

Fasting influences aquaporin expression, water transport and adipocyte metabolism in the peritoneal membrane

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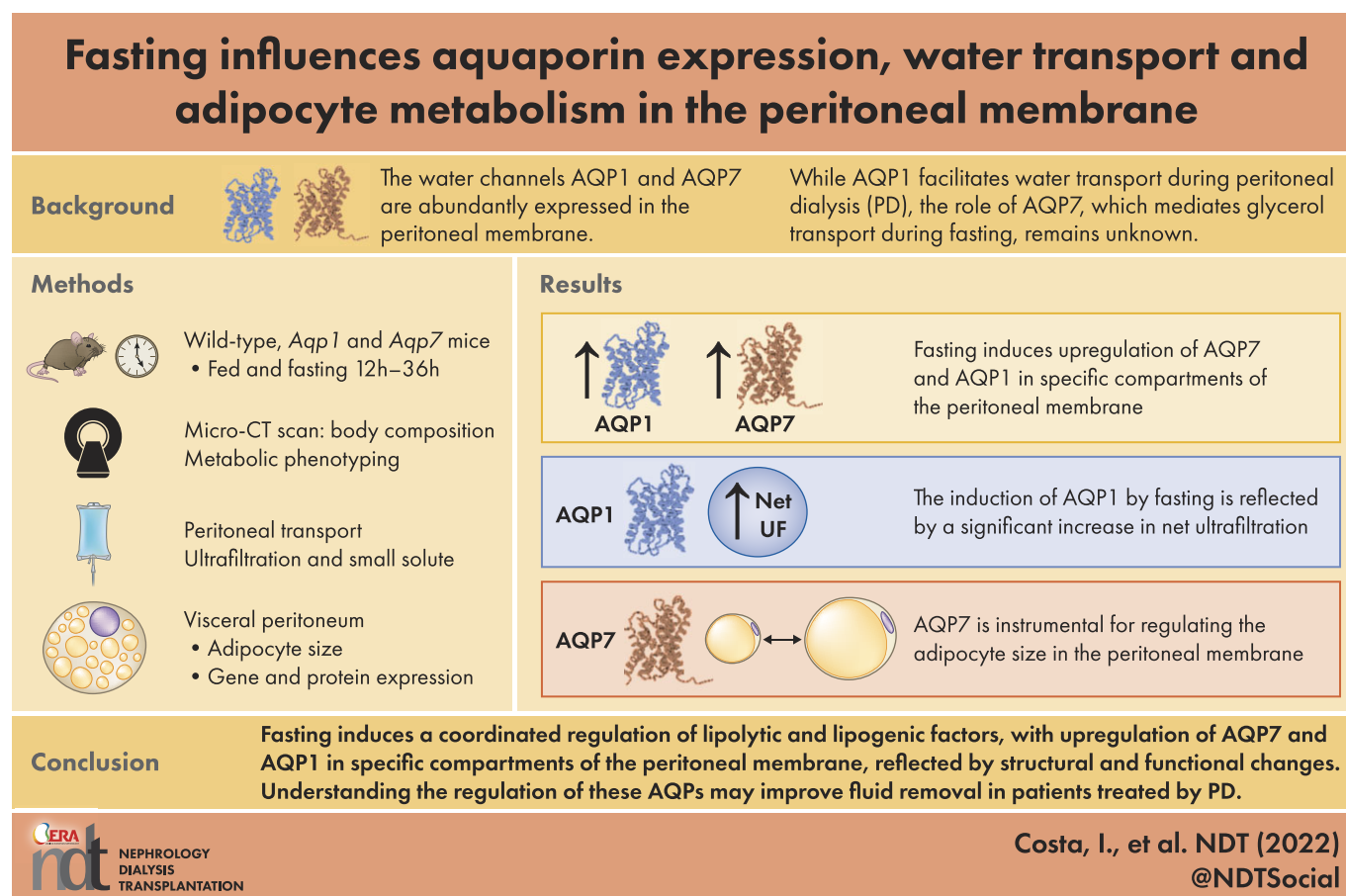
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GRAPHICAL ABSTRACT



KEY LEARNING POINTS

What is already known about this subject?

- Fasting induces a progressive, significant decrease in body weight, plasma glucose, insulin and triglycerides.
- The water channels aquaporin-1 (AQP1) and AQP7 are abundantly expressed in the peritoneal membrane.

What this study adds?

- This study demonstrates that fasting induces a coordinated regulation of lipolytic and lipogenic factors, with upregulation of AQP7 and AQP1 in specific compartments of the peritoneal membrane, reflected by structural and functional changes.
- Fasting increases ultrafiltration by upregulating AQP1 expression in the peritoneal membrane.
- AQP7 is instrumental for regulating the size of adipocytes in the peritoneum.

What impact this may have on practice or policy

- These results yield novel information on the specific roles of aquaporins and suggest that fasting may improve fluid removal during peritoneal dialysis.
- Factors modulating the expression of AQP7 may be relevant in patients who experience increased visceral abdominal fat during peritoneal dialysis.

ABSTRACT

Background. The water channels aquaporin-1 (AQP1) and AQP7 are abundantly expressed in the peritoneal membrane. While AQP1 facilitates water transport during peritoneal dialysis (PD), the role of AQP7, which mediates glycerol transport during fasting, remains unknown.

Methods. We investigated the distribution of AQP7 and AQP1 and used a mouse model of PD to investigate the role of AQP7 in the peritoneal membrane at baseline and after fasting.

Results. Single nucleus RNA-sequencing revealed that AQP7 was mostly detected in mature adipocytes, whereas AQP1 was essentially expressed in endothelial cells. Fasting induced significant decreases in whole body fat, plasma glucose, insulin and triglycerides, as well as higher plasma glycerol and corticosterone levels in mice, paralleled by major decreases in adipocyte size and levels of fatty acid synthase and leptin, and increased levels of hormone-sensitive lipase mRNAs in the peritoneum. Mechanistically, fasting upregulated the expression of AQP1 and AQP7 in the peritoneum, with increased ultrafiltration but no change in small solute transport. Studies based on *Aqp1* and *Aqp7* knockout mice and RU-486 inhibition demonstrated that the glucocorticoid induction of AQP1 mediates the increase in ultrafiltration whereas AQP7 regulates the size of adipocytes in the peritoneum.

Conclusions. Fasting induces a coordinated regulation of lipolytic and lipogenic factors and aqua(glycero)porins in the peritoneum, driving structural and functional changes. These data yield novel information on the specific roles of aquaporins in the peritoneal membrane and indicate that fasting improves fluid removal in a mouse model of PD.

Keywords: AQP7, aquaglyceroporin, glycerol, metabolism, peritoneal dialysis

INTRODUCTION

Peritoneal dialysis (PD) is the leading modality for self-dialysis in patients with kidney failure [1]. The technique is based on the removal of water, metabolic products and toxins from patients through diffusive and convective transport processes

across the peritoneal membrane. The endothelium lining the capillaries within the peritoneal membrane represents the rate-limiting step in solute and water transport. Computer simulations and studies in animal models and patients treated by PD have identified the water channel aquaporin-1 (AQP1) as the ultrasmall pore located in the endothelial barrier that facilitates the transport of water but not that of small solutes across the peritoneal membrane [2–4].

Since the identification of AQP1 as the archetypal water channel by Preston *et al.* [5], 13 mammalian aquaporin homologues have been identified along related proteins in plants, invertebrates and microorganisms [6–8]. The aquaporins are integral membrane proteins organized in tetramers, with each monomer containing six membrane-spanning α -helices surrounding a central pore. Structural and permeability characteristics define three subtypes of channels: the classic, water-specific aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8); the aquaglyceroporins (AQP3, AQP7, AQP9, AQP10), which are permeated by water and small solutes including glycerol; and the unorthodox or superaquaporins (AQP11 and AQP12), which share low homology with the other aquaporins and whose functions remain poorly defined [6, 8, 9].

We previously demonstrated that AQP1 and AQP7 are the most abundant aquaporins expressed in the mouse and human peritoneal membrane [3]. AQP1 is located in the capillary endothelium, and studies in *Aqp1* knockout (*Aqp1*^{−/−}) mice showed it mediates fast osmotic water transport and up to half the ultrafiltration (UF) during PD performed with hypertonic glucose dialysate [3, 10]. Modulation of AQP1 expression in the peritoneum, either by genetic factors or by pharmacological agents, drives significant changes in osmotic water transport and UF in mice and humans treated by PD [4, 11, 12]. Compared with the abundant evidence supporting the crucial role of AQP1 during PD, the potential role of AQP7 in the peritoneal membrane remains essentially unknown.

AQP7 is an aquaglyceroporin involved in the transport of water and glycerol [13, 14]. The AQP7 gene coding for AQP7 was cloned from rat testis and human adipose tissue. AQP7 is abundantly expressed in adipocytes (white and brown

adipose tissue) and in microvascular endothelial cells [15, 16], and its expression is negatively regulated by insulin [17, 18]. During lipolysis, typically associated with fasting, AQP7 facilitates efflux of glycerol by passive diffusion [13]. The glycerol efflux from the adipocytes is regulated by the translocation of AQP7 from the lipid droplets to the plasma membrane upon hormonal stimulation [19]. The critical role of AQP7 in regulating glycerol efflux has been substantiated by studies in *Aqp7*^{-/-} mice and by recent structural data [17, 18, 20, 21].

In the peritoneal membrane, the adipocytes release mediators, adipokines and growth factors that mediate interactions with other cell types [22]. A progressive increase in visceral fat has been described in relation to PD [23], potentially associated with inflammation and adverse metabolic outcomes [22]. Due to its abundant expression and its function as a regulated glycerol pathway, AQP7 may play an important role in the structure and transport properties of the peritoneal membrane—particularly during fasting. In this study, we analyzed the specific distribution of AQP7 and used well-established mouse models to assess the influence of fasting on the structure and function of the peritoneal membrane and the roles of AQP1 and AQP7 in that context.

MATERIALS AND METHODS

Single-nucleus RNA-sequencing for AQP1 and AQP7

Publicly available datasets were used to investigate the expression of the genes coding for AQP7 and AQP1 in mouse and human tissues, including mouse (ENCODE) and human (Human Protein Atlas) bulk RNA-sequencing (RNA-seq) [24, 25]. The expression of AQP7 and AQP1 in the adipose tissue was validated in a human white adipose tissue single nucleus RNA-seq (snRNA-seq) dataset under the series number GSE176171 [26], and a mouse epididymal white adipose tissue snRNA-seq dataset under the series number GSE160729 [27] (Supplementary data, Table S1). For analysis of snRNA-seq datasets, the R library Seurat version 4.1.0 [28] was used to read and process Rds objects provided by the authors [26, 27]. The DimPlot function was used to generate the Uniform manifold approximation and projection (UMAP) plot showing clustering of the cells into distinct populations based on the expression of specific markers. The FeaturePlot function was used to overlap cell clustering with gene expression, while violin plots were generated using the VlnPlot function, all part of the Seurat package.

Experimental animals

The experiments were conducted using 10- to 12-week-old male *Aqp1* (SV129 background) [29] and *Aqp7* (C57BL/6J background) [14] knock-out mice and their littermate controls. Wild-type mice used for fasting time-course and PD were of C57BL/6J background. Mice were housed in an air-conditioned room with a 12/12-h dark/light cycle and acclimatized to the new environment for 1 week before the experiment. Mice were allowed free access to standard laboratory diet (Scientific animal food & engineering, Augy,

France) and tap water. Fed mice were sacrificed to serve as control (time point 0); for fasted mice, food was removed 12, 24 or 36 h before sacrifice (each group, $n = 9$) [13]. Mice were allowed free access to tap water during the whole protocol. In a subgroup of mice, a single dose (25 mg/kg i.p.) of the glucocorticoid receptor antagonist RU-486 (Tocris Bioscience, Ellisville, MO, USA) was administrated 12 h before sacrifice [30]. The experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and approved by the ethics committee of the UCLouvain Medical School (Brussels, Belgium).

Micro-computed tomography analysis

Micro-computed tomography (micro-CT) scans were performed on mice anesthetized with isoflurane/oxygen to monitor changes in body composition and fat mass. Scanning was performed with Skyscan 1278 (Bruker, Kontich, Belgium) at 50 μm voxel resolution using a source voltage of 65 kV and a current of 770 μA as described [31]. Raw images were then reconstructed with an isotropic voxel size of ($51 \times 51 \times 51$) μm^3 . All analyses were performed using SkyScan software (CT Analyser version 1.17.7.2).

Peritoneal transport studies, blood and tissue sampling

At each time point, a peritoneal equilibration test (PET) with 2.5 mL of 3.86% glucose dialysate was performed to assess peritoneal transport parameters, as previously described [32]. Briefly, mice were submitted to a 2-h peritoneal equilibration test after instillation of 2.5 mL of 3.86% glucose dialysate. At the end of the dwell, the dialysate was recovered from the peritoneal cavity and the net UF volume was calculated. Blood samples were collected from the inferior vena cava and immediately separated the plasma by centrifugation. Plasma was stored at -20°C until analysis. Triglycerides, glucose and sodium were assayed using Dri Chem NX500i (Fujifilm, Tokyo, Japan). Protein concentration was assayed using Pierce[®] BCA protein assay kit (Thermo scientific, Rockford, IL, USA). The visceral peritoneum was snap-frozen in liquid nitrogen and stored at -80°C , or routinely fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (VWR, Brussels, Belgium).

Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assay kits were used to measure insulin (Mercodia, Uppsala, Sweden) and corticosterone (Enzo, Lausen, Switzerland) levels in serum samples. Assays were run in duplicate according to the manufacturer's protocols.

Glycerol analysis

A colorimetric assay kit (#MAK117, Sigma-Aldrich) was used to quantify glycerol levels released from adipocytes in serum, and performed as per the manufacturer's instructions.

RT-PCR and quantitative real-time RT-PCR

Total RNA from mouse visceral peritoneum was extracted with Trizol (Invitrogen, Merelbeke, Belgium) and using Aurum™ Total RNA fatty and fibrous tissue kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. DNase I treatment was performed to eliminate genomic DNA contamination. One microgram of RNA was used to perform the reverse transcriptase reaction with iScript™ cDNA Synthesis Kit (Bio-Rad). Four reference genes (*Gapdh*, *Actb*, *36b4*, *Cyc*) not influenced by fasting protocols were combined for the normalization. The primers are given in Supplementary data, Table S2. Changes in the mRNA levels of target genes were determined by semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) with an iCycler IQ system (Bio-Rad) using SYBR Green I detection as described previously [32]. The PCR conditions were 95°C for 3 min followed by 40 cycles of 30 s at 95°C, 15 s at 60°C and 1 min at 72°C. The relative changes in target mRNA between vehicle and treated groups were determined by using the relation $2^{-\Delta\Delta Ct}$.

Antibodies

The following antibodies were used in this study: rabbit anti-mouse AQP1 (Chemicon International, Temecula, CA, USA); rabbit anti-mouse AQP7 (Abcam, Cambridge, UK); rabbit IgG isotype controls (R&D systems, Wiesbaden-Nordenstadt, Germany); and mouse anti-mouse β -actin (Sigma, St Louis, MO, USA). The specificity of the antibodies was confirmed by western blot for AQP7 and AQP1 using wild-type and *Aqp7*^{-/-} and *Aqp1*^{-/-} mouse samples (Supplementary data, Fig. S1).

Western blotting

Immunoblotting was performed as described [3]. Proteins were extracted from visceral peritoneum, lysed in lysis buffer containing protease inhibitors (Complete Mini, Roche Diagnostics, Belgium) and followed by sonication and centrifugation at 10 000 r.p.m. for 10 min at 4°C. Proteins were diluted in Laemmli buffer and separated by SDS-PAGE as described [33]. Densitometry analysis was performed by scanning the blots and measuring the relative density of each band normalized to β -actin using the ImageJ software.

Tissue staining and immunohistochemistry

Samples from the peritoneum were fixed in 4% paraformaldehyde, embedded in paraffin and cut into 6 μ m sections. Hematoxylin and eosin staining and immunostaining were performed as described [3, 32]. Sections were scanned by SCN4000 scanner (Leica, Heerbrugg, Switzerland) and visualized by digital image hub (Leica). The areas of adipocytes in the visceral peritoneum (Hematoxylin and eosin staining) were calculated ($\pi \cdot \text{radius}^2$) in at least 50 cells per section, using ImageJ software. For immunofluorescence, sections of visceral peritoneum were dewaxed, rehydrated in serial ethanol dilutions and blocked with appropriate serum. The sections were then incubated for 1 h with primary antibody,

washed six times in phosphate-buffered saline, incubated for 1 h with the secondary (Alexa labelled) antibody, mounted in Prolong Gold anti-fade reagent (Invitrogen) and analyzed under a Zeiss LSM 510 Meta Confocal microscope (Carl Zeiss, Jena, Germany).

Data analysis

Results are expressed as mean $\pm$ SD. Differences between groups were assessed by unpaired *t*-test, one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test, two-way ANOVA (to assess genotype effect in fed and fasted states) and the Kolmogorov–Smirnov two-sample test (to compare distributions of the size of adipocytes in the different conditions), using GraphPad Software (San Diego, CA, USA). A *P*-value < .05 was considered statistically significant.

RESULTS

Distribution of AQP7 and AQP1 in human and mouse visceral adipose tissue

Both AQP1 and AQP7 mRNAs are highly expressed in human and mouse adipose tissue (Supplementary data, Fig. S2). Since traditional single cell expression studies on adipocytes are delicate due to the large size and fragility of these cells, snRNA-seq, which can capture adipocytes, has been used to investigate murine epididymal and human brown adipose tissue [26]. Analysis of snRNA-seq atlas of human visceral white adipose tissue revealed 17 cell populations including adipocytes, adipose cell progenitors, endothelial cells, mesothelial cells and several clusters of immune cells (Fig. 1A). AQP7 was mostly detected in the mature adipocyte cluster, characterized in humans by the expression of *ADIPOQ* and in mouse by the expression of *Lipe*, *Plin4* and *Pparg*, whereas AQP1 was strongly expressed in the cluster of microvascular endothelial cells, identified in human by the expression of *JAM2* and in mouse by expression of *Esam*, *Flt1* and *Vwf* (Fig. 1B and C; Supplementary data, Table S3).

Similarly, analysis of snRNA-seq of mouse epididymal adipose tissue showed seven different cell clusters, including mature adipocytes, fibro-adipogenic progenitors, mesothelial cells, endothelial cells and immune cells (Fig. 1D). Cells expressing *Aqp7* were mostly adipocytes, followed by fibro-adipogenic progenitors and endothelial cells, whereas cells expressing *Aqp1* were essentially identified as endothelial cells (Fig. 1E and F).

The expression of AQP7 and AQP1 in mouse and human peritoneum was confirmed by immunofluorescence, with AQP7 being mostly detected in the plasma membrane of the adipocytes, whereas AQP1 was essentially detected in the microvascular endothelium of the peritoneal membrane (Fig. 2).

Effect of fasting on biological parameters and expression of adipose-specific genes in mouse

We first assessed the effect of progressive fasting (12 h to 36 h) on body weight and metabolic parameters in mouse

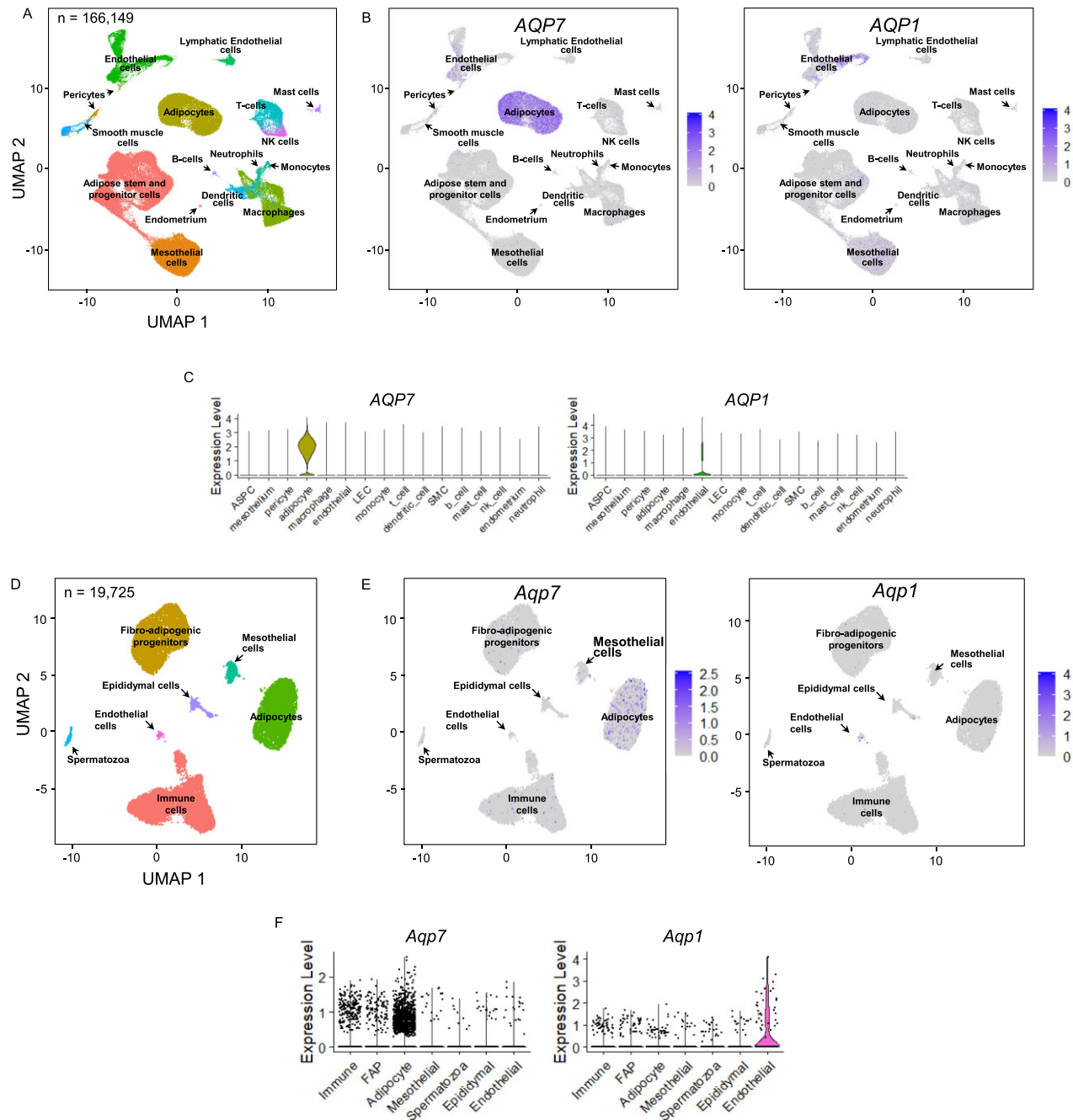


Figure 1: Differential expression of AQP7 and AQP1 mRNAs in white adipose tissue. (A–C) Human visceral white adipose tissue. (A) UMAP plot of human white adipose tissue showing 16 distinct cell populations. Feature (B) and violin (C) plots showing expression levels of AQP7 and AQP1 in the different cell clusters. Source: <https://drive.google.com/drive/folders/1rZ6Cn1vacb9ispdndNngB9VgZsnYmVop>, accessed on 9 February 2022. (D–F) Mouse epididymal white adipose tissue. (D) UMAP plot of mouse epididymal adipose tissue cells showing seven distinct cell populations. Feature (E) and violin (F) plots showing expression levels of *Aqp7* and *Aqp1* in the different cell clusters. Source: <https://osf.io/tsjqc/>, accessed on 9 February 2022.

(Fig. 3; Table 1). As expected, fasting induced a progressive loss of body weight (Fig. 3A), a time-dependent decrease in plasma glucose levels (Fig. 3B), an increase in plasma glycerol (Fig. 3C) and corticosterone (Fig. 3D) levels, and a dramatic fall in plasma insulin (Fig. 3E) and triglycerides (Fig. 3F) levels. These homeostatic changes (Table 1) were expected from lipolysis processes induced by fasting (Fig. 3G).

Changes in body composition were analyzed using micro-CT (Fig. 4): fasting induced a significant decrease in whole body fat mass (Fig. 4A) and visceral fat area (Fig. 4B), which was already significant after 12 h. The decrease in adipose tissue was reflected by structural modifications in the visceral peritoneum, with a major, time-dependent decrease in the diameter of the adipocytes (Fig. 4C). Conversely, fasting did not induce significant changes in inflammation or in the

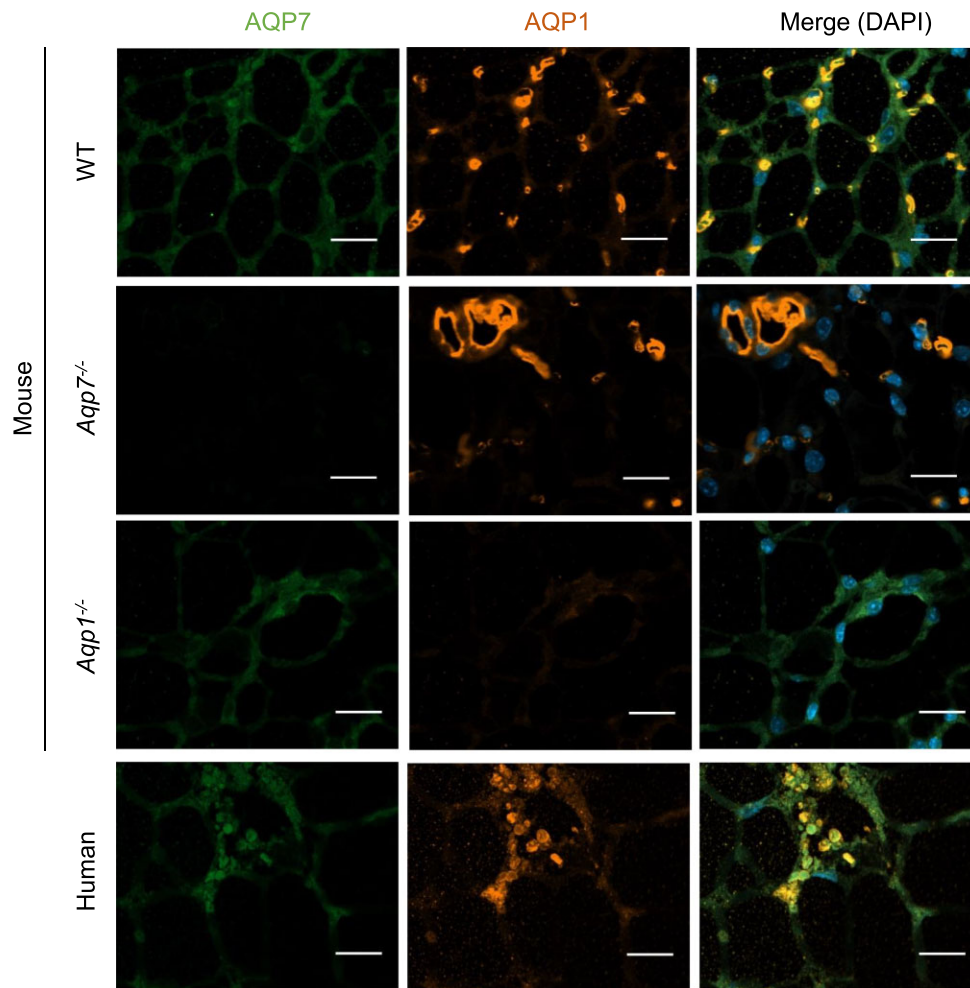


Figure 2: Cellular localization of AQP7 and AQP1 in the visceral peritoneum. Representative immunostaining for AQP7 (green channel) in the plasma membrane of adipocytes during fasting and for AQP1 (red channel) in the microvascular endothelial cells in mouse and human visceral peritoneum. *Aqp7^{-/-}* and *Aqp1^{-/-}* mice were used as negative controls. Original magnification, $\times 20$; scale bar 20 μm .

submesothelial thickness of the membrane (Supplementary data, Fig. S3).

The fasting-induced changes in the morphology of adipose tissue were reflected by the expression of key regulators of lipid metabolism within the visceral peritoneum, including upregulation of the hormone-sensitive lipase (HSL), regulating lipolysis, and significant decreases of the lipogenic factor fatty acid synthase (FAS) and of leptin, a major regulator of food intake (Fig. 4D). Together, these data demonstrate a coordinated regulation of lipolytic and lipogenic factors with changes in adipocyte morphology, suggesting that the visceral peritoneum plays a metabolic role during fasting.

Effect of fasting on the expression of aquaporins in the peritoneum

Considering the potential metabolic role of AQP7, we next analyzed the effect of fasting on AQP7 expression in the peritoneal membrane (Fig. 5). Fasting induced a progressive and significant increase in the expression of AQP7 and AQP1 mRNAs in the visceral peritoneum, reaching 146%

and 155% of the fed levels at 36 h, respectively (Fig. 5A). A 3- to 4-fold upregulation of the aquaporins in 36 h-fasted versus fed mice was confirmed at the protein level (Fig. 5B), with increased expression of AQP7 in adipocytes and AQP1 in microvascular endothelium of the peritoneal membrane (Fig. 5C). The specific changes in the expression of AQP1 or AQP7 observed during fasting in wild-type mice were also observed in *Aqp7^{-/-}* (for AQP1) or *Aqp1^{-/-}* (for AQP7) mice, respectively (Supplementary data, Fig. S4). No changes in the expression of *Aqp3* or *Aqp9* in the peritoneum were observed after fasting in wild-type, *Aqp1^{-/-}* and *Aqp7^{-/-}* mice (Supplementary data, Fig. 5).

Differential role of aquaporins in structural changes induced by fasting

To investigate whether AQP1 and AQP7 play a role in the structural changes observed in the visceral peritoneum, we used fasting protocols in *Aqp1^{-/-}* and *Aqp7^{-/-}* knock-out mice and their wild-type littermates (Fig. 6). Fasting induced a consistent decrease in the size of adipocytes in the four genotypes (Fig. 6A and B). The deletion of AQP1 had no impact on

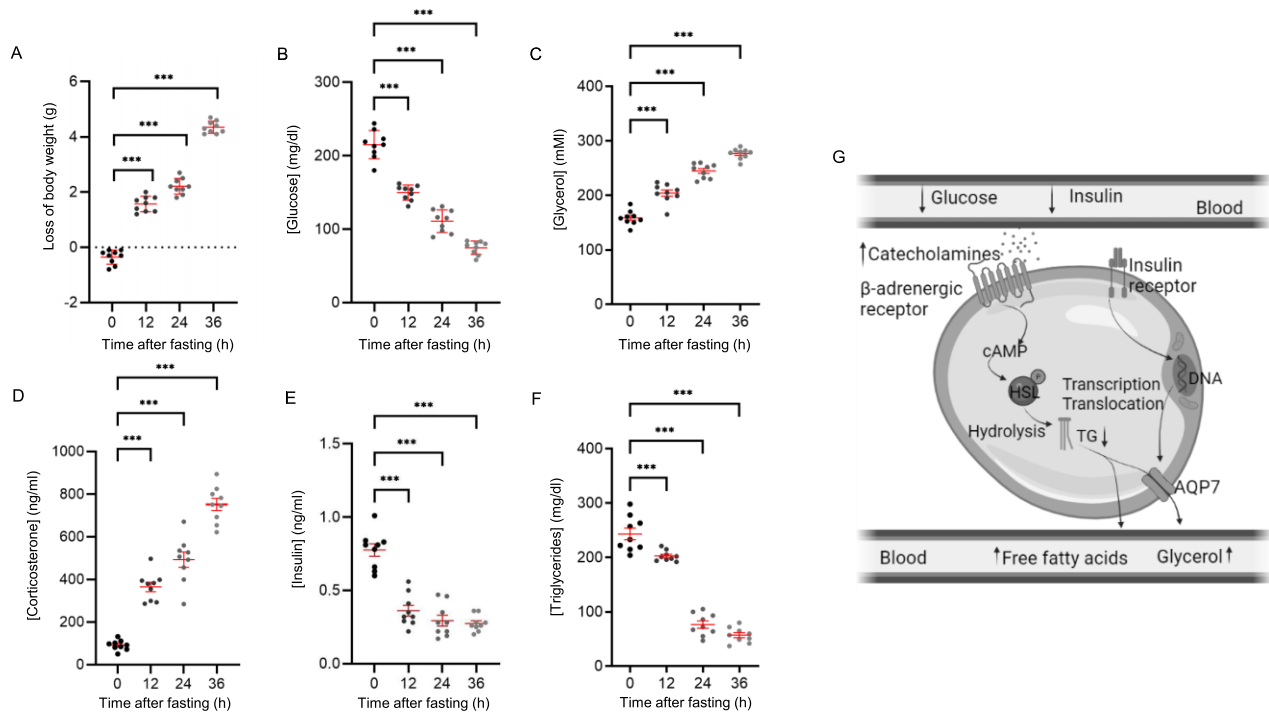


Figure 3: Effect of fasting on body weight and metabolic parameters of mouse. Compared with fed state, fasting (12 h to 36 h) induces a progressive loss of body weight (A), a decrease in plasma glucose levels (B), an increase in plasma glycerol (C) and corticosterone (D) levels, and a fall in plasma insulin (E) and triglycerides (F) levels. These changes are in line with the lipolysis processes occurring in adipocytes (G). During lipolysis, the triglycerides stored in the blood are hydrolyzed to produce free fatty acids and glycerol. Catecholamines are secreted in response to a low concentration of glucose in the blood. They activate the synthesis and activation of HSL, which hydrolyzes triglycerides. The fatty acids produced are secreted to the bloodstream. Created with BioRender.com. ($n = 9$ mice per group). The differences between groups were assessed by ANOVA followed by Bonferroni's multiple comparisons tests. The significance level is indicated as: *** $P < .001$ fasted versus fed groups.

Table 1: Effect of fasting on body composition, adipose tissue parameters, and metabolic parameters.

	Fed	Fasting		
		12 h	24 h	36 h
BW (g)	25.1 $\pm$ 0.9	23.2 $\pm$ 1.2	22.3 $\pm$ 1.3	21.6 $\pm$ 0.9
BW loss (g)		1.6 $\pm$ 0.3***	2.2 $\pm$ 0.3***	4.3 $\pm$ 0.2***
BW loss (%)		6.1%	8.8%	16.4%
Glucose (mg/dL)	215 $\pm$ 18	150 $\pm$ 10***	111 $\pm$ 15***	75 $\pm$ 9***
Glycerol (nM)	159 $\pm$ 13	204 $\pm$ 23***	245 $\pm$ 13***	277 $\pm$ 9***
Corticosterone (ng/mL)	92 $\pm$ 22	365 $\pm$ 63***	492 $\pm$ 101***	752 $\pm$ 81***
Insulin (ng/mL)	0.77 $\pm$ 0.12	0.36 $\pm$ 0.10***	0.29 $\pm$ 0.11***	0.28 $\pm$ 0.05***
Triglycerides (mg/dL)	243 $\pm$ 30	203 $\pm$ 9***	76 $\pm$ 19***	57 $\pm$ 13***
BUN (mg/dL)	30.3 $\pm$ 3.0	32.5 $\pm$ 3.2	33.9 $\pm$ 4.1	34.0 $\pm$ 5.4
Adipose tissue parameters				
Whole body fat (g)	2.95 $\pm$ 0.32	2.34 $\pm$ 0.16*	2.01 $\pm$ 0.32**	1.53 $\pm$ 0.35***
Visceral fat area (cm ²)	0.41 $\pm$ 0.06	0.26 $\pm$ 0.03**	0.15 $\pm$ 0.07***	0.06 $\pm$ 0.01***
Adipocyte size (μ m ²)	1130 $\pm$ 471	897 $\pm$ 466**	731 $\pm$ 330***	518 $\pm$ 231***

Results are mean $\pm$ SD values; $n = 9$ mice per group.

The differences between groups were assessed by ANOVA followed by Bonferroni's multiple comparisons tests. The significance level is indicated as: * $P < .05$; ** $P < .01$; *** $P < .001$ versus fed state.

BW, body weight; BUN, blood urea nitrogen.

the size of adipocytes in *Aqp1*^{-/-} mice, with only the fasting state having a significant contribution. Conversely, the deletion of AQP7 was reflected by a significant increase in the adipocyte size in *Aqp7*^{-/-} compared with *Aqp7*^{+/+} mice—with both the genotype and fasting state having significant effects (Fig. 6B). Analysis of the adipocyte size distribution (Fig. 6C) revealed that, in both fed and fasted conditions, adipocytes of larger

size were more frequent in *Aqp7*^{-/-} compared with *Aqp7*^{+/+} mice. While fasting induced a significant shift towards smaller adipocytes in all the groups, the genotype effect was only significant for *Aqp7* (larger adipocytes in the *Aqp7*^{-/-} vs *Aqp7*^{+/+} mice) (Fig. 6C). These results indicate a specific role of AQP7 in the plasticity of adipocyte size in the peritoneal membrane.

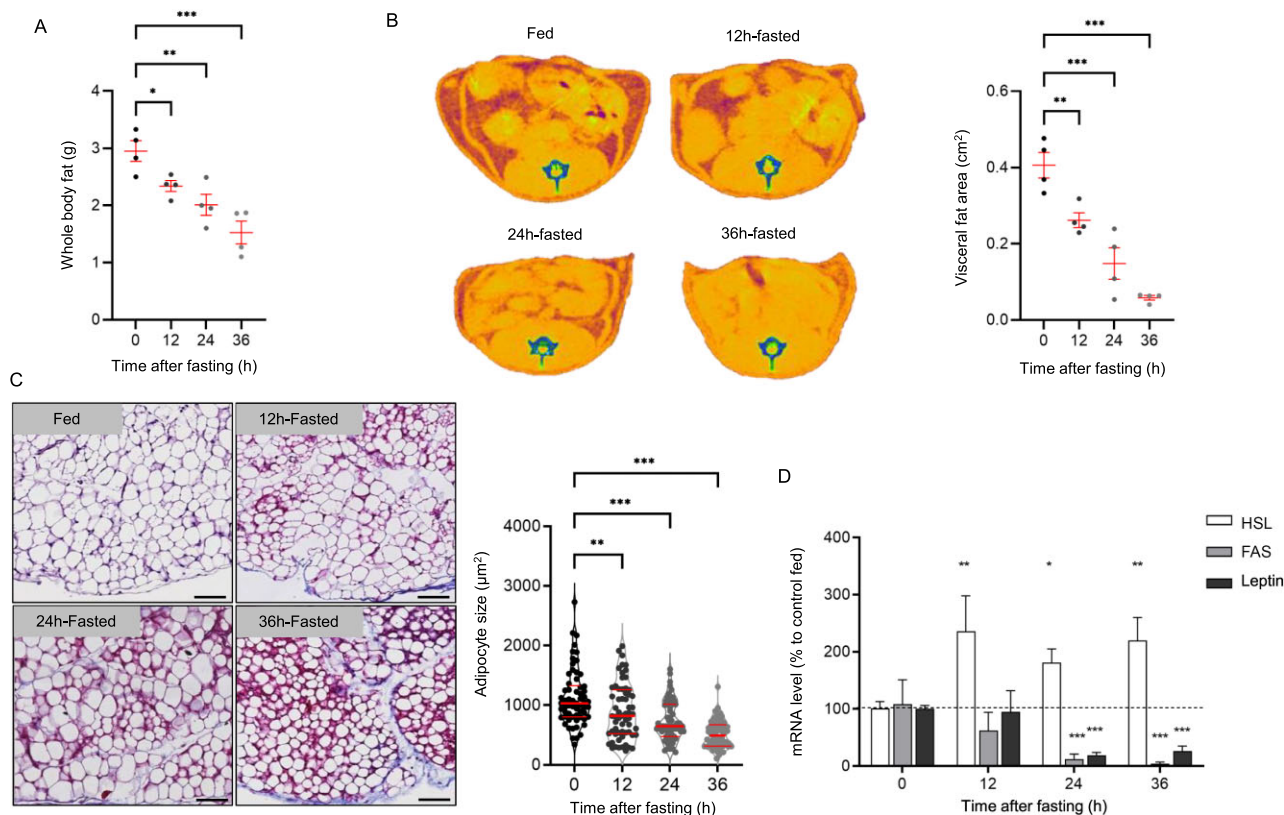


Figure 4: Effect of fasting on adipose tissue, morphology of adipocytes, and expression of adipose-specific genes in the peritoneum. (A, B) Changes in body composition analyzed using a micro-CT scan: the fasting-induced decrease in body weight is reflected by a decrease in whole body fat mass (A) and visceral fat area (B). Hematoxylin and eosin staining (C) indicates that fasting induces a progressive decrease in the size of adipocytes in the visceral peritoneum. Scale bar: 50 μ m. Magnification, $\times 40$. Quantitative RT-PCR (D) shows increased mRNA expression of HSL and decreased FAS and leptin in the visceral peritoneum from fasted compared with fed mice ($n = 4$ mice per group). The differences between groups were assessed by ANOVA followed by Bonferroni's multiple comparisons tests. The significance level is indicated as: * $P < .05$, ** $P < .01$, *** $P < .001$ fasted versus fed groups.

Effect of fasting on peritoneal transport parameters

Since fasting upregulated the expression of both AQP1 and AQP7 and caused a significant decrease in the size of adipocytes in the peritoneum, we examined whether it could affect peritoneal transport characteristics. The use of a standard PET with 2.5 mL of 3.86% glucose in the established mouse (wild-type) model of PD revealed that fasting induced a significant increase in the net UF, while the small solute transport remains unchanged (Table 2). Studies in *Aqp1* and *Aqp7* mice indicated that the fasting-induced increases in UF and sodium sieving were not observed in *Aqp1*^{-/-} mice, whereas they were preserved in *Aqp7*^{-/-} mice (Table 3). In *Aqp1* mice, significant effects of the genotype, fasting state and their interaction on the UF parameters were observed. In *Aqp7* mice, only the fasting state showed a significant effect on UF, while both the genotype and fasting variables showed significant effects on net UF/BW and body weight (Supplementary data, Table S4).

Effect of RU-486 on AQP7 and AQP1 expression during fasting

Finally, to investigate the mechanism driving the fasting-induced overexpression of aquaporins, we treated mice with

the glucocorticoid antagonist RU-486. Indeed, fasting was associated with induction of corticosterone (Fig. 3D), which in turn can modulate the expression of AQP1 via a glucocorticoid response element (GRE) [11]. *In silico* analysis revealed that GREs are present in both promoter regions of the human and mouse genes coding for AQP1 and AQP7 (Fig. 7A). Treatment with RU-486 significantly blunted the fasting-induced upregulation of AQP7 and AQP1 (Fig. 7B), and this was reflected by a significant effect on the net UF in the mouse model of PD (Fig. 7B). These data point to the essential role of corticosterone in mediating the increase in AQP1 expression and the effect on UF in fasting conditions.

DISCUSSION

Here, we combined molecular and functional analyses to assess the role and regulation of AQP7 in the peritoneal membrane, in comparison with that of AQP1. Since AQP7 is potentially involved in metabolism and transport of glycerol/water, we compared baseline and fasting situations in established mouse models. Our data reveal that fasting, reflected by the expected changes in metabolic parameters, body composition and adipocyte size, upregulates the expression of both AQP7 and AQP1 in the peritoneal membrane. These changes are

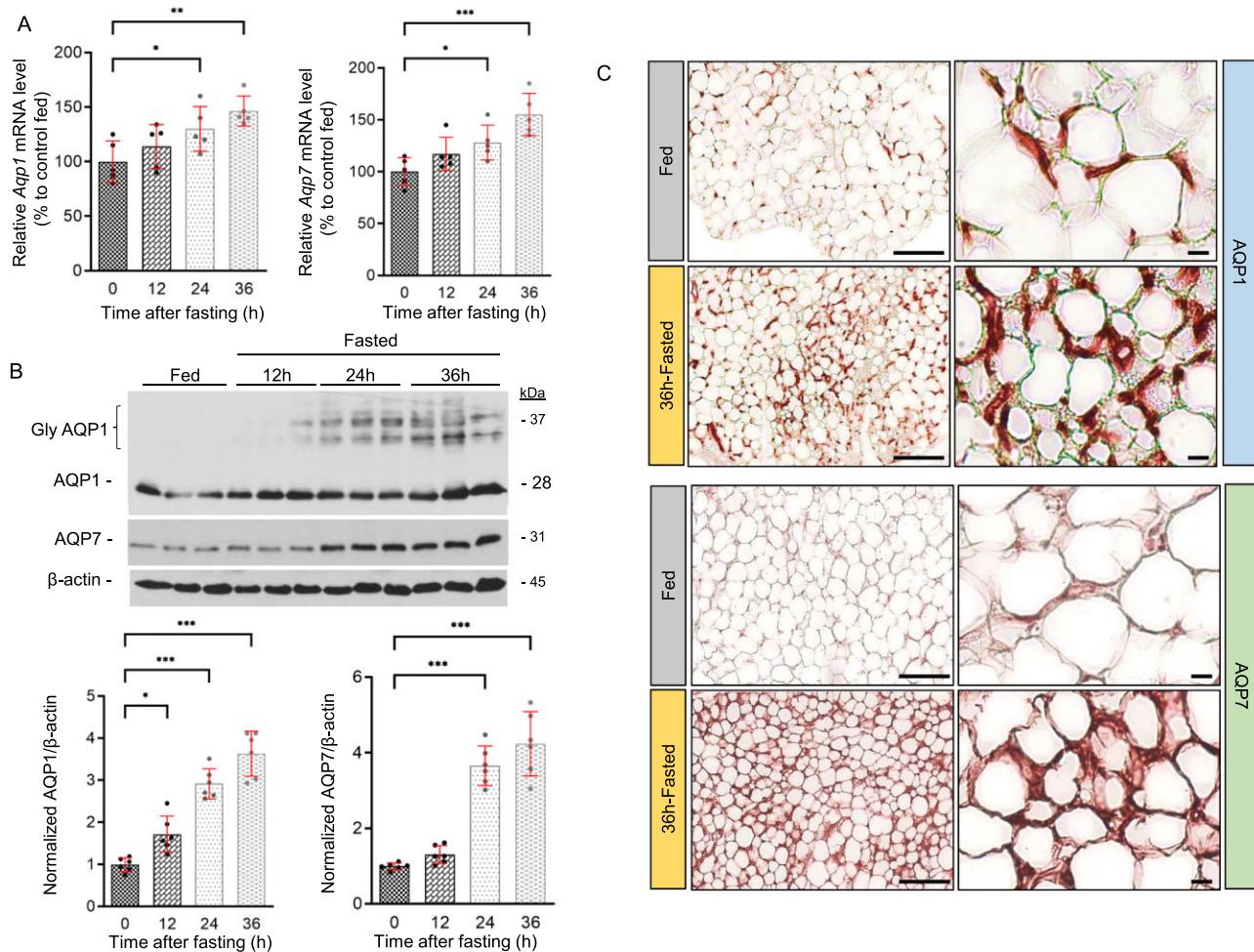


Figure 5: Effect of fasting on the expression of AQP1 and AQP7 in the peritoneum. **(A)** Quantitative RT-PCR: The expression of *Aqp1* and *Aqp7* in the visceral peritoneum is upregulated by fasting, compared with fed controls ($n = 5$ mice per group). **(B)** Representative immunoblots and densitometry analyses showing that fasting upregulates AQP1 and AQP7 in the visceral peritoneum. There were 20 μg of proteins in each lane. Differences between groups were assessed by ANOVA followed by Bonferroni's multiple comparisons tests. The significance level is indicated as: ** $P < .01$, *** $P < .001$ fasted versus fed groups. **(C)** Immunostaining for AQP1 (top panels): fasted mice show an increased density of AQP1-positive capillaries in the visceral peritoneum. Immunostaining for AQP7 (bottom panels): fasted mice show an increased density of AQP7-positive adipocytes in the visceral peritoneum. Scale bars: left, 50 μm , $\times 40$; right, 10 μm , $\times 80$.

paralleled by a significant increase in water transport and UF whereas the small solute transport remains unchanged. Using *Aqp1* and *Aqp7*-null mice and pharmacologic inhibitors, we showed that the fasting-induced increase in UF is mediated by the glucocorticoid induction of AQP1 but is unrelated to AQP7. In contrast, AQP7 is instrumental for the variations in adipocyte size in the peritoneum, in both fed and fasted conditions. These results yield novel information on the specific roles of aquaporins and suggest that fasting improves fluid removal in a mouse model of PD.

AQP1 is the most abundant water channel in the peritoneal membrane, located in the capillary endothelium where it forms an ultrasmall pore facilitating water transport and UF during a hypertonic dwell [3, 34]. Common genetic variants increasing AQP1 expression are associated with increased water transport and net UF during PD [4]. The expression of AQP1 in the rat peritoneum is increased by administration

of corticosteroids [11], mediated by GREs in the promotor region of *Aqp1* [11, 35].

Our data reveal that fasting induces a significant induction of AQP1 in the visceral peritoneum, reflected by an increase in water transport, in line with the induction of AQP1 in capillaries of the white adipose tissue by starvation in AQP7 knockout mice [36]. Fasting causes a strong increase in corticosterone, a classic metabolic response to increase fuel availability [37]. The effect of RU-486 indicates that the induction of AQP1 by fasting is mediated by endogenous steroids, confirming the relevance of GREs in regulating the expression of water channels. The induction of AQP1 and water transport by fasting in this mouse model may offer clinical perspectives, as poor fluid removal is linked to cardiovascular events, systemic inflammation, technical failure and death in PD [4]. However, this perspective should be balanced by the widely different body size and metabolic rate, and the disproportionately greater consequences of fasting in

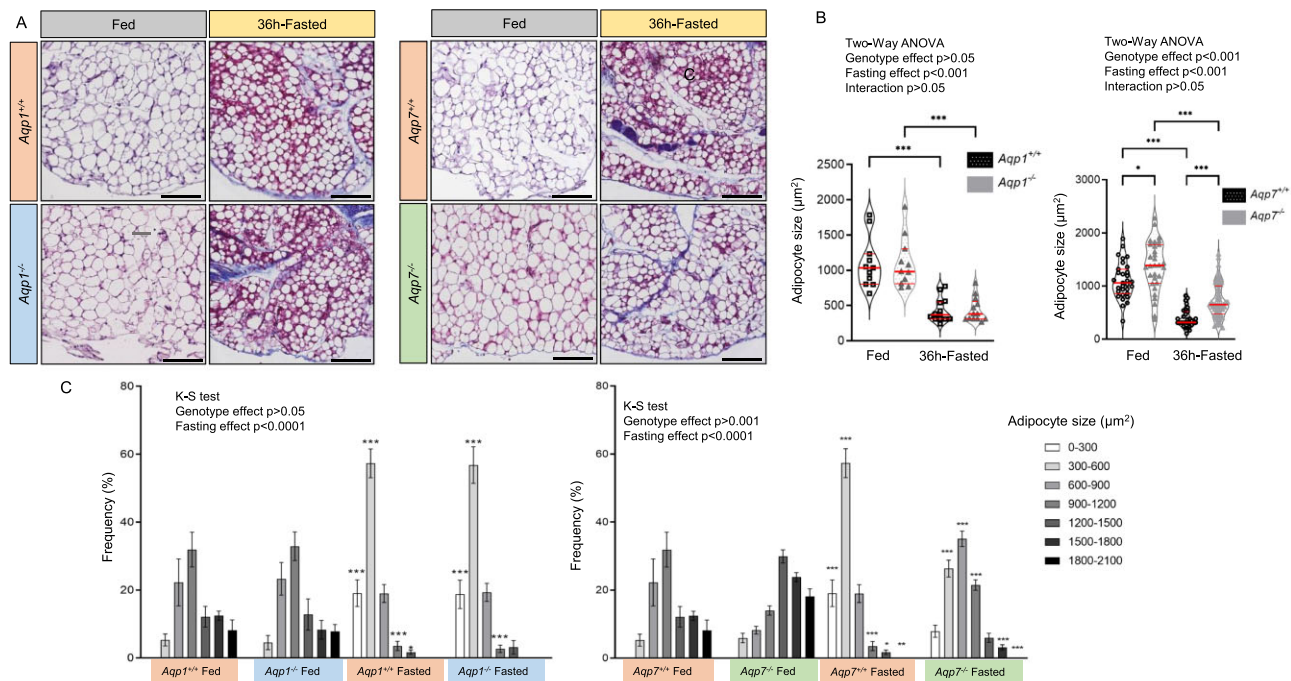


Figure 6: Effects of fasting and aquaporin genotype on the morphology of adipocytes in the visceral peritoneum. (A) Hematoxylin and eosin staining of the visceral peritoneum in fed and 36 h-fasted *Aqp1* and *Aqp7* mice. (B) Quantification of adipocyte size in the visceral peritoneum. Compared with fed animals, 36 h-fasting induces a significant decrease in the size of adipocytes in both genotypes. The visceral peritoneum of *Aqp7*^{-/-} mice shows bigger adipocytes than that of the *Aqp7*^{+/+} controls—both in fed and fasted state. (C) Distribution of adipocyte size in the visceral peritoneum in 36 h-fasted *Aqp1*^{-/-} and *Aqp7*^{-/-} mice and their wild-type controls. The analysis of the distributions shows significant differences between genotypes in both fed and fast state ($P < .001$) in *Aqp7* mice. Values are expressed in (%) as frequency occurrences. Scale bar: 50 μm. Magnification, ×40. Significant differences due to genotype, fasting or their interactions, were determined by two-way ANOVA (B), whereas differences in distribution were analyzed by Kolmogorov–Smirnov (KS) test (C). The significance level is indicated as: * $P < .05$, *** $P < .001$.

Table 2: Effect of fasting on peritoneal transport parameters.

Groups	BW (g)	Net UF/BW (μL/g)	Net UF (μL)	D120/D0 Glucose	ΔDNa (0–30 min) (mmol/L)
C57BL/6J Fed	25.1 ± 0.9	40.8 ± 4.2	1021 ± 79.9	0.46 ± 0.03	7.2 ± 0.8
36h-Fasted	21.6 ± 0.9***	56.0 ± 3.1***	1207 ± 74.0***	0.44 ± 0.03	8.8 ± 1.1**

Results are mean ± SD values; $n = 9$ mice per group.

Comparisons between the groups were performed using unpaired t -test. The significance level is indicated as: ** $P < .01$; *** $P < .001$ versus fed state.

BW, body weight; D120/D0 Glucose, dialysate glucose concentration at 120 min compared with baseline; ΔDNa (0–30 min), dip of sodium concentration in dialysate at 30 min compared with baseline.

mice compared with humans, notably for the magnitude of reduction in body fat [38]. Furthermore, it would only be possible to reduce food intake moderately in patients treated with PD, but certainly not to impose a severe fasting.

Next to AQP1, AQP7 is the most abundant AQP isoform expressed in the mouse peritoneal membrane [3]. Using snRNA-seq, we show that AQP7 is essentially enriched in mature adipocytes, whereas AQP1 is mostly detected in microvascular endothelial cells. These compartments play distinct roles in peritoneal physiology, with microvascular endothelial cells being the site of facilitated osmotic water transport whereas adipocytes participate in the adaptation to fasting by releasing free fatty acids and glycerol into the circulation [3, 39]. AQP7 facilitates glycerol transport in various cell types including adipocytes, a key process to regulate energy metabolism and cellular volume [20]. Several factors could account for the induction of AQP7 by fasting.

First, the significant decrease of AQP7 expression after RU-486 suggests a role for steroids, potentially related to a predicted GRE [40]. Second, decreased insulin levels during fasting, in order to prevent hypoglycaemia [41], may trigger the expression of AQP7 via a negative insulin response element (IRE) [20]. Third, fasting may cause robust transcriptomic changes, paralleled by changes of methylation patterns in the adipose tissue [42–44]. Recent data from epigenome-wide association studies suggest that methylation of AQP7 promoter is associated with adiposity and fat accumulation [45, 46]. Finally, catecholamines, activated during fasting to prevent the fall in glucose, stimulate lipolysis and allow the translocation of AQP7 from the lipid droplets to the plasma membrane for efficient efflux of glycerol [47].

The adipose tissue is the major energy storage organ where lipogenesis and lipolysis occur in response to energy requirements. In fasting condition, the increased sympathetic

Table 3: Effect of fasting on peritoneal transport parameters in AQP1 and AQP7 mice.

Groups		BW (g)	Net UF/BW ($\mu\text{L/g}$)	Net UF (μL)	D/D0 Glucose	ΔNa 0–30 min
<i>Aqp1</i> ^{+/+}	Fed	26.7 $\pm$ 1.8	40.1 $\pm$ 6.0	1046 $\pm$ 72.0	0.45 $\pm$ 0.04	7.5 $\pm$ 0.8
	36 h-Fasted	23.3 $\pm$ 2.9	54.3 $\pm$ 2.4 ^{§§}	1194 $\pm$ 75.0 [§]	0.46 $\pm$ 0.04	8.6 $\pm$ 0.7
<i>Aqp1</i> ^{-/-}	Fed	23.6 $\pm$ 2.9	21.7 $\pm$ 3.4 ^{***c}	508.1 $\pm$ 89.1 ^{***c}	0.48 $\pm$ 0.02	2.3 $\pm$ 0.8 ^{***c}
	36 h-Fasted	18.4 $\pm$ 1.9 ^{**§§§b}	25.1 $\pm$ 4.5 ^{***b}	465.3 $\pm$ 112.8 ^{***b}	0.47 $\pm$ 0.04	1.3 $\pm$ 0.8 ^{***§b}
<i>Aqp7</i> ^{+/+}	Fed	21.3 $\pm$ 2.0	46.9 $\pm$ 2.7	999.0 $\pm$ 92.2	0.48 $\pm$ 0.05	6.8 $\pm$ 0.7
	36 h-Fasted	18.1 $\pm$ 1.1 ^{§§}	63.8 $\pm$ 4.7 ^{§§§}	1155 $\pm$ 74.9 ^{§§}	0.44 $\pm$ 0.03	8.3 $\pm$ 1.0 ^{§§}
<i>Aqp7</i> ^{-/-}	Fed	23.9 $\pm$ 2.3 ^{*c}	41.4 $\pm$ 4.2 ^c	985 $\pm$ 89.8 ^c	0.44 $\pm$ 0.08	6.3 $\pm$ 0.7 ^c
	36 h-Fasted	20.7 $\pm$ 2.1 ^{*§§}	54.3 $\pm$ 7.7 ^{**§§§a}	1110 $\pm$ 85.7 ^{§a}	0.47 $\pm$ 0.02	7.8 $\pm$ 1.0 ^{§§}

Results are mean $\pm$ SD values; $n = 8$ mice per group.

Significant differences were determined by two-way ANOVA. The significance level is indicated as:

Genotype effect: * $P < .05$ versus WT; ** $P < .01$ versus WT; *** $P < .001$ versus WT.

Fasting effect: § $P < .05$ versus fed; §§ $P < .01$ versus fed; §§§ $P < .001$ versus fed.

Interaction effect: ^a $P < .05$ versus WT fed; ^b $P < .001$ versus WT-fed; ^c $P < .001$ versus WT-fasted.

BW, body weight; UF, ultrafiltration; D120/D0 Glucose, dialysate glucose concentration at 120 min compared with baseline; ΔNa (0–30 min), dip of sodium concentration in dialysate at 30 min compared with baseline; WT, wild type.

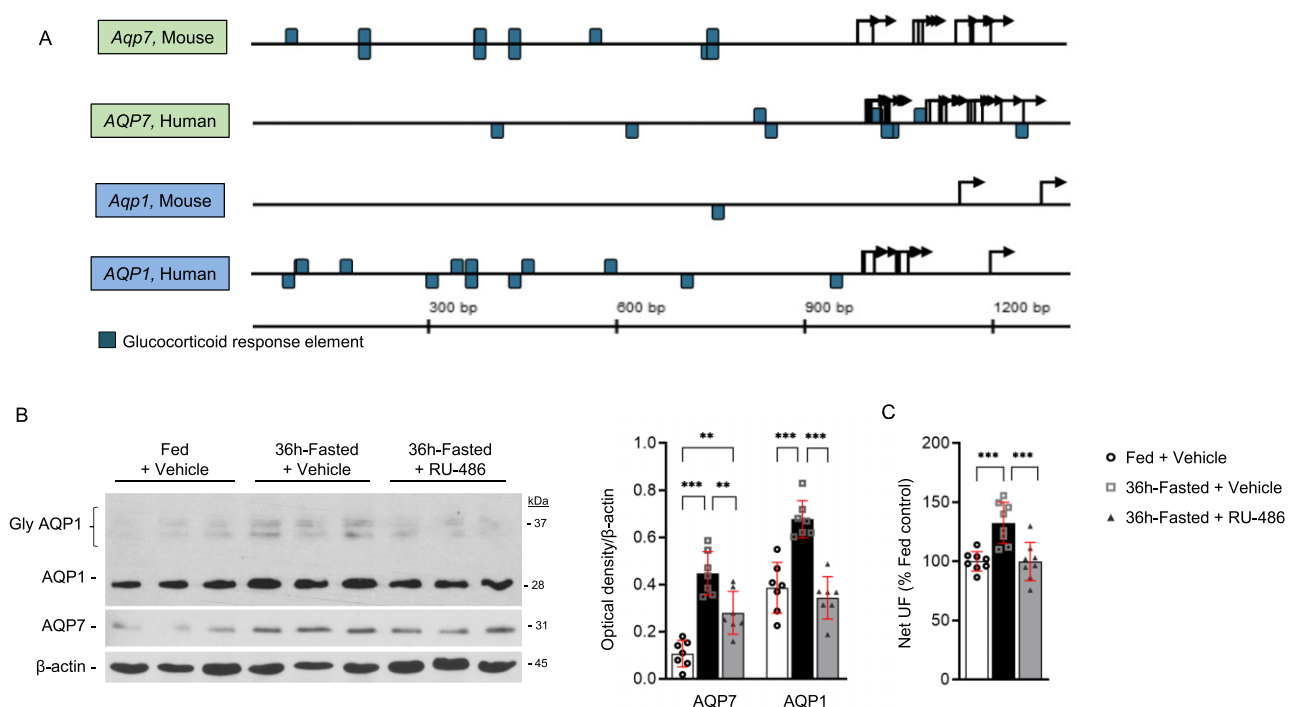


Figure 7: Effect of RU-486 on AQPs expression and ultrafiltration. (A) GREs in the promoter regions of genes coding for AQP1 and AQP7 in man and mouse. **(B)** Representative immunoblots and densitometry analyses for AQP7 and AQP1 in the visceral peritoneum from control mice, 36 h-fasted mice and 36 h-fasted mice treated by RU-486. Administration of RU-486 prevents the induction of AQP1 by fasting. There were 20 μg of proteins in each lane. **(C)** Treatment with RU-486 reduces significantly the ultrafiltration of 36 h-fasted mice ($n = 8$ mice per group). The differences between groups were analyzed by ANOVA followed by Bonferroni's multiple comparisons tests. The significance level is indicated as * $P < .05$, ** $P < .01$.

tone, via increased intracellular cAMP levels, phosphorylates the HSL, hydrolyzing TG to free fatty acid (FFA) and glycerol, a major substrate for gluconeogenesis in the liver [48]. The fasting protocol used here induced a remarkable decrease in visceral fat accumulation and a strong reduction in the size of adipocytes in the peritoneal membrane. Notably, this effect was not affected by the deletion of AQP1 (*Aqp1*^{-/-} mice), whereas it was strongly reduced (i.e. larger residual adipocytes) in *Aqp7*^{-/-} mice compared with their respective controls. In line with this, significantly larger adipocytes

were observed in the peritoneum of *Aqp7*^{-/-} mice, reflected by a higher body weight in the fed and fasted *Aqp7*^{-/-} compared with *Aqp7*^{+/+} mice. These results substantiate the involvement of AQP7 in regulation of lipid accumulation and adipose tissue, as suggested in mouse and human [13, 39, 46]. Identification of factors able to modulate the expression of AQP7 and thus impact on body composition may thus have a beneficial effect in patients who experience increased weight, body mass index and visceral abdominal fat during PD [23, 49].

In conclusion, these data indicate that a coordinated regulation of lipolytic and lipogenic factors operates in the visceral peritoneum during fasting, with upregulation of AQP7 and AQP1 in specific compartments, reflected by structural and functional alterations of the peritoneal membrane. With the limitations of inter-species differences, the fact that fasting increases AQP1-mediated water transport across the peritoneal membrane and induces a metabolic and lipolytic response via AQP7 may have a clinical relevance for patients treated by peritoneal dialysis.

SUPPLEMENTARY DATA

Supplementary data are available at [ndt](#) online.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

T.N. and O.D. conceptualized the overall study and supervised the work. I.P.D.C., N.H., S.U., T.N. and O.D. planned the overall experimental design. I.P.D.C. and N.H. performed the *in vivo* and molecular studies, analysed and interpreted the data. G.S. performed the analysis of single-nucleus RNA-sequencing for AQP1 and AQP7. All co-authors revised the draft manuscript and approved the submitted version.

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DATA AVAILABILITY STATEMENT

The data underlying this research will be shared upon reasonable request to the corresponding author.

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