqp7* in murine 3T3-L1 cells.[21,36] Another lipolytic factor, such as the adipocyte-derived hormone leptin, has been also shown to repress AQP7 protein expression via the PI3K/Akt/mTOR signaling cascade,[21] suggesting a negative feedback regulation in lipolytic states to restrict glycerol release from fat cells. Moreover, a recent study has shown that carboxymethyl chitin, a mucopolysaccharide with proposed anti-obesity properties, stimulates lipolysis by increasing HSL and AQP7 expression levels in 3T3-L1 adipocytes.[37] AQP7 appears to be one of the main channels for glycerol release from adipocytes. In this sense, it has been shown that adipocytes of *Aqp7*-deficient mice exhibit a higher intracellular glycerol content than those of wild-type mice.[38,39] In humans, the only subject homozygous for the *AQP7* missense mutation (G264V) identified so far exhibited normal body weight and TAG concentrations, but he did not show an exercise-induced increase in glycerol release in spite of elevated plasma noradrenaline concentrations.[29]

Human obesity is associated with an altered expression profile of aquaporins in adipose tissue.[20,40-43] Fat depot-specific differences in the gene expression of *AQP7* in human obesity have been reported. Thus, an overexpression of *AQP7* in omental adipose tissue suggests an increase in the overall lipolytic capacity, while a repression of *AQP7* in subcutaneous fat points to the promotion of an intracellular glycerol accumulation and a progressive adipocyte hypertrophy.[20,21,40-42] Human obesity-associated T2D is related to an additional alteration of the adipose expression profile of AQP3, AQP7 and AQP9 compared to obese normoglycemic subjects. These findings suggest that the regulation of aquaglyceroporins in human adipose tissue is more related to insulin resistance than to obesity.[21] Interestingly, it has been shown

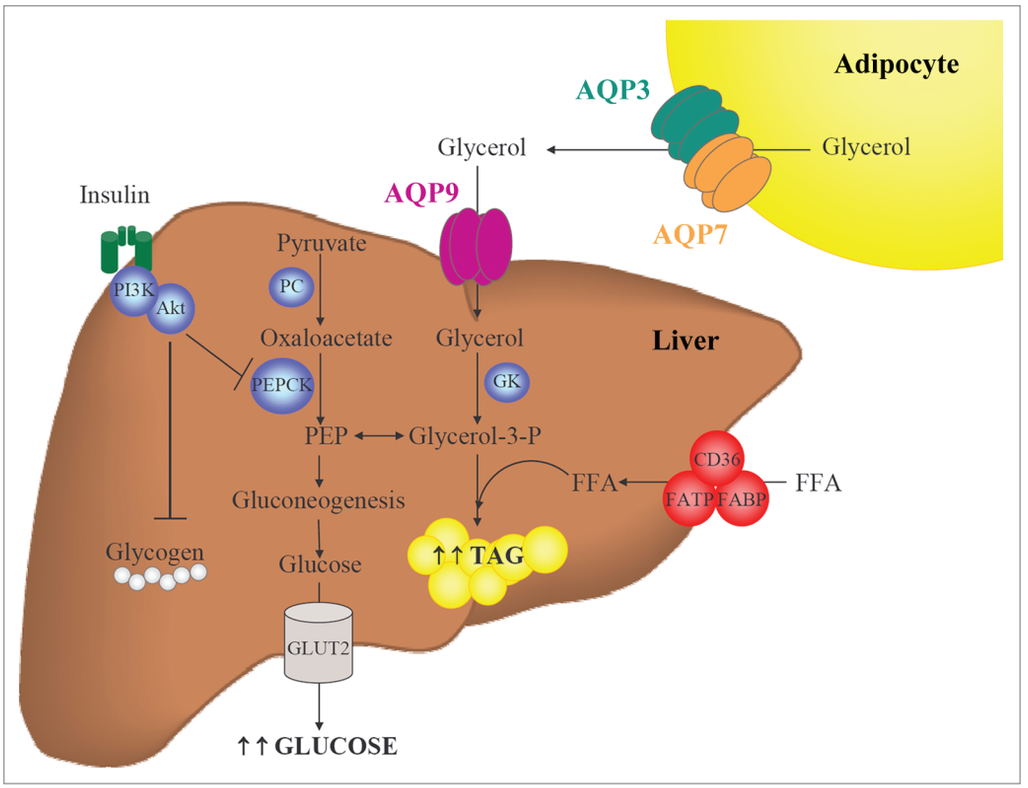

Figure 2. Participation of AQP9 in hepatic gluconeogenesis and steatosis. During fasting, adipocytes induce glycerol release through AQP3 and AQP7 while hepatocytes favor glycerol uptake through AQP9. Glycerol is phosphorylated by GK to produce glycerol-3-phosphate, a precursor of gluconeogenesis and a direct source of glycerol-3-phosphate for de novo synthesis of triacylglycerols. Coordinated regulation of adipose and hepatic aquaglyceroporins is necessary to maintain a correct balance between fat accumulation, hepatic gluconeogenesis and steatosis. FFA, free fatty acids; GK, glycerol kinase; GLUT, glucose transporter; PC, pyruvate carboxylase; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; TAG, triacylglycerols.

that the administration of the insulin-sensitizing agents thiazolidinediones (TZD), e.g., pioglitazone and rosiglitazone, augments the expression of adipose AQP7 in rodents without increasing glycerol release from adipocytes.[44,45] This apparent paradox results from the parallel increase of GK expression and activity induced by TZD in adipocytes, favoring the recycling of glycerol for TAG synthesis instead of stimulating the release of glycerol to the bloodstream.[26,45] Therefore, the undesirable increase of adipose AQP7 expression in insulin-resistant states, plausibly leading to elevated plasma glycerol levels and, consequently, to hepatic gluconeogenesis, can be overcome by an increased glycerol recycling in adipocytes using TZD.

Hepatic Gluconeogenesis and TAG Synthesis (AQP9)

The liver plays a central role in glycerol metabolism, since it is responsible for 70–90% of the whole-body glycerol metabolism.[15] AQP7 represents the main gateway for the delivery of fat-derived glycerol, while glycerol enters hepatocytes via AQP9, where it is converted into glycerol-3-phosphate, a precursor of gluconeogenesis and a direct source for TAG synthesis (**Fig. 2**).[8,18] Pyruvate also constitutes a source for glycerol-3-phosphate by the enzymatic activity of pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK).[15] Insulin inhibits gluconeogenesis by reducing the activity of PEPCK as well as by blocking glucogenolysis (i.e., the conversion of glycogen polymers to glucose monomers).[18] AQP9 constitutes the most important glycerol channel in hepatocytes and is localized at the sinusoidal plasma membrane that faces the portal vein.[46-48] However, AQP3 and AQP7 are also expressed in human hepatic cells, thereby demanding the need for a functional explanation for the redundancy of diverse glycerol channel in the same tissue.[21,49]

The coordinated regulation of adipose and hepatic aquaglyceroporins is required for the control of whole-body glucose homeostasis and lipid accumulation in rodents and humans.[21,28,42] In this sense, *Aqp3*-deficient mice develop nephrogenic diabetes insipidus,[50] *Aqp7* deletion leads to obesity[22,38,39] and *Aqp9* deficiency favors the onset of diabetes.[51] Insulin represses adipose *Aqp7* and hepatic *Aqp9* gene expression through the negative IRE in their gene promoters in rodents.[24,28,52] Murine *Aqp7* deficiency is associated with a failure of the antilipolytic action of insulin, due to impaired IRS-1-associated PI3K activity in adipocytes as well as a defective insulin-induced inhibition of hepatic gluconeogenesis, a result of low IRS-2-associated PI3K activity in hepatocytes.[38] Contrary to what is observed in rodents, the rare cases of human *AQP3* and *AQP7* deficiency do not resemble the phenotypic characteristics observed in transgenic knockout mice.

The individuals carrying a homozygous mutation in *AQP3* and *AQP7* are neither obese nor diabetic.[29,30] The regulation of aquaglyceroporins induced by insulin in human hepatocytes also appears to be different from the control that takes place in mice, since insulin upregulates the expression of aquaglyceroporins in human hepatic cells.[21] Insulin-mediated elevation of AQP3 and AQP7, two glycerol channels surrounding the lipid droplets, may reflect an increased intrahepatocellular TAG content, given that insulin induces hepatic steatosis through the activation of the expression of lipogenic genes.[53] On the other hand, insulin-induced AQP9 upregulation might favor glycerol influx into hepatocytes.[21] Obese patients with T2D exhibit a decreased hepatic AQP9, compatible with a compensatory mechanism aimed at reducing the glycerol entry into hepatocytes and further enhancing the development of hyperglycemia.[21,42,43]

Non-alcoholic fatty liver disease (NAFLD) is characterized by TAG accumulation in hepatocytes and ranges from simple fatty liver (steatosis) to non-alcoholic steatohepatitis (NASH) and to cirrhosis (irreversible, advanced scarring of the liver).[54] AQP9 has been proposed as a pharmacological therapy for NAFLD-NASH.[54] In this sense, our group provided evidence that hepatic *AQP9* mRNA was negatively correlated with intrahepatic lipid content in obese patients.[21] Nevertheless, Miranda and colleagues did not find any relationship between *AQP9* expression and the degree of hepatic steatosis or fibrosis in patients with morbid obesity.[43] In line with this observation, no apparent histological abnormalities were shown in hepatic sections of transgenic *Aqp9* knockout mice.[51] These contradictory observations raise some questions as regards the involvement of AQP9 in the etiopathology of NAFLD-NASH and deserve future studies to outline its potential contribution. Hepatocellular ballooning is one of the histological hallmarks of alcoholic steatohepatitis, while it is much less common in NASH. Acetaldehyde, an oxidation product derived from ethanol by the action of the hepatic enzyme alcohol dehydrogenase, induces a rapid increase in glycerol uptake and cell size in rat hepatocytes via AQP9, but prolonged exposure to acetaldehyde reduces AQP9 expression, suggesting a negative feedback regulation.[55] Exposure to ethanol, in spite of not modifying AQP9 expression, enhances the activity of GK and PEPCK, leading to increased formation of glycerol-3-phosphate, thereby contributing to alcoholic fatty liver.[55]

Pancreatic Insulin Secretion (AQP7)

Circulating glucose concentrations constitute the most physiologically relevant influence on the regulation of pro-insulin biosynthesis and insulin secretion in pancreatic β-cells.[56] Nonetheless, glycerol is also metabolized in the pancreas and shows the potential to stimulate insulin production and secretion.[57] AQP7 seems to embody the unique aquaglyceroporin that mediates the rapid entry of glycerol into β pancreatic cells of mice and rats, being localized in pancreatic islets but not in the pancreatic ducts or the acinar pancreas.[58-60] The increase in intracellular glycerol and the consequent activation of GK activity, in turn, stimulate the pro-insulin mRNA and insulin secretion, probably through their participation in the glycolysis and glycerol-phosphate shuttle activities in β cells (**Fig. 3**).[57,58] The glycerol-3-phosphate shuttle pathway constitutes a mechanism that allows the NADH synthesized in the cytosol by glycolysis to contribute to the oxidative phosphorylation pathway in the mitochondria to generate ATP.[19] Two enzymes are mainly involved in this shuttle, the cytosolic and the mitochondrial glycerol-3-phosphate dehydrogenases (GPD).[61] The cytosolic GPD converts dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate by oxidizing one molecule of NADH to NAD^+. In turn, glycerol-3-phosphate can be reconverted to DHAP by the mitochondrial GPD by reducing one molecule of FAD^+ to $FADH_2$. The electrons from $FADH_2$ feed into the oxidative phosphorylation pathway at coenzyme Q, thereby producing ATP. The raise in the cytoplasmic ATP/ADP ratio produced during glycolysis and the glycerol-phosphate shuttle closes the K_{ATP} channels and depolarizes the plasma membrane, allowing the opening of voltage-dependent calcium channels and a rapid influx of Ca^{2+}.[56,60,62] The consequent increase in cytosolic Ca^{2+} triggers the exocytosis of insulin-containing secretory granules.[60] In this sense, *Aqp7*-deficient mice show elevated glycerol content and increased GK activity in β cells, thereby promoting an increase in islet TAG levels.[58] Moreover, *Aqp7*-knockout mice show hyperinsulinemia and increased pancreatic insulin-1 and insulin-2 transcript levels.[38,58] In addition, *Aqp7* deficiency is associated with higher basal and high glucose-induced insulin secretion, probably as a consequence of the increased pancreatic intracellular glycerol content.[38,58] Interestingly, the increased insulin secretion of *Aqp7*-deficient mice is accompanied by a reduction in the islet cell number and total β cell mass, indicating a more efficient insulin biosynthesis and secretion in these cells.

Diabetes occurs when there is inadequate insulin production in response to the body's demand for the hormone.[56] Type 1 diabetes is associated with absolute insulin deficiency, which occurs when β cells are destroyed by autoimmunity. In insulin-resistant states, such as obesity and type 2 diabetes, expansion of the β-cell mass occurs in response to the increased demand, but diabetes does not occur unless there is concomitant β-cell dysfunction.[56] AQP7, which controls the pancreatic intracellular glycerol content, appears to play a key role in regulating pro-insulin biosynthesis and insulin secretion.[58-60] Human obesity and obesity-associated T2D have been associated with an altered gene expression profile of AQP7 in insulin-sensitive tissues, such as adipose tissue and liver.[21,40-43] Therefore, further studies analyzing the expression or regulation of AQP7 in pancreatic β cells are needed to shed light on its contribution to the development of T2D.

Energy Production in Skeletal Muscle and Heart (AQP7)

Glycerol levels in the interstitial fluid of human skeletal muscle are much higher than plasma levels and approach the concentrations found in adipose tissue.[63] AQP7 is the aquaglyceroporin responsible for glycerol uptake in murine type 2 myofibers,[33] while both AQP3 and AQP7

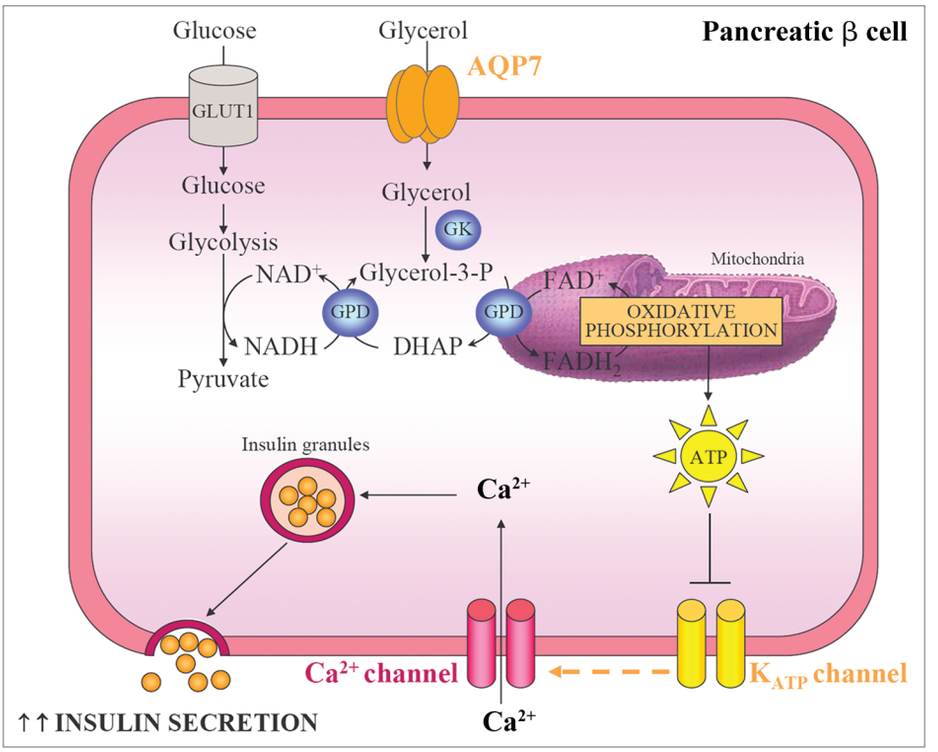

Figure 3. Pancreatic AQP7, glycerol phosphate shuttle and insulin secretion. AQP7 mediates glycerol uptake in pancreatic β cells. Glycerol is phosphorylated to glycerol-3-phosphate by GK and, subsequently, glycerol-3-phosphate is converted to DHAP by the mitochondrial GPD. This reaction reduces equivalents into the mitochondrion for use in oxidative phosphorylation and is called the glycerol-3-phosphate shuttle. The increase in intracellular ATP:ADP ratio induces the closure of ATP-sensitive K^+ channels, the cell membrane depolarization and the opening of voltage-sensitive Ca^{2+} channels. The raise in intracellular Ca^{2+} enhances the exocytosis of insulin-containing granules. GK, glycerol kinase; GLUT, glucose transporter; GPD, glycerol-3-phosphate dehydrogenase.

constitute the glycerol gateways in human type I and type II myofibers.[64,65] Glycerol is converted to glycerol-3-phosphate by GK and it is readily used for TAG synthesis, but it can also be diverted to form glycolytic intermediates that are, in turn, converted to glycogen or lactate.[63] Given the elevated glycerol levels in the interstitial fluid, it seems plausible that changes in aquaglyceroporins and/or GK activity in skeletal muscle may exert important influences on fuel deposition in this tissue.

The heart requires FFA and glucose for energy production in order to maintain its contractility.[66] The oxidation of FFA supplies 60–90% of myocardial ATP, while glucose and lactate provide 10–40% of the cardiac energy production.[19,66] Interestingly, it has been recently described that the murine heart also utilizes glycerol as energy substrate via the glycerol-3-phosphate shuttle pathway, a mechanism that allows the NADH synthesized in the cytosol by glycolysis to contribute to the oxidative phosphorylation pathway in the mitochondria to generate ATP for the contraction of myofilaments (**Fig. 4**).[19] The mitochondrial creatine kinase catalyzes the reaction transforming ATP and creatine into phosphocreatine and ADP.[66] Phosphocreatine rapidly diffuses from the mitochondria to the myofibrils, where myofibrillar creatine kinase catalyzes the restauration of ATP from phosphocreatine. ATP is used by myofibrillar ATPase and other ATP-consuming reactions, such as sarcolemmal and sarcoplasmic reticulum pumps to carry out the contractile work.

AQP7 is apparently the sole glycerol facilitator in the murine heart, and it is localized in cardiac capillaries, fibroblasts and cardiomyocytes.[19,67] Plasma glycerol is introduced into cardiomyocytes by AQP7 and converted to glycerol-3-phosphate by the enzymatic activity of GK (**Fig. 4**). The subsequent entrance into the glycerol-3-phosphate shuttle will lead to increased ATP production and the contraction of myofibrils. In this sense, it has been shown that transgenic mice lacking *Aqp7* gene show reduced cardiac glycerol content, lower expression of enzymes related to the glycerol-3-phosphate shuttle, such as GK and GPD2, as well as lower intracellular ATP levels, suggesting a possible disturbance of cardiac glycerol metabolism and energy production.[19] Surprisingly, under basal conditions, *Aqp7*-knockout mice develop normal cardiac phenotypes. However, *Aqp7*-deficient mice develop cardiac hypertrophy and defective left ventricular contraction following an isoproterenol challenge and subsequently, exhibit a higher mortality than wild-type counterparts, which suggests an impaired myocardial adaptation to pressure overload.[19,68] In humans, the adipose tissue constitutes the major expression site of AQP7,[69] but Sjöholm and colleagues showed that the heart displays the second tissue with highest expression of *AQP7*, both by microarray and real-time PCR analyses.[70] Therefore, further studies are needed to better assess the pathophysiological relevance of cardiac AQP7 in humans.

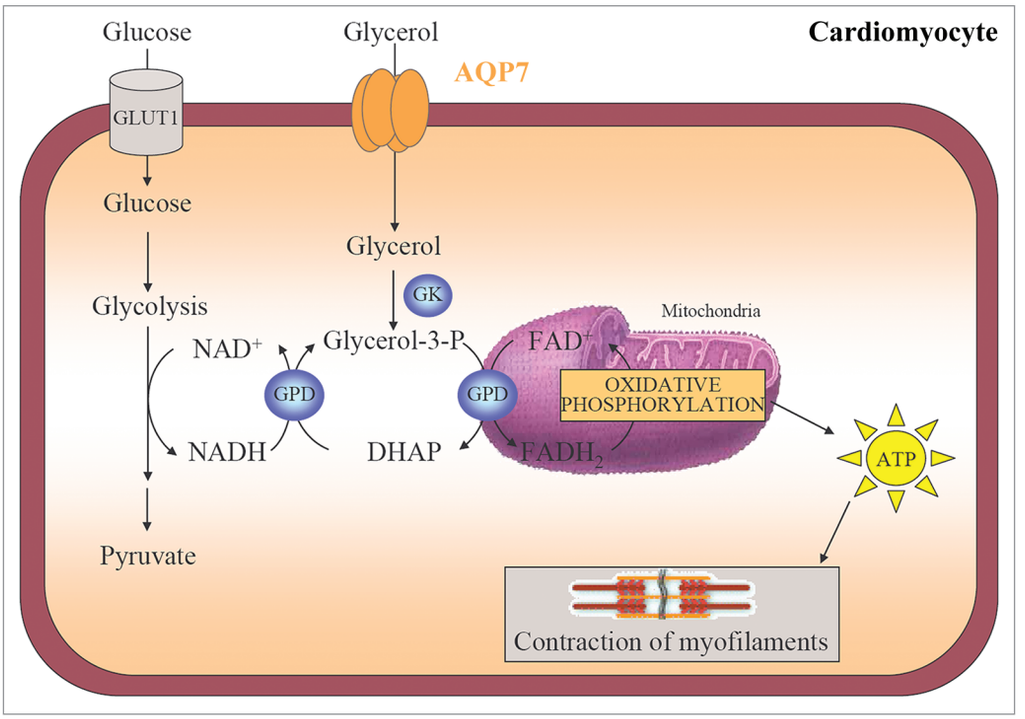

Figure 4. Cardiac AQP7, the glycerol phosphate shuttle and ATP production. AQP7 facilitates glycerol uptake by cardiomyocytes. Glycerol is phosphorylated to glycerol-3-phosphate by GK that enters in the glycerol-3-phosphate shuttle to trigger ATP production in the mitochondrial oxidative phosphorylation. The transfer of ATP to the cytosol is achieved by the creatine kinase energy shuttle and is used for the contraction of myofilaments. DHAP, dihydroxyacetone phosphate; GK, glycerol kinase; GPD, glycerol-3-phosphate dehydrogenase.

Human Genetic Studies

The functional importance of glycerol channels in mammalian physiology and pathophysiology has been extensively studied by analyzing the phenotype of transgenic knockout mice lacking different aquaglyceroporins.[10,11] Until now, information regarding the role of aquaglyceroporins in humans has been scarce. The rare cases of homozygous mutations in the coding region of the *AQP3* and *AQP7* genes identified so far do not resemble the same evident phenotypic characteristics observed in mice, since they were neither diabetic nor obese.[29,30] In the Japanese population, the frequency of missense mutations (R12C, V59L, G264V) and silence mutations (A103A, G250G) of *AQP7* was not associated with obesity or T2D, despite the absence of glycerol transport in a subject carrying the G264V mutation.[29] Nevertheless, Ceperuelo-Mallafré and colleagues reported one case homozygous for the G264V mutation with overweight and T2D.[71]

The detection of several single nucleotide polymorphisms (SNPs) within the genes encoding aquaglyceroporins also represents an important approach to investigate their cardiometabolic effects. One single variation (G105C) in the *AQP3* gene was found in 11 patients (32%) with Menière's disease, an episodic cochleovestibular dysfunction of unknown etiology.[72] Prudente and colleagues identified one SNP (A953G) in the promoter region of the *AQP7* gene, resulting in an impaired ability to bind the CCAAT/enhancer binding protein β (C/EBPβ) transcription factor.[41] Furthermore, it was shown that the *AQP7* gene expression was lower in adipose tissue of obese subjects. In addition, the A953G variant in the *AQP7* promoter was associated with T2D in 977 Caucasians.[41] Recently, an intronic A/T (rs2414539) SNP in the *AQP9* gene has been associated with femoral neck bone mineral density in postmenopausal women and may represent one of the susceptibility genes for phenotypes related to bone mass.[73]

Finally, altered gene and protein expression levels of aquaglyceroporins have been reported in several human diseases. To date, the impaired coordination of adipose and hepatic aquaglyceroporins have been extensively studied in human obesity and obesity-associated T2D.[20,21,40-43] A strong increase in *AQP9* transcripts was observed in synovial tissues from patients with osteoarthritis and rheumatoid arthritis, suggesting its relation to the pathogenesis of hydrarthrosis and inflammatory synovitis.[74] Interestingly, *AQP3* mRNA levels are highly expressed in several human cancer cell types, including skin, esophageal, lingual, lung, colorectal and gastric carcinomas.[75-79] In this regard, AQP3 appears to play a critical role in basal and epidermal growth factor (EGF)-induced cancer cell migration and proliferation.[77,80]

Conclusion

In summary, given the versatile functions of aquaglyceroporins, additional and unexpected roles of these glycerol channels are sure to emerge in the coming years. Likewise, further investigations in novel mutations, SNPs or differential expression levels of aquaglyceroporins are needed to broaden our understanding of the implications of these glycerol pores in the onset of human diseases.

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