Liver-specific Aquaporin 11 knockout mice show rapid vacuolization of the rough endoplasmic reticulum in periportal hepatocytes after amino acid feeding

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Rojek A, Füchtbauer EM, Füchtbauer A, Jelen S, Malmendal A, Fenton RA, Nielsen S. Liver-specific Aquaporin 11 knockout mice show rapid vacuolization of the rough endoplasmic reticulum in periportal hepatocytes after amino acid feeding. Am J Physiol Gastrointest Liver Physiol 304: G501–G515, 2013. First published December 28, 2012; doi:10.1152/ajpgi.00208.2012.—Aquaporin 11 (AQP11) is a protein channel expressed intracellularly in multiple organs, yet its physiological function is unclear. Aqp11 knockout (KO) mice die early due to malfunction of the kidney, a result of hydropic degeneration of proximal tubule cells. Here we report the generation of liver-specific Aqp11 KO mice, allowing us to study the role of AQP11 protein in liver of mice with normal kidney function. The unchallenged liver-specific Aqp11 KO mice have normal longevity, their livers appeared normal, and the plasma biochemistries revealed only a minor defect in lipid handling. Fasting of the mice (24 h) induced modest dilatation of the rough endoplasmic reticulum (RER) in the hepatic periphery. Refeeding with standard mouse chow induced rapid generation of large RER-derived vacuoles in Aqp11 KO mice hepatocytes. Similar effects were observed following oral administration of pure protein or large doses of various amino acids. The fasting/refeeding challenge is associated with increased expression of markers of ER stress Grp78 and GADD153 and decreased glutathione levels, suggesting that ER stress may play a role in the development of vacuoles in the AQP11-deficient hepatocytes. NMR-based metabolome analysis of livers from mice subject to amino acid challenge showed decreased amount of extractable metabolites in the AQP11-deficient livers and particularly a decrease in glucose levels. In conclusion, in the liver, deletion of Aqp11 results in disrupted RER homeostasis and increased sensitivity to RER injury upon metabolic challenge with amino acids.

ER stress; glutathione; ammonia; oxidative stress

Generation of the liver-specific Aqp11 KO mice. The targeting construct (see Fig. 1) for creating the “floxed” Aqp11 gene, with loxP sites flanking exons 2 and 3, was generated by insertion of PCR-amplified genomic DNA segments spanning over the Aqp11 gene sequence into a modified pKOScrambler(FRT-loxP) vector. The left arm covering exon 1 was 3.9 kb, the middle part covering exons 2 and 3, and intron 2 was 2.8 kb, and the right arm covering sequences downstream of exon 3 was 3.4 kb. The targeting construct was sequenced, which revealed a single nucleotide difference compared with the Aqp11 cDNA sequence that does not affect the AQP11 protein amino acid sequence (silent mutation). The targeting construct was linearized and electroporated into CJ7 embryonic stem (ES) cells derived from 129S1/Sv mice (23). G418-resistant clones were selected and expanded. The clones with homologous recombination were identified by Southern blot with probes flanking the targeting construct sequence (probe 1 and probe 2). The neomycin phosphotransferase expression cassette was deleted from the floxed Aqp11 allele by transient transfection of targeted ES cells with a Flp-recombinase expression plasmid. The neomycin-sensitive clones were validated by Southern blotting with a 905 bp probe generated by amplification of a genomic fragment using primers “Southern-L”: TATTTGCCTCCCTTCCTACCATC, and “Southern-R”: TCTT-ATGTTCTTTCTTCCCTC.
Chimeric mice were generated by injection of the ES cells into B6D2F1 mouse blastocysts (25). Chimeric males were bred with C57BL/6 females, and agouti offspring (indicating germ-line transmission of the manipulated 129S1/Sv ES cells) were analyzed for the presence of Aqp11<sup>flx/flx</sup> mutation by PCR using genomic tail DNA and the primers “genotyping L1”: 5′-TGTATACAGGTGCTCT- GAAGGAG and “genotyping R”: 5′-CAGTTGGGAAACCTGCTGAA- GTTAG. The expected product for the wild-type allele is 182 bp and 234 bp for the Aqp11<sup>flx/flx</sup> allele.

We obtained live-deleted Aqp11 KO mice by breeding mice carrying the Aqp11<sup>flx/flx</sup> allele with a liver-specific Cre-recombinase expressing mouse strain, B6.Cg-Tg(Alb-cre)21Mgn/J (The Jackson Laboratory). The truncated Aqp11<sup>flx/flx</sup> allele lacking exons 2 and 3 was detected as a 404 bp PCR product in the liver cells using primer pair “genotyping L2”: 5′-ATCTCCCCAGCCCTCATTTCTTAAC, and “genotyping R”: 5′-CAGTTGGGAAACCTGCTGAA-GTTAG. For experiments, Aqp11<sup>flx/flx</sup>/Alb-cre<sup>+</sup> mice were used, and Aqp11<sup>flx/flx</sup>/ Alb-cre<sup>−</sup> siblings were used as controls. All mice used in experiments were on a mixed C57BL/6-129S1/Sv genetic background.

**Animal studies.** All mouse experiments were approved by the Danish Ministry of Justice. Aqp11<sup>flx/flx</sup>/Alb-cre<sup>+</sup> mice or Aqp11<sup>flx/flx</sup>/ Alb-cre<sup>−</sup> siblings were housed in standard cages, with free access to standard mouse chow (Altromin 1320) and drinking water. For a metabolic challenge, the mice were fasted for 24 h, with free access to water. The following day, food pellets (Altromin 1320) were offered to the mice, and the time when the mouse started to feed registered. Mice were killed between 30 min and 48 h following refeeding. For metabolic challenge with different substances, the mice obtained between 0.5 and 1 ml of solution by oral gavage, and precisely 2 h following administration, the mice were anesthetized by intraperitoneal injection of pentobarbital and the chest opened, and a blood sample aspirated from the right ventricle. For metabolic challenge, the mice were fasted for 24 h, with free access to water. For measurement of blood glucose levels in response to fasting and refeeding, we used an Accu-Chek Sensor (Roche, Mannheim, Germany) to measure glucose in a drop of blood from the tip of the tail. Serum was diluted with 1 ml of solution by oral gavage, and precisely 2 h following administration, the mice were anesthetized by inhaled isoflurane and organs fixed by perfusion (see below). The following substances were administered: 1) glucose, the mice obtained ~10 g/kg body weight (BW); 2) rapeseed oil, the mice obtained ~8 g/kg BW; 3) skimmed milk powder suspension (dissolved in water), the mice obtained ~16 g/kg BW; 4) individual amino acids: glycine, alanine, glutamine, glutamate, arginine, lysine, the mice obtained ~15 mmol/kg BW; 5) pyruvate; the mice obtained ~15 mmol/kg BW; 6) urea solutions 2 and 10%, the mice obtained ~15 or ~100 mmol/kg BW; 7) ammonium acetate solution, the mice obtained ~15 or 7 mmol/kg BW; 8) pyridoxine hydrochloride (150 mg/kg BW); 9) estradiol-17β (1 mg/kg BW); 10) testosterone (1 mg/kg BW); 11) cortisol (5 mg/kg BW); 12) sucrose (150 g/kg BW); 13) glucose (dissolving 0.5 g oil red O in 100 ml 100% propylene glycol). The solution was filtered by Whatman filter paper prior to use to remove solids. For immunohistochemistry: mouse anti-GADD 153 antibody (B-3) sc-7351 from Santa Cruz Biotech diluted 1:100 and secondary antibody HRP-conjugated goat anti-mouse IgG (DAKO) diluted 1:200.

**Sample preparation and immunoblotting.** For preparing membrane-enriched pellets containing AQP11, the livers were homogenized in dissection buffer (0.3 M sucrose/25 mM imidazole/1 mM EDTA, pH 7.2, containing 8.5 μl leupeptin, and 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 10,000 × g for 10 min to remove whole cells, nuclei, and large organelles. The supernatant was centrifuged at 100,000 × g for 1 h, and the membrane pellet dissolved in 1× Laemmli buffer [2% SDS, 0.1 M dithiothreitol (DTT), 10% glycerol, 50 mM Tris-HCl pH 6.8, 0.2 mg/ml bromphenol blue] and heated at 65°C for 15 min. For preparing protein samples for analysis of LDL receptor, we homogenized livers in dissection buffer and centrifuged them at 4,000 × g for 10 min to remove whole cells and nuclei. The supernatant was mixed with 4× Laemmli buffer (8% SDS, 0.4 M DTT, 40% glycerol, 200 mM Tris-HCl pH 6.8, 0.8 mg/ml bromphenol blue) and heated at 65°C for 15 min. For preparation of proteins from serum, blood was obtained from the tip of the tail (5 drops), allowed to coagulate for a minimum of 30 min, and centrifuged at 3,000 × g for 10 min. Serum was diluted with 1× Laemmli buffer 1:4,000 and heated for 15 min at 65°C.

The protein samples were run on 10 or 12% polyacrylamide gels and electrottransferred to PVDF membrane (Amersham). Membranes were blocked with 5% milk in PBS-T (0.1 M PBS with 0.1% vol/vol Tween) and incubated overnight at 4°C with appropriate primary antibodies. After being washed with PBS-T, membranes were incubated with appropriate secondary antibody and the antigen-antibody interactions visualized with an ECL chemiluminescence kit (Amer sham Bioscience, UK) and Hyperfilm (Amersham Bioscience).

**PGNaseF treatment.** Protein samples were heated at 95°C in 0.5% SDS and 1% β-mercaptoethanol for 10 min. The denatured proteins were incubated overnight at 37°C in a 50 μl reaction containing 0.1% SDS, 0.2% β-MeEiOH, 1% Triton-X 100, 5 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, 25 mM NaCl, 0.5 mM DTT, pH 7.9, and 1,000 units of PGNaseF. The reactions were stopped by mixing with 4× Laemmli buffer and heating at 65°C for 15 min.

**Total protein glycosylation analysis.** Glycosylation profile of proteins isolated from AQP11-deficient and control livers were analyzed with the GlycoProfile III fluorescent glycoprotein detection kit (Wexford, according to the manufacturer’s protocol. In brief, the liver protein samples 4,000 g supernatant fraction were separated on 10 or 12% polyacrylamide gels, the proteins fixed by incubation in 3% acetic acid with 50% methanol for 1 h, the gel was washed for 1 h in two changes of pure water, and glycans oxidized by incubation with oxidation reagent for 20 min in the dark; after being washed the gel was incubated for 90 min with glycoprotein staining reagent in the dark. After overnight washing in water the gel was photographed under UV illumination. The detection limit of this method is 5 ng carbohydrates. In parallel an identical gel was stained with Coomassie stain to visualize total protein profile in the samples.

**Tissue fixation and processing for light and electron microscopy.** Mice were anesthetized with pentobarbital and perfused with ice-cold 1% glutaraldehyde in PBS for 5 min through the left ventricle of the heart. The livers were dissected out and postfixed for at least 24 h in 1% glutaraldehyde in PBS. For light microscopy, the median lobe of the liver was dissected and washed for 30 min in PBS, dehydrated through a series of ethanol and xylene, and embedded in paraffin. Sections (2 μm) were cut on a rotary microtome (Leica Microsystems, Herlev, Denmark) deparaffinized in xylene, rehydrated, and stained with hematoxylin and eosin. Sections were then dehydrated and mounted in DPX mounting medium. For electron microscopy, tissues were postfixed with 1% OsO<sub>4</sub> in veronal acetate buffer, pH 7.2, dehydrated in graded alcohols, and embedded in Epon. Sections 50 nm thin were stained with uranyl acetate and lead citrate.

**Lipid staining with oil red O.** The oil red O stain was prepared by dissolving 0.5 g oil red O in 100 ml 100% propylene glycol. The solution was filtered by Whatman filter paper prior to use to remove solids.
undissolved particles. Postfixed tissue was incubated in 30% sucrose overnight and snap-frozen, and 20 μm thick sections cut on a cryomicrotome. Sections were rinsed in three changes of distilled water, air-dried, and placed in 100% propylene glycol for 5 min. Sections were stained in oil red O solution for 8–10 min at 60°C, differentiated in 85% propylene glycol for 2 min, and rinsed in running tap water. Finally, the sections were briefly stained in hematoxylin and mounted using Glycergel mounting medium (DAKO).

Glycogen staining. A carmine stock solution was prepared (2.5% carmine, 1.25% potassium carbonate, 6.25% potassium chloride, 7% ammonium hydroxide). Deparaffinized tissue sections were stained in freshly prepared working carmine solution (10 ml of stock carmine solution, 15 ml ammonium hydroxide 28%, and 15 ml methanol) for 30 min, placed in differentiating solution (20 ml ethanol, 10 ml methyl alcohol, 25 ml H2O2) for 3 s, rinsed quickly in 70% ethanol, and dehydrated in a graded alcohol series. Sections were cleared in three changes of xylene and mounted in DPX mounting medium.

Tissue fixation and processing for immunohistochemistry. Mice were anesthetized with pentobarbital and perfused with ice-cold 3% paraformaldehyde in PBS for 5 min through the left ventricle of the heart. The livers were dissected out and postfixed for 24 h in the same fixative. After being washed in PBS for 30 min the tissues were dehydrated in a graded alcohol series and xylene and embedded in paraffin. We cut 2-μm sections on a rotary microtome (Leica Microsystems, Herlev, Denmark) and deparaffinized them in xylene and 99% ethanol. Endogenous peroxidase activity was blocked with 0.5% H2O2 in absolute methanol for 10 min and rehydrated in a graded ethanol series. The sections were boiled in a target retrieval solution overnight and snap-frozen, and 20 μm thick sections cut on a cryomicrotome. Sections were rinsed in three changes of distilled water, air-dried, and placed in 100% propylene glycol for 5 min. Sections were stained in oil red O solution for 8–10 min at 60°C, differentiated in 85% propylene glycol for 2 min, and rinsed in running tap water. Finally, the sections were briefly stained in hematoxylin and mounted using Glycergel mounting medium (DAKO).
GAATACAGCCGAGACCTGA and RT-GADD153-R GGGACATGACCACCTGTGT, and for a reference gene β-2-microglobulin-L GCTATCCAGAAAAACCCCTCAA and β-2-microglobulin-R CATGTCTGGATCCCTGATTGCGGT. The cycling conditions were 30 s at 95°C, 30 s at 60°C, and 1 min at 72°C. We calculated the relative initial template quantity in each reaction by comparing the Ct values against the titration curve of six 2× serial dilutions of template mix.

Isolation of solutes from the livers and NMR analysis. Six male Aqp11\textsuperscript{flx/flx}/Alb-cre\textsuperscript{+} mice and six Aqp11\textsuperscript{flx/flx}/Alb-cre\textsuperscript{−} sibling controls were administered oral glycine (15 mmol/kg BW). Two hours later the mice were killed by decapitation, the liver was removed, and the liver lobes were dissected, weighed and snap-frozen in liquid nitrogen. To extract water-soluble metabolites, the liver samples were homogenized in ice-cold acetonitrile-H\textsubscript{2}O 4:1 (4 ml/g tissue). The homogenates were centrifuged for 10 min at 10,000 g. The pellets were extracted with an additional three times with four volumes of the original tissue weight of the indicated solvent mixture, and all the supernatants were pooled. The supernatants were incubated at −20°C overnight to assure complete precipitation of proteins, thawed on ice, and centrifuged at 10,000 g for 30 min; 1 ml supernatant was lyophilized and stored at −80°C until analysis. Immediately before the NMR measurements, samples were rehydrated in 650 μl of 50 mM phosphate buffer (pH 7.4) in D\textsubscript{2}O, and 600 μl of the solution was transferred to a 5 mm NMR tube. The buffer contained 50 mg/l of the chemical shift reference 3-(trimethylsilyl)-propionic acid-D4, sodium salt (TSP), and 100 mg/l Na\textsubscript{3}H.

NMR measurements were performed at 25°C on a Bruker Avance 500 spectrometer (Bruker Biospin), operating at a 1H frequency of 500.13 MHz, equipped with a 5 mm HX double resonance probe. 1H NMR spectra were acquired using a single 90°-pulse experiment or a single 90°-pulse experiment with a Carr-Purcell-Meiboom-Gill (CPMG) delay added to attenuate broad signals from high-molecular-weight components. The total CPMG delay was 40 ms, and the spin-echo delay was 200 μs. In both experiments the water signal was suppressed by presaturation of the water peak during the relaxation delay of 5 s. A total of 128 transients of 32 K data points spanning a spectral width of 10 ppm were collected, corresponding to a total experiment time of 15 min. For assignment purposes a two-dimensional 1H-1H TOCSY spectrum with 80 ms mixing was acquired. Spectral width of 10 ppm were collected, corresponding to a total experiment time of 15 min. For assignment purposes a two-dimensional 1H-1H TOCSY spectrum with 80 ms mixing was acquired. In both experiments the water signal was suppressed by presaturation of the water peak during the relaxation delay of 5 s. A total of 128 transients of 32 K data points spanning a spectral width of 10 ppm were collected, corresponding to a total experiment time of 15 min.

Glutathione assay. Glutathione (GSH) was measured by a method described by Senft et al. (22), with minor modifications. In brief, mice were killed by decapitation, 0.25 g liver tissue was homogenized in 5 ml of ice-cold redox quenching buffer (20 mM HCl, 5 mM DTPA, 10 mM ascorbic acid) containing 5% TCA, samples centrifuged at 14,000 g for 15 min, and supernatants stored at −80°C until analysis. The GSH assays consisted of paired reactions, labeled A and B. Liver extract (20 μl) or appropriate GSH standard dilution in RQB-TCA buffer were added to the tubes, and reaction volume adjusted to 150 μl with RQB buffer. In reaction A GSH was sequestered by addition of 20 μl 7.5 mM N-ethylmaleimide in RQB, (20 μl of RQB was added to reaction B), and pH in the reactions neutralized by addition of 250 μl 1 M phosphate buffer pH 7. After 5 min, 1 ml of 0.1 M phosphate buffer pH 6.9 and 150 μl of 5 mg/ml o-phthalaldehyde (OPA) was added to each reaction and the reactions incubated for 30 min. OPA is nonfluorescent until it reacts as a heterobifunctional reagent with a primary amine in the presence of a GSH thiol group. The fluorescence was measured at 365-nm excitation and 430-nm emission using an EnSpire multiplate reader (Perkin Elmer). Fluorescence in reaction A represents the background non-GSH-dependent fluorescence that was subtracted from the paired reaction B. GSH concentration in samples was calculated by comparing to the standard curve. The data are presented as relative of the GSH concentration in unchallenged control mice.

Statistical analysis. Data are presented as means ± SE. Statistical significance was determined by ANOVA or t-test. P < 0.05 was considered statistically significant.

RESULTS

Generation of a floxed Aqp11 mouse model and liver-specific Aqp11 KO mice. The strategy employed to generate a floxed Aqp11 gene, allowing Aqp11 gene deletion in a conditional manner is demonstrated in Fig. 1A. Cre-recombinase-mediated recombination of the loxP sites will lead to mutation of the Aqp11\textsuperscript{flx} allele into the Aqp11\textsuperscript{flx/ko} allele lacking exons 2 and 3. Translation of the Aqp11\textsuperscript{flx/ko} allele is predicted to produce a 245 amino acids protein with only the initial 207 amino acids of the full-length 271 amino acids AQP11 protein, followed by 38 random amino acids. Importantly, the targeting strategy deletes the last AQP11 transmembrane domain and one of the conserved pore forming (NPA) boxes, thus is expected to result in an inactive channel.

To generate liver-specific Aqp11 KO mice, the mice carrying the Aqp11\textsuperscript{flx} allele were bred with the B6.Cg-Tg(Alb-cre)-21Mgn/J mice strain. This strain shows strong expression of Fig. 2. Light microscopic image of hematoxylin/eosin stained liver from unchallenged control (A and C) and liver-specific Aqp11 KO mouse (B and D). C and D: higher magnification of A and B. The perihepatic hepatocytes in the KO mouse show increased eosinophilic staining of hepatocyte cytoplasm and decreased size of hepatocyte nuclei (arrows in D). CV, central vein; PV, portal vein.
Cre-recombinase in albumin-producing cells, i.e., hepatocytes, from the seventh postnatal day (19). Figure 1B shows the result of PCR performed with genomic DNA isolated from different organs of a liver-specific Aqp11 KO mouse. Two PCR products, a 234 bp fragment corresponding to the Aqp11flx allele, and 404 bp fragment corresponding to the truncated Aqp11delE2/H110013 allele were amplified from the liver DNA sample of liver-specific Aqp11 KO mouse. The presence of both bands is anticipated and results from the isolated DNA being derived from different cell types e.g., hepatocytes, endothelial cells, Kupffer cells, etc., of which only the hepatocytes express the Cre-recombinase. However, it cannot be ruled out that recombination of the Aqp11 gene is not effective in all hepatocytes and that some hepatocytes still retain the functional Aqp11flx allele. Additionally, Aqp11 gene recombination was detectable in the gall bladder. Only the Aqp11flx allele (234 bp PCR product) was detected in the kidneys and other organs from the liver-specific Aqp11 KO mouse, confirming that the model is liver specific.

Immunoblotting of membrane proteins from unchallenged control mouse liver showed a 22 kDa unglycosylated protein band that was absent from the liver-specific Aqp11 KO mouse sample, suggesting that this band represents AQP11 protein (Fig. 1C) and indicating that our model does result in AQP11 protein deficiency in the liver. Unfortunately, this antibody was not suitable for immunohistochemistry.

The liver-specific Aqp11 KO mice appear to have a normal life span of more than 1 yr, are fertile (both females and males), show a normal growth rate compared with controls, and do not show any behavioral abnormalities.
Liver ultrastructure in liver-specific Aqp11 KO mice: unchallenged mice.

In unchallenged mice, the AQP11-deficient liver cells do not show any vacuolization by light microscopy (Fig. 2); however, in some mice the cytoplasm of the hepatocytes in the periportal zone of the liver lobulus appears more eosinophilic than in the perivenous hepatocytes. This is in contrast to more uniform staining of the whole liver lobulus of the control mice.

Effects of fasting and refeeding.

To challenge the liver-specific Aqp11 KO mice, the mice were subjected to 24 h fasting. During fasting, the liver uses up its glycogen stores and, to maintain the systemic glucose level, generates glucose in the process of gluconeogenesis. The hepatocytes are also actively taking up and metabolizing the lipids released by adipose tissue. Blood glucose levels did not differ significantly between the liver-specific Aqp11 KO and control mice following 24 h fasting [control 4.7 ± 0.3 mmol/l (n = 12) vs. KO 5.1 ± 0.2 mmol/l (n = 22)], indicating that the liver-specific Aqp11 KO mice can effectively maintain glucose levels under fasting conditions. Following 24 h fasting, the morphology of the livers was examined by light microscopy. In KO mice, the cytoplasm of the hepatocytes surrounding the portal veins appears markedly more eosinophilic than in the perivenous hepatocytes (Fig. 3, B and D). In a few animals, vacuolization of hepatocytes in close proximity to the portal vein was observed, appearing as translucent areas in the cells. Similar results were obtained with fasting periods varying from 8 to 48 h (not shown). In contrast, livers from fasted control mice show uniform staining of perivenous and periportal hepatocytes (Fig. 3, A and C).

Thirty minutes after initiation of refeeding of fasted mice with standard mouse chow, blood glucose levels were 8.2 ± 0.3 mmol/l (n = 8) in controls vs. 8.3 ± 0.3 mmol/l (n = 15) in KO mice. One hour after initiation of refeeding, blood glucose levels were 8.1 ± 0.4 mmol/l (n = 8) in controls vs. 8.7 ± 0.3 mmol/l (n = 15) in KO mice. These results indicate that the AQP11-deficient liver is capable of taking up the nutrients supplied by the portal vein and effectively controlling glucose levels.
blood glucose levels within the normal range during the post-prandial period.

Refeeding of the liver-specific Aqp11 KO mice with standard mouse chow induced rapid development of giant vacuoles in the hepatocytes from the periportal area (Fig. 4, B and D), whereas no vacuoles were observed in the livers of refed control mice (Fig. 4, A and C). Sporadically, undamaged cells with a normally stained basophilic cytoplasm and no vacuoles were observed in the periportal area of KO mice (arrowheads in Fig. 4B), which may be cells expressing functional AQP11 protein due to unsuccessful recombination of both Aqp11flx alleles. The severity of the vacuolization varied between mice, with some having damage to only a few cells in the closest proximity to the portal vein, while in other mice the area of vacuolization extended over several “rows” of cells. The size and shape of the vacuoles varied in different cells (Fig. 4D), with some cells containing multiple relatively small round vacuoles, while other cells contain few large irregularly shaped vacuoles. In between the vacuoles glycogen granules can be distinguished (Fig. 4D), but very few lipid droplets (Fig. 4F). In contrast, the periportal hepatocytes from control mice show multiple round lipid droplets in the cytoplasm (Fig. 4E).

Examination of hepatocyte ultrastructure by electron microscopy revealed that in the liver-specific Aqp11 KO mice fasted for 24 h the hepatocytes surrounding the portal vein displayed dilatation of the RER space of varying degrees (Fig. 5) from very little, e.g., cell 1 in Fig. 5A, to severe, e.g., cells 3 and 4. A small amount of electron-dense material is present inside the dilated RER lumen. Other organelles in the cells appear normal, and we observed no mitochondrial damage as reflected by regular mitochondrial cristae and similar density of mitochondrial matrix in neighboring cells with and without RER dilatation. In liver-specific Aqp11 KO mice subject to 24 h fasting and fixed 2 h after refeeding of standard mouse chow, the periportal hepatocytes are filled with giant vacuoles (Fig. 6). Ribosomes are visible on the cytoplasmic leaf of the vacuole membrane, indicating that the vacuoles originate from the dilated RER. The perinuclear cistern in these cells appears slightly dilated; however, the nuclear pore complexes prevent distension of the space between the outer and inner nuclear membrane. Additionally, the smooth endoplasmic reticulum in association with glycogen stores appears slightly dilated, but the mitochondria appear normal, without any signs of swelling or damage, even in cells with very severe vacuolization.

To investigate possible long-term liver damage and the fate of the vacuolated cells, the liver-specific Aqp11 KO mice were fixed at different time points after refeeding was initiated. The vacuolization of the periportal hepatocytes can be observed as early as 30 min following refeeding (Fig. 7A). Subsequently, the vacuoles gradually increase in size over time, with the cells

Fig. 7. Light microscopic image of hematoxylin/eosin stained liver from liver-specific Aqp11 KO mouse. The mice were fasted for 24 h, then allowed free access to mouse chow, and fixed after a different period of time: 30 min (A), 1 h (B), 2 h (C), 4 h (D), 6 h (E), 8 h (F), 12 h (G), and 24 h (H) from the moment the mice began consuming the chow. The perivenous hepatocytes at the early time points show increased eosinophilic staining, and granular cytoplasm. One hour after the feeding begins the cells in close proximity to the PV start to show vacuoles. At later time points the vacuoles increase in size. At 12 h some of the damaged cells contain a droplet of cytoplasm (arrows). At 24 h the liver appears again normal without any vacuolated cells. The few cells with basophilic staining of the cytoplasm (probably cells where AQP11 protein is still present due to lack of Cre-recombination) are indicated with arrowheads.
becoming increasingly translucent, likely reflecting complete loss of normal cytoplasm components and organelles (Fig. 7, A–G). It is plausible that as the vacuoles increase in size, the membranes of neighboring vacuoles join to form larger vacuoles. In some specimens, cells with multiple small round vacuoles and cells with a single giant vacuole were visible in close proximity to one another, likely reflecting different stages of the process of degeneration (Fig. 7E). The nucleus originally located in the center of the cell and surrounded by multiple small vacuoles is, at later time points, often pushed aside to the cell border by a single giant vacuole (Fig. 7, E and F). Twelve hours after refeeding the majority of affected perportal hepatocytes have a small drop of cytoplasm often detached from the nucleus (Fig. 7G). Compared with the early stages of the process (30 min–2 h), relatively few vacuolated cells are present in the perportal area at later time points, suggesting that some of the initially injured cells recover and only a fraction continues to develop the giant vacuoles and lethal cell injury. Alternatively, some of the most severely vacuolated cells might have already deteriorated and their remnants been cleared. At 24 h post-refeeding there are no degenerated hepatocytes in the perportal area (Fig. 7H). No macrophages were observed in the affected area of the liver, so the mechanism of cell remnants clearing is unclear but may occur via phagocytosis by neighboring cells.

Blood chemistry of liver-specific Aqp11 KO mice. To accompany the observed morphological analysis of the liver, a variety of biochemical assays were performed on plasma from male liver-specific Aqp11 KO mice and sibling controls under the three different conditions: 1) unchallenged metabolic state (free access to chow and water), 2) 24 h fasting (free access to water), and 3) 24 h fasting followed by 2 h refeeding with chow (Table 1). In general, the results of the blood chemistries were unremarkable. The marker of hepatocyte damage, alanine aminotransferase (ALT) was not elevated in the KOs compared with controls. ALT levels were similar between the groups, even in mice subject to fasting/refeeding, indicating that the plasma membrane of the vacuolated cells is intact at the time points examined. Total bilirubin levels were normal, and levels of a marker of bile duct epithelium damage, alkaline phosphatase, were similar in KOs and controls, consistent with the vacuolization only affecting the hepatocytes and that cell death occurs in a controlled manner by apoptosis rather than necrosis. Albumin and total plasma protein levels were in the normal range in all three tested conditions, suggesting that the synthesis and secretion of proteins by the liver of KO mice appear normal. Ammonia levels were not significantly different between the genotypes, and apart from a modestly reduced urea concentration in the KO under basal conditions, no major differences in urea levels in the fasted and fasted/refed mice were observed. Similarly, uric acid levels, representing the metabolism of purines, were similar in KOs and controls.

In contrast, several tests relating to lipid metabolism showed small but statistically significant differences between the KOs and the controls, suggesting the handling of lipids by the liver-specific Aqp11 KO mice is different. For example, HDL levels in the unchallenged groups were significantly increased in KOs compared with controls, whereas triglycerides, free glycerol, and free fatty acids levels were significantly lower in the fasted KO compared with fasted controls.

Effects of various nutritional stresses in liver-specific Aqp11 KO mice. To further investigate the mechanisms leading to the vacuolization of the perportal hepatocytes in liver-specific Aqp11 KO mice, we examined if any particular component of the chow (carbohydrate, lipid, or protein) alone can induce vacuolization. Oral administration of glucose (Fig. 8, A–D) or

Table 1. Plasma biochemistry in liver-specific AQP11 KO mice and control mice in unchallenged state, after 24 h fasting, and following 24 h fasting/refeeding with standard chow

<table>
<thead>
<tr>
<th></th>
<th>Not Challenged</th>
<th>Fasted 24 h</th>
<th>Fasted 24 h, Refed 2 h</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>con, n = 8†</td>
<td>KO, n = 15†</td>
<td>con, n = 19†</td>
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<tr>
<td>ALP, U/l</td>
<td>91 ± 8</td>
<td>79 ± 5</td>
<td>96 ± 9</td>
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<td>ALT, U/l</td>
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<td>Total protein, g/l</td>
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<td>Albumin, g/l</td>
<td>25.5 ± 0.6</td>
<td>25.6 ± 0.2</td>
<td>27.4 ± 0.18</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>2.81 ± 0.11</td>
<td>3.19 ± 0.13</td>
<td>3.19 ± 0.18</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.90 ± 0.08</td>
<td>2.23 ± 0.09*</td>
<td>2.29 ± 0.13</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>0.41 ± 0.01</td>
<td>0.43 ± 0.02</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.04 ± 0.12</td>
<td>0.92 ± 0.08</td>
<td>1.42 ± 0.16</td>
</tr>
<tr>
<td>Glycerol, µmol/l</td>
<td>295 ± 18</td>
<td>268 ± 9</td>
<td>387 ± 14</td>
</tr>
<tr>
<td>Free fatty acids, mmol/l</td>
<td>0.73 ± 0.07</td>
<td>0.64 ± 0.04</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td>Ketone bodies, mmol/l</td>
<td>0.29 ± 0.03</td>
<td>0.21 ± 0.02*</td>
<td>0.93 ± 0.07</td>
</tr>
<tr>
<td>Total bilirubin, µmol/l</td>
<td>2.1 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Uric acid, µmol/l</td>
<td>83 ± 6</td>
<td>89 ± 6</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>Ammonia, µmol/l</td>
<td>133.3 ± 11.2</td>
<td>138.8 ± 12.2</td>
<td>169.5 ± 12.9</td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>11.8 ± 0.6</td>
<td>10.2 ± 0.3*</td>
<td>13.0 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical significance was determined by t-test between groups control (con) and knockout (KO). *p value < 0.05. †the number of samples is lower for some measurements, due to exclusion of samples with unacceptably high hemolysis. AQP, aquaporin; ALT, alanine phosphatase; AST, aspartate aminotransferase; HDL, high density lipoprotein; LDL, low density lipoprotein.
triglyceride (Fig. 8, E and F) loads (following 24 h fasting) did not trigger liver vacuolization in KO mice, and no defects in periportal hepatocyte glycogenesis were apparent, as observed by normal glycogen staining (Fig. 8, C and D). In contrast, oral administration of casein suspension to KO mice fasted for 24 h resulted in vacuolization of periportal hepatocytes similar to vacuolization induced by fasting and refeeding with mouse chow (Fig. 8H), suggesting that the generation of vacuoles in periportal hepatocytes following fasting is efficiently triggered by intake of protein.

Mice challenged with amino acids, ammonium acetate, pyruvate, or urea. We speculated that the vacuolization of the hepatocytes triggered by protein could in principle be a result of any particular amino acid, or any other downstream product of protein catabolism, and thus examined the effects of each of these components individually on their ability to induce vacuolization in liver-specific Aqp11 KO mice.

The mice obtained an oral load of various amino acids: glycine, alanine, glutamate, glutamine, arginine, and lysine (15 mmol/kg BW). The amino acids were administered at a dose that did not induce any visible behavioral effects in the KO or control mice. The different amino acids tested produced liver vacuolization of a variable degree, with the most severe effect observed after administration of glutamine, glutamate, glycine > alanine > arginine > lysine (Fig. 9). The response to glycine was dose dependent, with high doses (13.3 and 4.0 mmol glycine/kg BW) producing severe vacuolization, whereas lower doses (1.3 and 0.4 mmol glycine/kg BW) resulted in very few vacuolated cells (not shown). Intraperitoneal administration of isoosmolar glutamine or glycine solutions in doses corresponding to those used during oral gavage resulted in similar liver damage, indicating that the route of administration of the amino acids is not critical (not shown). Sibling control mice showed no liver damage in response to similar treatments.

The administration of glutamine, glutamate, or glycine resulted in more severe damage to hepatocytes than observed after fasting/reefeding the mice with standard mouse chow. Qualitatively, up to one-third of the liver section observed showed severe damage under these conditions. The structure of the liver was investigated at various time points following the administration of glycine. Similarly to fasting/reefeding with standard mouse chow, no vacuolated cells were detectable 24 h after glycine treatment (not shown). ALT values in plasma measured at 2 and 6 h after glycine administration were similar in the controls and KOs, [at 2 h: KO 34.0 ± 2.1 (U/l), n = 7 vs. Con 31.2 ± 1.9 (U/l), n = 9; at 6 h: KO 40.0 ± 2.7 (U/l), n = 13; vs. Con 43.8 ± 5.1 (U/l), n = 16], suggesting once again that the severe injury caused by administration of glycine leads to apoptosis and efficient phagocytosis of cell remnants, rather than cytonecrosis.
In mice treated with ammonium acetate (7 mmol/kg BW), the liver showed some degree of vacuolization, but this was not as severe as in mice treated with amino acids e.g., glutamine or glycine (Fig. 10). Administration of urea (15 mmol/kg BW) did not result in liver vacuolization (Fig. 10). Even after a high dose (100 mmol/kg BW), only a small number of vacuolated cells were observed (not shown). Pyruvate (a ketoacid produced during deamination of alanine), resulted in minor vacuolization in the cells in the closest proximity to the portal vein (Fig. 10).

### Glycosylation of proteins synthesized by the liver

The RER is the organelle where the initial steps of protein N-glycosylation occur, thus we analyzed the glycosylation profile of proteins in liver homogenates from liver-specific Aqp11 KO and control mice. In unchallenged AQP11-deficient liver, there was no apparent defect in protein glycosylation. The general banding pattern of proteins in liver homogenates (assessed by Coomassie blue-stained gels) and both the profile and abundance of protein-bound glycans were similar for the AQP11-deficient and control livers (Fig. 11, A and B). To confirm these
results and to assess if there are any measurable defects in the synthesis or glycosylation of specific proteins synthesized by hepatocytes, we performed immunoblotting for two selected glycoproteins: 1) a secreted protein, transferrin, and 2) a protein localized at the hepatocyte membrane, the LDL receptor. No differences in relative abundance or the molecular weight of transferrin (in serum) or the LDL receptor (in liver homogenates) were observed between the liver-specific Aqp11 KO and control mice (Fig. 11, C and D). Together, these results indicate that the rate of synthesis and the glycosylation of proteins by the hepatocytes are not severely affected in the liver-specific Aqp11 KO mice in an unchallenged state, but we cannot exclude the possibility that a defect might be present in a small subfraction of cells that is masked by the normally functioning liver cells.

Expression of other hepatic aquaporins and ER stress markers. We assessed the mRNA levels of hepatic aquaporins AQP9 and AQP8 by quantitative RT-PCR and found no statistically significant difference between control and AQP11-deficient mice, Fig. 12, A and B, indicating no compensatory regulation of other hepatic aquaporins AQP8 and AQP9 in AQP11-deficient livers. We also analyzed the expression of key markers of ER stress. Quantitative RT-PCR of Grp78 (BiP) showed an increase of KO and control mice (Fig. 11, C and D). Together, these results indicate that the rate of synthesis and the glycosylation of proteins by the hepatocytes are not severely affected in the liver-specific Aqp11 KO mice in an unchallenged state, but we cannot exclude the possibility that a defect might be present in a small subfraction of cells that is masked by the normally functioning liver cells.
induced in response to a variety of cellular stresses, including ER
mice livers (not shown). GADD153 is a transcription factor that is
AQP11-deficient liver (Fig. 12, showing vacuolar damage and no staining in unchallenged
GADD153 revealed increased nuclear staining in the hepatocytes
twice the KO and control mice in each treatment group; data from one of the experiments are presented in Fig. 13.

NMR analysis of solutes isolated from livers of mice subject to
glycine load. We speculated whether the dilatation of RER
cisternae results from accumulation of osmotically active solutes in the cistern lumen. To identify potential differences in
solutes from livers of liver-specific Aqp11 KO and control mice 2 h after administration of glycine, we utilized NMR metabolomic analysis. Two types of 1D 1H NMR spectra were acquired: regular spectra showing signals from all types of metabolites and CPMG spectra where the signals from fatty acids and larger metabolites are attenuated. Analysis of the liver extracts revealed a decreased amount of extractable metabolites in the AQP11-deficient livers, and particularly a decrease in α- and β-glucose levels (identified by a signal decrease at 5.22 and 4.65 ppm, respectively, as well as at many other resonances between 3.9 and 3.2 ppm) (Fig. 14). No solutes markedly accumulated in the liver of the liver-specific Aqp11 KO mice compared with controls.

AQP11 is expressed, among other places, in the kidney and
liver where its molecular function is unknown. Mice lacking
AQP11 show an intriguing phenotype of cellular injury with
dilatation of RER cisterns, named vacuolar or hydropic degeneration.
The most prominent injury occurs in the kidney, where
the proximal tubule (PT) cells show severe vacuolization from
birth (18, 24), resulting in abnormal kidney development, generation of PT-derived cysts, and kidney failure and death
within 1–2 mo of age.

GSH levels. The antioxidant GSH levels in tissue can be
used as a marker of oxidative stress. In the liver, GSH levels
were assessed by a fluorometry-based assay in unchallenged, fasted, refed, and glycine-treated liver-specific Aqp11 KO and
control mice. We detected a substantial GSH depletion in
response to feeding glycine or fasting, which was partially
corrected after refeeding (Fig. 13), indicating that the meta-
bolic challenge generates oxidative stress in the liver. The
relative amount of GSH was similar in the KO and control
mice in each treatment group; data from one of the experiments are presented in Fig. 13.

Dilatation of ER cisterns, named vacuolar or hydropic
degeneration. The most prominent injury occurs in the kidney, where
the proximal tubule (PT) cells show severe vacuolization from
birth (18, 24), resulting in abnormal kidney development, generation of PT-derived cysts, and kidney failure and death
within 1–2 mo of age.

Grp78 mRNA following refeeding of mice fasted for 24 h;
however, no statistical significant difference was observed
between the KO and control mice (Fig. 12C). This result indicates
that the fasting/refeeding challenge induces ER stress in hepatocytes, which may cause severe impairment of RER function and
vacuolation in AQP11-deficient peripoortal hepatocytes. Quanti-

cative RT-PCR of another marker of ER stress, the growth arrest
and DNA-damage-inducible protein GADD153 (CHOP) showed only
a mild increase of mRNA in fasted KOs compared with controls
(Fig. 12D). Immunohistochemistry with an antibody against
GADD153 revealed increased nuclear staining in the hepatocytes
showing vacuolar damage and no staining in unchallenged
AQP11-deficient liver (Fig. 12, E and F) or in liver from control
mice livers (not shown). GADD153 is a transcription factor that is
induced in response to a variety of cellular stresses, including ER

Fig. 12. Relative abundance of AQP8 (A) and AQP9 (B) mRNA in the livers
of unchallenged, fasted and refed mice, liver-specific Aqp11 KO and control
mice. Although significant changes in mRNA levels were detected in response
to different treatments, no significant differences were measured between
genotypes in mice subject to the same treatment. C: relative abundance of
Grp78 (Bip) mRNA increases after fasting/refeeding in liver-specific Aqp11
KO and control mice to similar levels, indicating that this treatment causes ER
stress both in control as well as AQP11-deficient livers. D: relative abundance of
GADD153 (CHOP) mRNA increases during fasting and refeeding in both
controls and AQP11-deficient livers. GADD153 is significantly increased in
fasting liver-specific Aqp11 KO compared with control. *P < 0.05. E and F: the
immunohistochemical staining of GADD153 (CHOP). Positive staining
was detected in the cell nuclei of vacuolated cells (F, arrowheads), while no signal was detected in unchallenged AQP11-deficient livers (E).

Fig. 13. Relative GSH levels in livers from unchallenged, fasted, refed and
glycine treated liver-specific Aqp11 KO and control mice measured by a
fluorometry-based assay; n = 6–8 for liver-specific Aqp11 KO, and n = 4 for
controls. There is highly significant decrease of GSH in fasted, fasted/refed,
and glycine treated mice compared with controls, but no statistically significant
differences between the AQP11-deficient and control livers in each treatment
group.
membrane integrity is likely to result in water and ion movement, maintaining the unique composition of RER luminal fluid or RER membranes. Reduced GSH in the cytosol (11) disturbs processes that are present in its oxidized form (GSSG) compared with mostly micromolar in the cytosol, and a higher fraction of GSH concentrations of calcium are in millimolar range compared to the ER lumen. During metabolic challenge these solutes might accumulate in the ER lumen at concentrations high enough to cause osmotic swelling of this organelle. Our analysis of the metabolome indicated a decrease of total metabolites in the ER lumen. Moreover, GSH depletion does not seem to be the direct cause of RER vacuolation in the AQP11-deficient livers. The positions of the glucose signals are indicated. Signals that are significantly different are highlighted in black ($P < 0.05$, Bonferroni). The inset shows relative signal values for total metabolite, $\alpha$- and $\beta$-glucose for each sample.

Fasting/refeeding with standard chow or administration of large doses of various amino acids to the liver-specific Aqp11 KO mice resulted in severe, degeneration of perportal hepatocytes, accompanied by an increased nuclear staining of the ER stress marker GADD153 in the damaged cells. The vacuoles in the damaged cells are derived from the RER compartment, consistent with previous observations in kidneys of Aqp11 KO mice (18, 24). Other organelles in the hepatocytes appear undamaged, with mitochondria showing no swelling or other degenerative changes even in severely vacuolated cells. We speculate that the molecular mechanism of RER damage in the AQP11-deficient PTs and the peripoortal hepatocytes might be the same. However, it is plausible that PT cells are more sensitive to induction of RER vacuolization, resulting in persistent severe cell damage leading to neophren dysfuntion and anomalous development, while severe vacuolization of hepatocytes takes place only transiently after a metabolic challenge and is followed by rapid regeneration of the liver, allowing relatively undisturbed function of this organ.

The exact molecular mechanisms leading to RER cistern dilatation in the absence of AQP11 is at present unknown, but our current data suggest a number of potential scenarios that require further investigation:

**Solute accumulation in the RER.** The RER lumen provides a unique environment necessary for proper folding and disulfide bridge formation of newly synthesized proteins, e.g., luminal concentrations of calcium are in millimolar range compared with micromolar in the cytosol, and a higher fraction of GSH is present in its oxidized form (GSSG) compared with mostly reduced GSH in the cytosol (11). Disturbance of processes that maintain the unique composition of RER luminal fluid or RER membrane integrity is likely to result in water and ion movement and osmotic swelling of the RER. AQP11 can transport water (27); however, it cannot be ruled out that it can also transport small solutes and serve as their exit pathway from the ER lumen. During metabolic challenge these solutes might accumulate in the RER lumen at concentrations high enough to cause osmotic swelling of this organelle.

**Effect of oxidative stress.** Oxidative stress has previously been linked to ER stress. Altered oxidative states cause membrane lipid peroxidation, resulting in damage to and increased permeability of the biomembranes especially the mitochondrial and RER membranes. RER dilatation has been observed in liver following hypoxia (5) and in the regenerating liver of rats subject to partial hepatectomy and infused with high glucose/high insulin, a treatment inducing oxidative stress (13). Administration of an antioxidative drug prevented the cell injury and vacuolar degeneration of the RER (28). Our analysis demonstrated decreased GSH levels in the mice in response to different metabolic challenges, such as fasting or glycine treatment, and increased expression of ER stress-related genes GRP78 and GADD153, indicating that fasting and refeeding and amino acid challenge itself cause oxidative stress and ER stress in the liver. However, GSH depletion does not seem to be the direct cause of RER vacuolation in the AQP11-deficient hepatocytes, as the fasted mice, while presenting lowest GSH levels, show only mild RER dilatation. Nevertheless, the GSH depletion and the presumed underlying oxidative stress might be a factor affecting RER function and predisposing the AQP11-deficient hepatocytes to develop giant vacuoles following refeeding or catabolism of amino acids.
them for synthesis of new cellular proteins and as precursors of secondary metabolites. Surplus amino acids cannot be stored and are metabolized into other chemical components such as carbohydrates (glycogen) and lipids. Ammonia is released during these reactions, which is detoxified to urea in the perportal hepatocytes. During prolonged fasting the perportal zone of the liver activates gluconeogenic pathways with amino acids derived from breakdown of proteins serving as an important substrate for glucose production. Thus, intake of a high-protein meal after a period of fasting may result in a rapid deamination of the supplied amino acids with an acute rise in intracellular ammonia levels, leading to altered cellular and/or RER homeostasis. This hypothesis is supported by the finding that the most severe RER vacuolation in AQP11-deficient livers was observed after administration of the gluconeogenic amino acids glycine, glutamine, and glutamate. The fact that lysine induces very little RER vacuolation might result from slower metabolic conversion and release of amine groups, whereas arginine might in fact enhance the rate of ammonia transformation to urea (7). Although direct oral administration of ammonium ions to the liver-specific Aqp11 KO mice resulted in relatively minor damage of perportal hepatocytes, it is possible that rapid deamination of large doses of glucogenic amino acids increases the intracellular ammonia levels in the perportal hepatocytes to greater levels than uptake of ammonium after ammonium acetate feeding. Correspondingly, the early PT cells, which show severe vacuolization in total Aqp11 KO mice, are the site of reabsorption of the filtered amino acids and produce majority of tubular ammonia (6).

Effect of protein translation/folding/glycosylation. The RER is involved in protein translation and translocation, protein folding, and the initial steps of N-glycosylation of secretory, lysosomal, and many integral membrane proteins. Defects in these processes could trigger ER stress, which can manifest as RER swelling; however, the molecular mechanisms of this are currently unknown. For example, RER swelling was observed in cells treated with eeyarestatin I, an inhibitor of protein translocation (16); upon accumulation of misfolded mutants protein in thyroid gland cells (21); after simultaneous inhibition of the RER resident chaperone Hsp90 and the proteasome (17); and in syndromes related to hereditary defects in RER-dependent protein glycosylation in AQPI1- mutants protein in thyroid gland cells (21); after simultaneous inhibition of the RER resident chaperone Hsp90 and the proteasome (17); and in syndromes related to hereditary defects in RER-dependent protein glycosylation in AQP11-deficient liver. AQP11-deficient hepatocytes have very little electron-dense material inside the RER lumen at the early stages of cistern dilatation, and no fixable material is detectable in the giant vacuoles. Furthermore, production of secretory proteins, e.g., albumin and transferrin, is unaffected in liver-specific Aqp11 KO mice. Together, our observations do not provide any evidence for defective protein synthesis and secretion in liver-specific Aqp11 KO mice in the unchallenged state; however, analysis of protein production rates at a single cell level are needed to definitively exclude a defect protein synthesis and secretion in the perportal hepatocyte population.

ER stress is an important factor in the outcome of many acute and chronic diseases of the liver, such as chronic viral hepatitis, insulin resistance, fatty liver disease, alcoholic liver disease, and liver ischemia. The newly developed liver-specific Aqp11 KO mouse is potentially a valuable model for studying the mechanism of ER stress in the liver and the cellular ER stress response pathways and potentially provide a new model for testing the pharmacological treatment of ER stress associated with many diseases, which in the future might improve the treatment of and help prevent liver disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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