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INVITED EDITORIAL

Aquaporin in diabetes: more underwater enemies?

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This editorial refers to 'Aquaporins enriched in endothelial vacuole membrane regulate the diameters of microvasculature in hyperglycaemia', by C. Chen *et al.*, https://doi.org/10.1093/cvr/cvae085.

Diabetes mellitus (DM), characterized by high concentrations of blood glucose due to resistance to insulin, inadequate insulin secretion, or both, affects millions of people worldwide, regardless of country, age, group, or sex. In 2021, 529 million people lived with diabetes worldwide, with a global age-standardized total diabetes prevalence of 6.1%.[1] Type 2 diabetes mellitus (T2DM), the most common form of diabetes, accounts for approximately 90% of all cases of diabetes worldwide with a high incidence rate in recent years and an expected increase in global prevalence by almost 50% resulting in 700 million people affected by 2045.[2]

This long-term chronic disease is one of the leading causes of death but also of disability worldwide, not least because of its secondary vascular complications. The DM-related vascular disease includes both macrovascular atherosclerosis and microvascular disease related to endothelial dysfunction, basement membrane thickening, and microthrombosis[3] leading to end-organ damage including in the retina. In addition, the prevalence of DM-related microvascular complications is two to three times higher than the expected rate of macrovascular complications (with almost 20% of people with T2D likely to develop microvascular complications during their lifetime). Diabetes is the leading cause of blindness in the working-age population due to the high prevalence (2–33%) of diabetic retinopathy, resulting from an impaired microcirculation,[4] a debilitating condition with currently no specific available treatment.

Mammalian aquaporins (AQPs) belong to a family of 13 isoforms (AQP0–AQP12) of water channels, the first of which (AQP1) was discovered by the Nobel Laureate, P. Agre and his colleagues in 1992.[5] Their main function is to induce rapid transmembrane bidirectional transport of water; as such, they are involved in transepithelial fluid transport, brain water balance, cell migration with rapid changes in cell volume, cell proliferation, or epidermal hydration. AQP1 is the main isoform expressed in cardiovascular (CV) tissues of mammals, while lower expression of AQP8 is only observed in the myocardium. AQP8-null mice display no specific cardiovascular phenotype so the role of this isoform in the heart and vessels remains uncertain.[6] In contrast, AQP1 is strongly expressed in all CV resident cell types including cardiac myocytes, smooth muscle, and endothelial cells. Deletion of the *Aqp1* gene in mice results in abnormal cardiovascular morphology characterized by microcardia (despite no change in capillary density) and a significant reduction in the thickness of arterial walls in the aorta.[7]

Relatively little is known on the regulation of *AQP1* gene expression, despite previous descriptions of up-regulation by hyperosmolality through Tonicity Enhanced Binding Protein (TonEBP) in several cell types[8] and glucocorticoids through a glucocorticoid-response element in peritoneal capillary endothelial cells.[9] Importantly, a common SNP (rs2075574) in the *AQP1* promoter drives an approximately 27% reduction in *AQP1* mRNA (and 37% lower protein) expression in the human peritoneum and has been associated with an impaired efficiency of peritoneal dialysis (due to decreased endothelial AQP1-dependent ultrafiltration) and adverse outcome in several cohorts of patients with end-stage renal disease.[10] Pre-specified subgroup analysis suggested a slightly (non-significant) higher risk of composite of death or peritoneal dialysis failure (i.e. transfer to conventional haemodialysis) in diabetics with this specific SNP.

In the current issue of the journal, Chen *et al.*[11] examined the expression of AQP1 in the retina of diabetic patients and the role of *aqp1a.1* and *aqp8a.1* (corresponding to human AQP1 and -8 orthologs) in the vascular development of zebrafish embryos under high glucose (*Figure 1*).

First, the authors confirmed a slight reduction in the diameter of retinal arterioles and venules in DM patients compared with healthy individuals. They correlated this with a lower expression of *AQP1* mRNA in diabetic compared with healthy retinas. Exposure of human retinal microvascular endothelial cells (ECs) to high glucose produced a dose-dependent decrease in *AQP1* mRNA. Likewise, transgenic (Tg) zebrafish embryos with endothelial-specific expression of enhanced green fluorescent protein (EGFP) exposed to hyperglycaemic milieu during development (i.e. between Days 2 and 3 after fertilization) showed a reduced diameter of the inner optic circle (also called the circumferential vein) and of the intersegmental vessels, associated with dysfunctional perfusion of the same vessels. Transcriptomic data using single-cell RNA-seq and qPCR analyses of the EGFP-expressing ECs confirmed a reduced expression of *aqp1a.1* and *aqp8a.1* (among other genes) in high glucose. Specific *aqp1a.1* and *aqp8a.1* gene deletion using CRISPR/Cas9 or down-regulation (using morpholino) and treatment with $HgCl_2$ (a non-specific inhibitor of AQP water conductance) resulted in a slight reduction in the diameter of the arterial and venous vessels of the embryos, associated with an alteration in microperfusion (only upon gene deletion). In contrast, Tg embryos overexpressing aqp1a.1 and aqp8a.1 developed larger blood vessels (particularly in the venous network). In addition, Tg embryos with mosaic expression of *aqp1a.1* in ECs exposed to a hyperglycaemic milieu recovered the decrease in vascular diameter specifically in vessel sections expressing *aqp1a.1*. Embryos genetically deficient in *aqp1a.1* and *aqp8a.1* had smaller and less intracellular vacuoles in ECs, which are required for vessel lumenization. In a model of embryoid body using a human embryonic stem cell line, deletion of *AQP1* did not impair differentiation to ECs, but reduced tube

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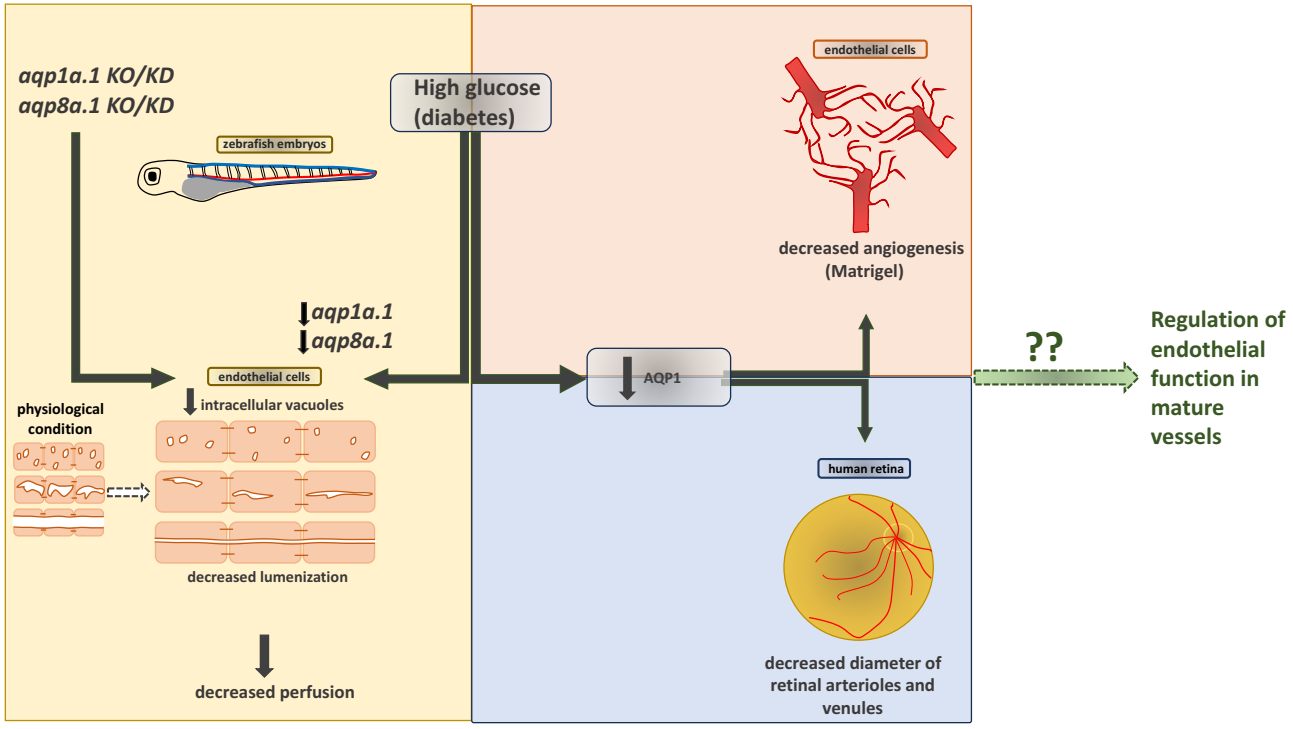

Figure 1 Role of aquaporins in embryonic vessel development and angiogenesis. (Left) In zebrafish embryo, aqp1a.1 and aqp8a.1 expression promote the formation of intracellular vacuoles that ultimately fuse together to ensure vessel lumenization. Aqp1a.1 and aqp8a.1 gene deletion or down-regulation, as well as embryo exposure to high glucose decrease vacuole formation and vessel lumenization, resulting in reduced vessel diameter and impaired perfusion. (Right) Retinas from diabetic patients show smaller arterioles and venules, together with decreased AQP1 abundance. Exposure of human retinal microvascular endothelial cells to high glucose decreases AQP1 abundance, paralleled with decreased sprouting and tube formation on Matrigel. These effects of high glucose on angiogenesis and embryonic vessel formation may differ from those on AQP1 and endothelial function in mature vessels, where additional roles of AQP1—beyond water transport—may adversely influence vascular biology (see text for details).

formation from differentiated ECs on Matrigel. Mechanistically, the authors propose that *aqp1a.1* and *aqp8a.1* in (zebrafish) endothelial cells regulate vascular development through the formation of intracellular vacuoles and their subsequent coalescence for lumenization. As AQP1 (but not AQP8) is identically expressed and down-regulated by high glucose in human EC, they propose gene therapy as a potential approach to re-express AQP1 and correct vascular dysregulation in diabetes.

With a detailed phenotypic analysis in transgenic zebrafish embryos, the authors build on previous demonstrations of the role of vacuole coalescence in lumen formation during vessel development,[12] while pointing to the critical role of vacuolar *aqp1a.1* and *aqp8a.1* expression and function. The inhibitory effect of the (non-specific) aquaporin inhibitor, $HgCl_2$ [that, in mammalian Aqp1, blocks water passage by covalent modification of a critical Cysteine residue (Cys189) in the pore of monomeric channels], suggests a mechanism that somehow involves the transport function of the channel, rather than alternative effects through, e.g. protein–protein interactions with other (unidentified) key molecular players. If water transport is involved, this is probably to support vacuole growth by swelling, until multi-vacuole coalescence. What creates and sustains the osmotic gradient to drive water influx to the vacuoles, though, remains unknown. In essence, such process would be very similar to the build-up of lamellipodia at the leading edge of migrating cells, where the role of Aqp1 was described almost 20 years ago.[13]

It is important to note that, in the present study, all the effects of *aqp1a.1* and *aqp8a.1* modulation were examined on vascular formation during embryo development, including under high glucose, so that such effects may not be extrapolated to pre-formed, mature vessels. The reported effects on blood flow and perfusion, again, should be interpreted as a direct consequence of altered maturation, resulting in smaller vessel diameters. This may be different from other effects of high glucose (or aquaporin modulation) on endothelial function in fully formed vessels. Likewise, the present study of AQP1 modulation in human endothelial cells was limited to their angiogenic capacity (with a reduction in sprouting and tube formation on Matrigel upon *AQP1* deletion). Nevertheless, incubation of human retinal microvascular endothelial cells in high glucose did reduce *AQP1* expression in the present study; if applicable to other vascular beds, this could impact other AQP1-dependent functions, such as ultrafiltration in peritoneal dialysis, *a fortiori* under long dwells with glucose solutions. Notably, in the study reported above,[10] lower *AQP1* expression associated with rs2075574 had no impact on vascular density or vessel diameter in the peritoneum, contrary to the present data in zebrafish embryos. Also, previous studies showed that high glucose and hyperosmolality increased AQP1 expression in other macro- and microvascular endothelial cells, as well as tube formation on Matrigel.[14] Finally, if high glucose reduces endothelial AQP1 and angiogenesis, the authors' observation of lower AQP1 in diabetic patients with retinal vasculoproliferative disease seems counterintuitive.

AQP1 (unlike AQP8, not expressed in EC) could be involved in many other mechanisms of glucose toxicity on the endothelium, particularly in diabetic vasculopathy. For example, AQP1 may promote a shift of insulin signalling away from physiological nitric oxide (NO) signalling and towards mitogenic and pro-inflammatory pathways in endothelial cells exposed to high glucose.[15] In addition to osmotic-driven water movements, AQP1 also mediates oxidant signalling. We recently confirmed AQP1 as a bona fide

'peroxiporin' by its property to facilitate the transmembrane passage of hydrogen peroxide (H_2O_2) through its water channel. In cardiac myocytes, AQP1 modulates H_2O_2-dependent signalling to a pro-hypertrophic response, and genetic deletion of *Aqp1* or pharmacologic blockade of Aqp1 *in vivo* prevents the development of myocardial hypertrophy and fibrosis.[16] A similar property of AQP1 in endothelial cells may promote oxidant stress in the endothelium by facilitating the intracellular penetration of H_2O_2 generated extracellularly from NADPH (NOX)-derived superoxide anions.[15] The resulting endothelial dysfunction would impair vasorelaxation and promote vascular remodelling towards atherosclerosis and ensuing inflammatory and thrombotic complications. Therefore, changes in AQP1 abundance (or function) may have radically different impacts on embryonic vessel development and endothelial function in mature vessels and it is unclear, then, if increasing AQP1 expression in hyperglycaemic conditions would truly be beneficial. Overall, the multifaceted role of AQP1 in the vasculature mandates future research to orient its modulation for the treatment of diabetes and its cardiovascular complications.

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