Research Communication

Cholesterol Can Modulate Mitochondrial Aquaporin-8 Expression in Human Hepatic Cells

Mauro Danielli[1]
Alejo M. Capiglioni[1]
Julieta Marrone[1]
Giuseppe Calamita[2]
Raúl A. Marinelli[1]*

[1]Instituto de Fisiología Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina
[2]Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi di Bari "Aldo Moro," Bari, Italy

Abstract

Hepatocyte mitochondrial aquaporin-8 (mtAQP8) works as a multifunctional membrane channel protein that facilitates the uptake of ammonia for its detoxification to urea as well as the mitochondrial release of hydrogen peroxide. Since early oligonucleotide microarray studies in liver of cholesterol-fed mice showed an AQP8 downregulation, we tested whether alterations of cholesterol content *per se* modulate mtAQP8 expression in human hepatocyte-derived Huh-7 cells. Cholesterol loading with methyl-β-cyclodextrin (mβCD):cholesterol complexes downregulated the proteolytic activation of cholesterol-responsive sterol regulatory element-binding protein (SREBP) transcriptions factors 1 and 2, and the expression of the target gene 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). Under such conditions, mtAQP8 mRNA and protein expressions were significantly reduced. In contrast, cholesterol depletion using mβCD alone increased SREBP-1 and 2 activation and upregulated HMGCR and mtAQP8 mRNA and protein expressions. The results suggest that cholesterol can regulate transcriptionally human hepatocyte mtAQP8 expression likely via SREBPs. The functional implications of our findings are discussed. © 2017 IUBMB Life, 00(0):000–000, 2017

Keywords: *cholesterol; mitochondrial aquaporin-8; hepatic cells*

Introduction

Aquaporin-8 (AQP8) is a multifunctional membrane channel that facilitates the passage of ammonia and hydrogen peroxide in addition to water (1). AQP8, as a nonglycosylated 28-kDa protein, is expressed in the inner mitochondrial membrane of hepatocytes and certain other cells (2). Hepatocyte mitochondrial AQP8 (mtAQP8) was initially thought to be relevant for the mitochondrial transport of water, nevertheless, further detailed studies suggest that mtAQP8 does not play an important role in the facilitation of water transport across mitochondrial membranes (reviewed in (3)). In contrast, there is convincing evidence that mtAQP8 facilitates the uptake of ammonia and its detoxification to urea in rat hepatocytes (4–7), and the ammonia exit in response to acidosis in human renal proximal tubule cells (8). Besides, mtAQP8 could also function as a peroxiporin for the mitochondrial release of hydrogen peroxide in hepatic (9, 10) and nonhepatic cells (11).

Sterol regulatory element-binding proteins (SREBPs) are key membrane-bound transcription factors in the regulation of hepatic cholesterol synthesis (12). When cellular cholesterol is low, proteolytically activated SREBPs translocate to the nucleus and bind to sterol response elements located in the promoter of genes encoding key enzymes of the cholesterol biosynthetic pathway, for example, the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). When cellular cholesterol content rises, SREBPs no longer are processed proteolytically and gene transcription is reduced (12). Oligonucleotide microarray studies, published before the mitochondrial localization of hepatocyte AQP8 was documented (2), reported that cholesterol feeding in mice downregulates a number of liver genes including AQP8 (13, 14). Besides, a recent transomics study suggests mouse

Abbreviations: *mtAQP8, mitochondrial aquaporin-8; SREBP, sterol regulatory element binding protein; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; mβCD, methyl- β-cyclodextrin*

Volume 00, Number 00, Month 2017, Pages 00–00

**Address correspondence to: Raul A. Marinelli, Instituto de Fisiología Experimental, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 570, 2000 Rosario, Santa Fe, Argentina. Tel: 54-341-4305799. Fax: 54-341-4399473.*
E-mail: rmarinel@unr.edu.ar or marinelli@ifise-conicet.gov.ar

Received 21 November 2016; Accepted 15 February 2017
DOI 10.1002/iub.1615
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com)

liver AQP8 as a cholesterol-related gene (15). Here, we tested whether alterations of cholesterol content are able to modulate mtAQP8 expression at message and protein levels in human hepatic cells.

Materials and Methods

Materials and Reagents

Dulbecco's modified Eagle's medium (DMEM), L-glutamine, fetal calf serum, Pen-Strep antibiotic mixture, 0.25% Trypsin-EDTA and TRIzol reagent were all from Invitrogen (Carlsbad, CA). Lipoprotein deficient serum from fetal calf, cholesterol, methyl-β-cyclodextrin (mβCD) and Triton X-100 were from Sigma-Aldrich (St Louis, MO). Prolong and 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) were obtained from Molecular Probes (Eugene, OR). Horseradish peroxidase-conjugated secondary antibodies were from Thermo Scientific (Rockford, IL). Protease inhibitors phenyl-methylsulfonyl fluoride was from Sigma-Aldrich and Leupeptin from Chemicon, Millipore (Darmstadt, Germany). Sucrose was purchased from MP Biomedicals, (Solon, OH). Materials for immunoblotting were obtained as follows: Polyscreen polyvinyl difluoride transfer membrane from Perkin Elmer (Boston, MA); enhanced chemiluminescence detection system (ECL) from Thermo Scientific (Rockford, IL); Amersham Hyperfilm ECL film from GE Healthcare Limited (Buckinghamshire, UK).

Cell Culture

Huh-7 (ATCC) cells were cultured in T75 flasks in DMEM (4.5 g/L, high glucose formulation), supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal calf serum and 100 I.U. penicillin/100 μg streptomycin at 37°C in a 5% CO_2 atmosphere. Media was changed every other day, and cells were trypsinized after reaching confluence.

Cholesterol Loading and Depletion

To prepare the mβCD:cholesterol complexes (16), cholesterol was dissolved in a methanol/chloroform (1:1) mixture at the concentration of 100 mM. Then, 100 μL of this solution was dried under N_2, and 4 mL of 10 mM mβCD phosphate buffered saline (PBS) solution was added to the dried cholesterol. The suspension was sonicated in bath sonicator at 4 °C for 5 min and incubated with agitation at 37 °C overnight. The suspension containing the complexes was adjusted to pH 7.4 and filtered through a 0.22 μm syringe filter immediately before being used. Cholesterol concentration in the prepared solution was about 300 mg/mL, and it was added to semiconfluent cell cultures at 32, 64 and 128 μM concentration. For cholesterol depletion (16), semiconfluent cell cultures were incubated in DMEM 10% lipoprotein deficient serum for 4 h and, after that, incubated in the same media containing 2 mM mβCD.

Preparation of Cellular Fractions

Huh-7 cells were harvested in sucrose 0.3 M (pH 7.4) and sonicated to obtain total cell lysates. A fraction of cell lysates were subjected to low-speed centrifugation to obtain postnuclear supernatants which were then centrifuged at 6,200*g* for 10 min, yielding the mitochondrial pellet. Mitochondria were washed twice before being resuspended in 0.3 M sucrose with protease inhibitors. The postmitochondrial supernatant was spun at 100,000*g* to obtain the microsomal fraction which was resuspended in 0.3 M sucrose with protease inhibitors. Proteins were determined according to Lowry et al. (17) using bovine serum albumin as standard.

Cellular Cholesterol Content

Huh-7 cells were cultured and treated in six-well plates. Cells were washed and scraped with cold PBS. Lipids were extracted using the method developed by Folch et al. (18). After extraction of the organic phase, the solvent was evaporated under N_2 stream. Cholesterol was determined using a colorimetric method (Wiener Lab, Rosario, Argentina) and normalized to the protein level of corresponding cell lysate.

Immunoblotting

Western blot analyses were performed as described (9, 10). We used affinity-purified antibodies from Santa Cruz Biotechnology (Santa Cruz, CA) against human AQP8, HMGCR, SREBP 1 and 2. Membranes were reprobed with rabbit antibodies against Prohibitin (Abcam, Cambridge, UK) or with mouse antibodies against β-actin (Sigma) at a final concentration of 1 μg/mL as loading control.

Confocal Immunofluorescence Microscopy

Cells were grown on glass coverslips and, at the end of each experiment, washed with cold PBS and fixed with 4% paraformaldehyde at room temperature. Fixed cells were permeabilized and blocked with 0.3% Triton X-100/bovine serum albumin 1% in PBS pH 7.4 for 30 min, and incubated 2 h with monoclonal rabbit affinity-purified AQP8 antibody (5 μg/mL) (Millipore, Temecula, CA). We found that this antibody is able to recognize nonglycosylated mtAQP8 but not glycolylated AQP8 of plasma or intracellular membranes. After washing, coverslips were incubated with Alexa 488-conjugated goat anti-rabbit secondary antibody (Molecular Probes) for 1 h. Coverslips were washed, incubated with DAPI (50 μM) for 10 min and mounted with Prolong. Fluorescence was detected by using confocal laser microscopy (Zeiss LSM 880 AxioObserver Confocal Laser Scanning Microscope). Images were collected with the same confocal settings in each set of experiments. With these settings no autofluorescence was detected. Controls omitting primary or secondary antibodies revealed no labeling.

Real-Time RT-PCR

Total RNA from Huh-7 cells was isolated by the TRIzol reagent, and cDNA was produced by using SuperScript III RNase H reverse transcriptase (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. SYBR Green-based real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis was used to assess transcript levels; triplicate amplification reactions were performed with a StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA) with Platinium Taq DNA polymerase (Invitrogen). Primers

used for detection of AQP8 were 5′-CCACGCTGGGGAATATCA-3′ and 5′-GAGGAGCATCACCAGGTTG-3′, for detection of HMGCR were 5′-GTCATTCCAGCCAAGGTTGT-3′ and 5′-GGGA CCACTTGCTTCCATTA-3′ and those for house-keeping gene glyceraldehyde 3-phosphate dehydrogenase were 5′-CAATGA CCCCTTCATTGACC-3′ and 5′-TTGATTTTGGAGGGATCTCG-3′. The cDNA fragments amplified by each pair of primers were of 92, 228 and 159 bp for AQP8, HMGCR and glyceraldehyde 3-phosphate dehydrogenase, respectively. At the end of these experiments, specificity of each reaction was confirmed with a dissociation curve starting from 55 °C to 95 °C with continuous fluorescence measure. Relative levels of the AQP8 and HMGCR mRNA normalized to glyceraldehyde 3-phosphate dehydrogenase were calculated based on the $2^{-\Delta\Delta Ct}$ method.

Lactate Dehydrogenase Assay

Lactate dehydrogenase activity was used as a measure of cell viability by measuring the enzyme leakage with a commercial assay kit (Wiener Lab, Rosario, Argentina).

Statistical Analysis

Data are expressed as means ± SE. Significance was determined using Student's *t*-test or the one-way ANOVA, Tukey test. $P < 0.05$ was considered as statistically significant.

Results

mtAQP8 Expression in Cholesterol-Loaded Cells

The incubation of Huh-7 cells with increasing concentrations of cholesterol/mβCD complexes augmented intracellular cholesterol content in a dose-dependent manner (Fig. 1A). Cell viability as it determined by lactate dehydrogenase release was unaffected (data not shown). As is well known that cholesterol represses HMGCR gene transcription, its mRNA and protein expressions were used as positive control for cholesterol effect. Protein expressions of HMGCR (assessed in microsomal fractions) and mtAQP8 (assessed in mitochondrial fractions) were reduced dose-dependently by cholesterol (Fig. 1B). Confocal immunofluorescence studies showed AQP8 labeling in cytoplasmic structures compatible with mitochondria. Consistent with the immunoblotting studies, the fluorescence intensity of mtAQP8 staining was decreased in cholesterol-loaded Huh-7 cells (Fig. 1C). Figure 1D shows that HMGCR and mtAQP8 mRNA expressions were also significantly downregulated. Since liver HMGCR and presumably AQP8, are cholesterol-responsive SREBP target genes (13, 14), we checked whether SREBP-1 and SREBP-2 were accordingly regulated by cellular cholesterol content. As expected, the expressions of proteolytically activated, nuclear SREBP-1 and SREBP-2 were downregulated in cholesterol-loaded Huh-7 cells (Fig. 1E). Together, these data suggest that cholesterol loading transcriptionally downregulates mtAQP8 expression in Huh-7 cells likely via SREBPs.

mtAQP8 Expression in Cholesterol-Depleted Cells

The treatment of Huh-7 cells with mβCD diminished intracellular cholesterol content down to nearly 50% (Fig. 2A). Cell viability as determined by lactate dehydrogenase release was unaffected (data not shown). Cholesterol depletion significantly increased HMGCR (assessed in microsomal fractions) and mtAQP8 (assessed in mitochondrial fractions) protein expressions (Fig. 2B). Consistent with the immunoblotting studies, the fluorescence intensity of mtAQP8 staining was increased in cholesterol-depleted Huh-7 cells (Fig. 2C). Figure 2D shows that HMGCR and mtAQP8 mRNA expressions were also significantly upregulated. Accordingly, the expressions of proteolytically activated, nuclear SREBP-1 and SREBP-2 were upregulated in cholesterol-depleted cells (Fig. 2E). Together, these data suggest that cholesterol depletion transcriptionally upregulates mtAQP8 expression in Huh-7 cells likely via SREBPs.

Discussion

Using human hepatic cells, we found that cholesterol loading decreased, whereas cholesterol depletion increased, protein and mRNA expressions of mtAQP8. This agrees with early oligonucleotide microarray reports indicating an AQP8 downregulation in mice fed cholesterol-rich diets (13, 14). Our data, together with the presence of putative sterol response elements in the AQP8 gen promoter [SABiosciences' proprietary database (DECODE, DECipherment of DNA Elements)] and the increased hepatic AQP8 mRNA in transgenic mice with SREBP overexpression (19) strongly suggest that cholesterol regulates transcriptionally the hepatic mtAQP8 expression via SREBPs.

Hepatocyte cholesterol is known to activate or repress its biosynthetic genes by means of SREBP transcription factors. Other key cholesterol-related transcription factor, the liver X receptor, participates in the cholesterol metabolism by mainly controlling genes involved in its excretion and degradation to bile acids (12, 14). Thus, contrarily to SREBPs, liver X receptor agonists do not modify the hepatic expression of HMGCR and other cholesterol biosynthetic genes. Interestingly, they do not either modify AQP8 mRNA expression (14). Thus, our data might suggest that mtAQP8 plays a role in the SREBP-controlled cholesterol biosynthesis. The mtAQP8 is able to work as a peroxiporin by mediating the diffusional exit of mitochondrial hydrogen peroxide in human hepatic cells (9); and the hydrogen peroxide has been described to stimulate cholesterogenesis in liver cells due to increased expression of HMGCR via SREBPs 1 and 2 (20). Moreover, insulin, which is known to induce hepatocyte cholesterol synthesis, has been reported to stimulate the mitochondrial production of hydrogen peroxide (21). Thus, mitochondrial-derived hydrogen peroxide may act as a signalling molecule in cholesterol synthesis, as it does in several other biochemical pathways (22, 23). In this context, it is tempting to speculate that when cellular cholesterol is low, the SREBP-dependent mtAQP8 upregulation could facilitate the release of mitochondrial hydrogen peroxide which would contribute to stimulate cholesterogenesis. The opposite would be expected when cellular cholesterol level is high.

We previously described that the knockdown of mtAQP8 expression in human hepatic cells decreases H_2O_2 mitochondrial

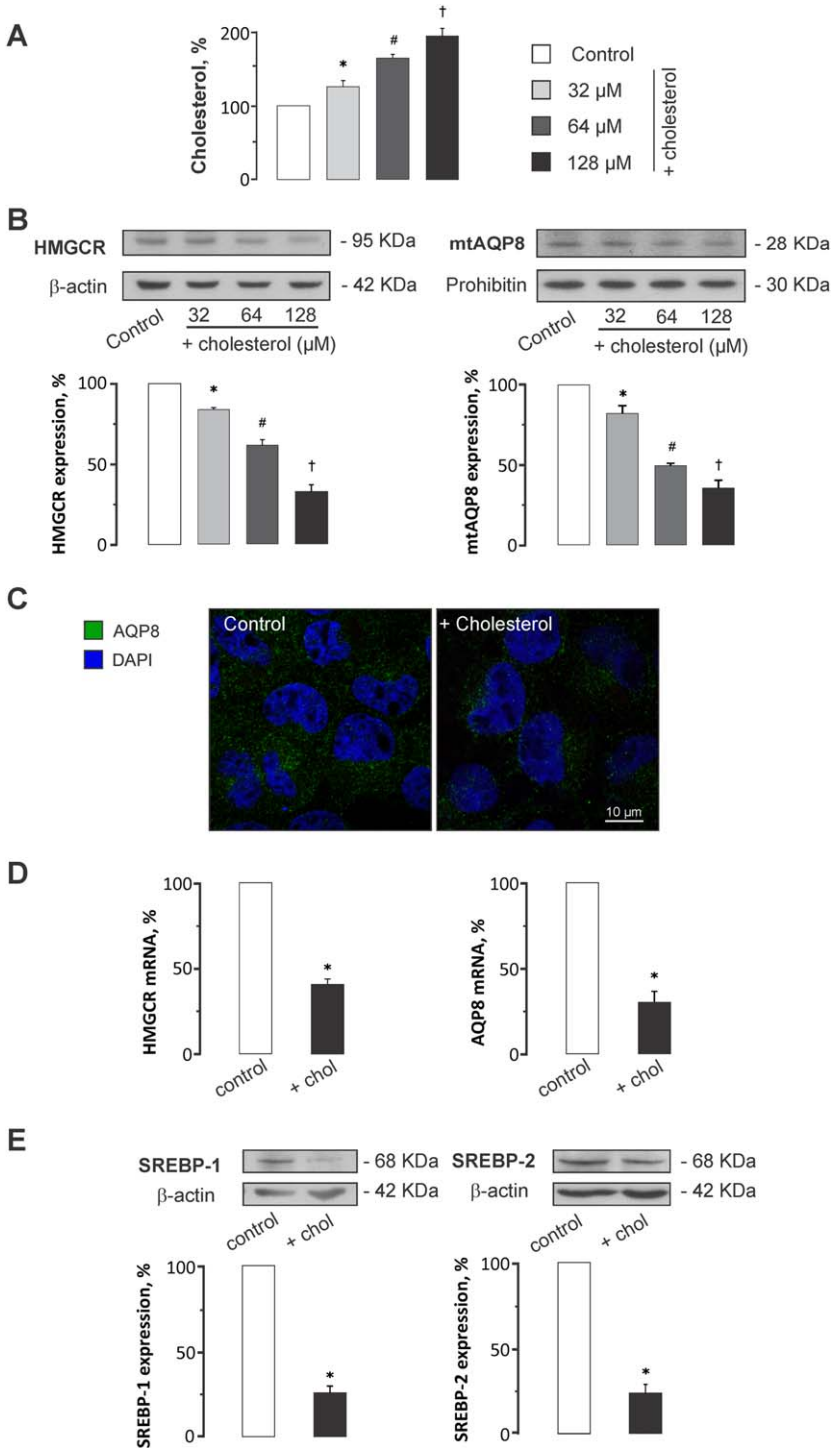

FIG 1

*Expression of mtAQP8 in cholesterol-loaded cells. (A) Cellular cholesterol content in human hepatocyte-derived Huh-7 cells after treatment with mβCD:cholesterol complexes for 24 h. (B) Representative immunoblottings for HMGCR (in microsomal fraction) and mtAQP8 (in mitochondrial fraction) proteins with corresponding densitometric analysis at increasing cellular cholesterol content for 24 h. Actin and the inner mitochondrial membrane protein prohibitin were used as controls for equal protein loading. (C) Confocal immunofluorescence microscopy for mtAQP8 (green) in cells incubated with 128 μM cholesterol for 24 h. Nuclei were stained with DAPI (blue). (D) Messenger RNA expression of HMGCR and mtAQP8 assessed by real-time RT-PCR in cells incubated with 128 μM cholesterol (chol) for 8 h. Data were normalized to housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase. (E) Protein expressions of nuclear/active forms of SREBP-1 and SREBP-2 in cells incubated with 128 μM cholesterol (chol) for 6 h. Data are means ± SE of three or four independent experiments. *$P < 0.05$ from controls and cholesterol 64 and 128 μM (A and B) or from controls (D and E); #$P < 0.05$ from controls and cholesterol 32 and 128 μM; †$P < 0.05$ from controls and cholesterol 32 and 64 μM.*

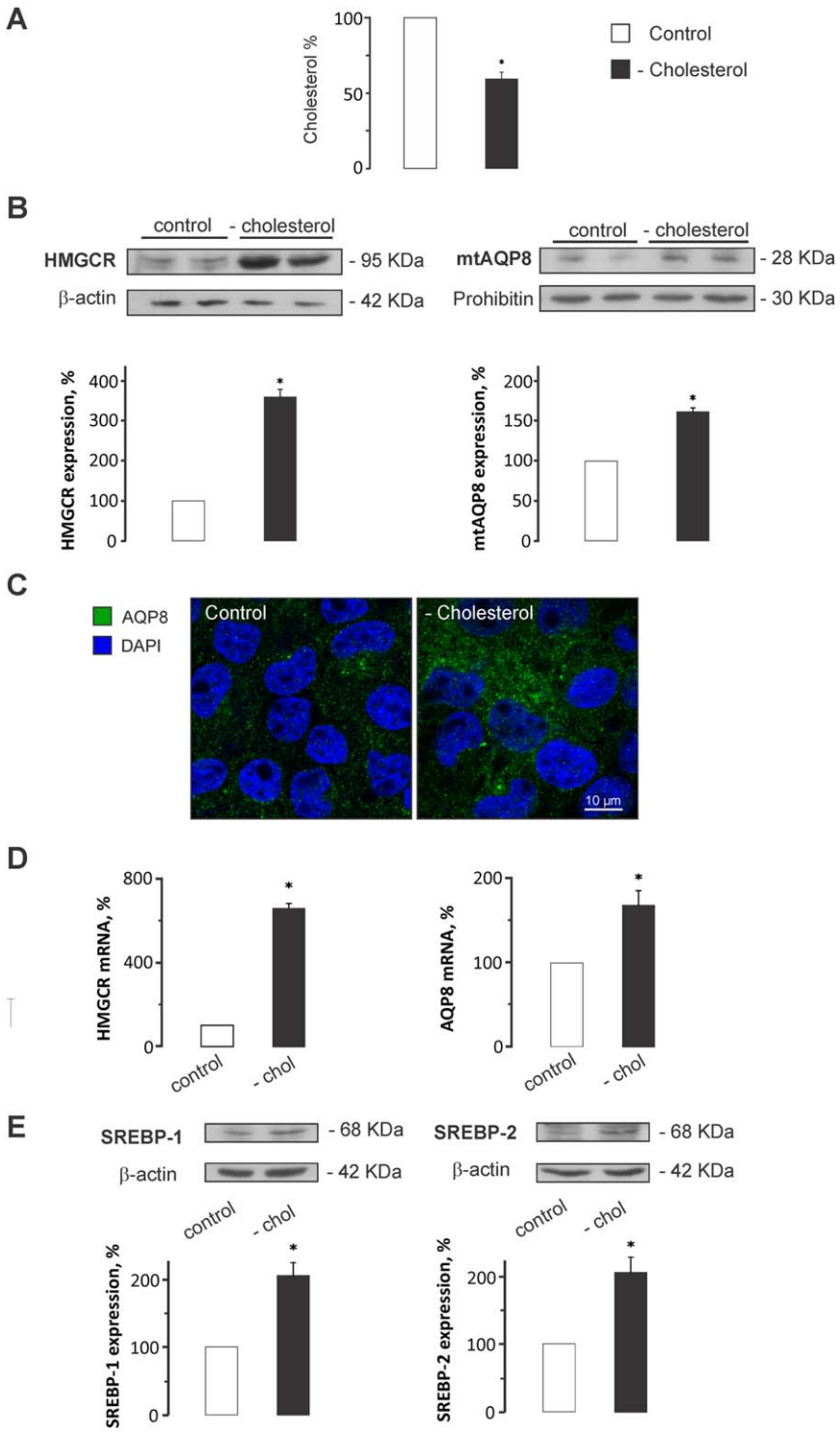

FIG 2 *Expression of mtAQP8 in cholesterol-depleted cells. (A) Diminished intracellular cholesterol content in human hepatocyte-derived Huh-7 cells after treatment with mβCD for 24 h. (B) Representative immunoblottings for HMGCR (in microsomal fraction) and mtAQP8 (in mitochondrial fraction) proteins with corresponding densitometric analysis in cholesterol-depleted cells for 24 h. Actin and the inner mitochondrial membrane protein prohibitin were used as controls for equal protein loading. (C) Confocal immunofluorescence microscopy for mtAQP8 (green) in cholesterol-depleted cells for 24 h. Nuclei were stained with DAPI (blue). (D) Messenger RNA expression of HMGCR and mtAQP8 assessed by real-time PCR in cholesterol (chol)-depleted cells for 8 h. Data were normalized to housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase. (E) Protein expressions of nuclear/active forms of SREBP-1 and SREBP-2 in cholesterol (chol)-depleted cells for 6 h. Data are means ± SE of three or four independent experiments. *$P < 0.05$ from corresponding control.*

release and consequently increases mitochondrial reactive oxygen species (including H_2O_2) assessed with 2′,7′-dichlorodihydrofluorescein diacetate (9). In line with that, reactive oxygen species in cholesterol-loaded Huh-7 cells, where mtAQP8 is downregulated, significantly increased in mitochondria ($+45 \pm 12\%$; n: 3; $P < 0.05$) and decreased in cytosol ($-19 \pm 2\%$; n: 3; $P < 0.05$). These data support our speculation; nevertheless, the elucidation of the role of mtAQP8 in cholesterol metabolism requires further *ad hoc* studies.

In certain nonhepatic cells, plasma membrane AQP8 transports NOX-generated H_2O_2 contributing to intracellular signal transduction pathways (24, 25). On the contrary, in hepatocytes, plasma membrane AQP8 is exclusively expressed on the bile canalicular domain (1). Thus, AQP8 cannot be involved in the intracellular transport of NOX-generated H_2O_2 at sinusoidal plasma membranes.

On the other hand, hepatocyte mtAQP8 can facilitate the mitochondrial uptake of ammonia and its metabolism into urea (4–6). Ureagenesis and hepatic lipid metabolism have been suggested to play important interactive roles (26). In this context, it is worth mentioning that high-cholesterol, high-fat diets downregulate hepatic ammonia-derived ureagenesis (27, 28), which is in line with our finding that high-cholesterol induced mtAQP8 downregulation.

In conclusion, our results suggest that cholesterol regulates transcriptionally human hepatocyte mtAQP8 expression via SREBPs, a novel finding that may be relevant to hepatic cholesterol homeostasis.

Acknowledgements

We thank Veronica Livore for her assistance in cell culture. This work was supported by Grant PICT 1217 (R.A. Marinelli) from Agencia Nacional de Promoción Científica y Tecnológica, Argentina.

References

[1] Calamita, G., Delporte, C., and Marinelli, R. A. (2015) Aquaporins in the liver, salivary glands and pancreas. In Aquaporins in Health and Disease: New Molecular Targets for Drug Discovery (Soveral, G., Nielsen, S., and Casini, A., eds). pp. 183–205, CRC Press, Boca Raton, FL.

[2] Calamita, G., Ferri, D., Gena, P., Liquori, G. E., Cavalier, A., et al. (2005) The inner mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water. J. Biol. Chem. 280, 17149–17153.

[3] Gena, P., Fanelli, E., Brenner, C., Svelto, M., and Calamita, G. (2009) News and views on mitochondrial water transport. Front. Biosci. 1, 352–361.

[4] Soria, L. R., Fanelli, E., Altamura, N., Svelto, M., Marinelli, R. A., et al. (2010) Aquaporin-8-facilitated mitochondrial ammonia transport. Biochem. Biophys. Res. Commun. 393, 217–221.

[5] Soria, L. R., Marrone, J., Calamita, G., and Marinelli, R. A. (2013) Ammonia detoxification via ureagenesis in rat hepatocytes involves mitochondrial aquaporin-8 channels. Hepatology 57, 2061–2071.

[6] Soria, L. R., Marrone, J., Molinas, S. M., Lehmann, G. L., Calamita, G., et al. (2014) Lipopolysaccharide impairs hepatocyte ureagenesis from ammonia: involvement of mitochondrial aquaporin-8. FEBS Lett. 588, 1686–1691.

[7] Molinas, S. M., Soria, L. R., Marrone, J., Danielli, M., Trumper, L., et al. (2015) Acidosis-induced downregulation of hepatocyte mitochondrial aquaporin-8 and ureagenesis from ammonia. Biochem. Cell Biol. 93, 417–420.

[8] Molinas, S. M., Trumper, L., and Marinelli, R. A. (2012) Mitochondrial aquaporin-8 in renal proximal tubule cells: evidence for a role in the response to metabolic acidosis. Am. J. Physiol. Renal Physiol. 303, F458–F466.

[9] Marchissio, M. J., Francés, D. E. A., Carnovale, C. E., and Marinelli, R. A. (2012) Mitochondrial aquaporin-8 knockdown in human hepatoma HepG2 cells causes ROS-induced mitochondrial depolarization and loss of viability. Toxicol. Appl. Pharmacol. 264, 246–254.

[10] Marchissio, M. J., Francés, D. E. A., Carnovale, C. E., and Marinelli, R. A. (2014) Evidence for necrosis, but not apoptosis, in human hepatoma cells with knockdown of mitochondrial aquaporin-8. Apoptosis 19, 851–859.

[11] Chauvigné, F., Boj, M., Finn, R. N., and Cerdà, J. (2015) Mitochondrial aquaporin-8-mediated hydrogen peroxide transport is essential for teleost spermatozoon motility. Sci. Rep. 5, 7789.

[12] DeBose, Y. J., and Boyd, R. A. (2011) Regulation of cholesterol and fatty acid synthesis, Cold Spring Harbor Perspect. Biol. 3, 1–13.

[13] Vergnes, L., Phan, J., Strauss, M., Tafuri, S., and Reue, K. (2003) Cholesterol and cholate components of an atherogenic diet induce distinct stages of hepatic inflammatory gene expression. J. Biol. Chem. 278, 42774–42784.

[14] Maxwell, K. N., Soccio, R. E., Duncan, E. M., Sehayek, E., and Breslow, J. L. (2003) Novel putative SREBP and LXR target genes identified by microarray analysis in liver of cholesterol-fed mice. J. Lipid Res. 44, 2109–2119.

[15] Williams, E. G., Wu, Y., Jha, P., Dubuis, S., Blattmann, P., et al. (2016) Systems proteomics of liver mitochondria function. Science 352, aad0189.

[16] Han, J., Hajjar, D. P., Tauras, J. M., and Nicholson, A. C. (1999) Cellular cholesterol regulates expression of the macrophage type B scavenger receptor, CD36. J. Lipid Res. 40, 830–838.

[17] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

[18] Folch, J., Lees, M., and Sloan-Stanley, G. H. (1956) A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

[19] Horton, J. D., Shah, N. A., Warrington, J. A., Anderson, N. N., Park, S. W., et al. (2003) Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc. Natl. Acad. Sci. USA 100, 12027–12032.

[20] Giudetti, A. M., Damiano, F., Gnoni, G. V., and Siculella, L. (2013) Low level of hydrogen peroxide induces lipid synthesis in BRL-3A cells through a CAP-independent SREBP-1a activation. Int. J. Biochem. Cell Biol. 45, 1419–1426.

[21] Pomytkin, I. A. (2012) H_2O_2 signalling pathway: a possible bridge between insulin receptor and mitochondria. Curr. Neuropharmacol. 10, 311–320.

[22] Finkel, T. (2012) Signal transduction by mitochondrial oxidants. J. Biol. Chem. 287, 4434–4440.

[23] Sies, H. (2014) Role of metabolic H_2O_2 generation: redox signaling and oxidative stress. J. Biol. Chem. 289, 8735–8741.

[24] Vieceli Dalla Sega, F., Zambonin, L., Fiorentini, D., Rizzo, B., Caliceti, C., et al. (2014) Specific aquaporins facilitate Nox-produced hydrogen peroxide transport through plasma membrane in leukaemia cells. Biochim. Biophys. Acta 1843, 806–814.

[25] Bertolotti, M., Farinelli, G., Galli, M., Aiuti, A., and Sitia, R. (2016) AQP8 transports NOX2-generated H_2O_2 across the plasma membrane to promote signaling in B cells. J. Leukoc. Biol. 100, 1071–1079.

[26] Madiraju, A. K., Alves, T., Zhao, X., Cline, G. W., Zhang, D., et al. (2016) Argininosuccinate synthetase regulates hepatic AMPK linking protein catabolism and ureagenesis to hepatic lipid metabolism. Proc. Natl. Acad. Sci. USA 113, 3423–3430.

[27] Barber, T., Viña, J. R., Viña, J., and Cabo, J. (1985) Decreased urea synthesis in cafeteria-diet-induced obesity in the rat. Biochem. J. 230, 675–681.

[28] Thomsen, K. L., Grønbæk, H., Glavind, E., Hebbard, L., Jessen, N., et al. (2014) Experimental nonalcoholic steatohepatitis compromises ureagenesis, an essential hepatic metabolic function. Am. J. Physiol. Gastrointest. Liver Physiol. 307, 295–301.