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Conflicts of interest
The authors disclose the following: A. J. Thompson is an Advisory board member for Gilead Sciences, Abbvie, Bristol-Myers Squibb (BMS), and Merck; a speaker for Gilead Sciences, Abbvie, Merck, BMS, Roche Diagnostics; and receives research/grant support (to institution) from Gilead, Abbvie. J. Howell discloses no conflicts.

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2017.03.025

Gallstones: Bad Company for the Steatotic Liver

See "Activation of the hypoxia inducible factor 1α subunit pathway in steatotic liver contributes to formation of cholesterol gallstones," by Asai Y, Yamada T, Tsukita S, et al, on page 1521.

Gallstones are very frequent worldwide with a prevalence ranging from 10% to 15% in Western countries to <5% in Africa, with the geographic variations being associated with genetic and environmental factors.[1] Although asymptomatic in more than 80% of patients, gallstone disease incurs one of the highest health care costs among digestive diseases and hospitalization is frequent as a consequence of its complications.[1–3] In Western countries, cholesterol stones largely predominate, and exogenous and genetic risk factors have been carefully defined, including female sex, age, and number of pregnancies.[1,2] Several epidemiologic studies have definitively demonstrated an association between cholesterol gallstones and nonalcoholic fatty liver disease (NAFLD).[4] Cholesterol gallstones share common risk factors with NAFLD, including obesity, diabetes mellitus, hypertension, hyperlipidemia, insulin resistance, sedentary lifestyle, and the metabolic syndrome.[1–5] Other than being more prevalent, gallstones are also more symptomatic and complicated when they occur in conjunction with NAFLD[2,5] and, therefore, they are unwanted company. Much less clear are the pathogenetic mechanisms linking NAFLD and gallstones. In the different conditions leading to NAFLD, one or more of the following mechanisms could predominate: (i) hepatic cholesterol oversecretion in bile as consequence of insulin resistance,[6] (ii) supersaturated bile and rapid phase transition owing to increased concentrations of mucins or other pronucleating agents,[7] (iii) gallbladder hypomotility, which frequently occurs in diabetics or patients with obesity,[8] and (iv) intestinal dysbiosis perturbing the cholehepatic bile salt (BS) circulation.[9] The article by Asai et al[10] in this issue of *Gastroenterology* proposes a novel and intriguing pathogenetic mechanism linking liver steatosis with gallstones. Indeed, the authors[10] propose that, in steatotic livers, hypoxia up-regulates the expression of hypoxia-inducible factor 1 alpha subunit (HIF1A), which reduces the expression of aquaporin (AQP)-8 and concentrates biliary lipids by suppressing water secretion from hepatocytes. In addition, inflammation and mucin deposition in the gallbladder wall, associated with experimental liver steatosis, was reduced in iHIF knockout mice. An effect of HIF1A knockdown was that gallstone formation was markedly decreased. In sum, this article is suggesting that steatotic hepatocytes secreted more concentrated bile on the basis of suppressed water secretion and this significantly affects gallbladder wall integrity and function, thus favoring gallstone formation.

How can more concentrated bile favor gallstone formation? Pioneering studies in the field have clearly demonstrated the importance of bile concentration for nucleation time and cholesterol saturation index.[11,12] Indeed, nucleation time is shortened if bile is concentrated whereas, conversely, nucleation time is prolonged by serial in vitro dilution of bile.[11] In addition, more concentrated bile may affect gallbladder motility by increasing the movement of biliary compounds into gallbladder epithelial cells, by increasing the permeability of the gallbladder epithelium to cholesterol, and by favoring its accumulation in muscle membranes, thus reducing contraction.[13] Increased concentration of secondary BS may alter phospholipid acyl groups and, therefore, cholesterol solubility within the micelle/vesicle.[13] Finally, higher BS concentrations reaching the gut lumen may perturb the microbiota and, as a consequence, the enterohepatic BS circulation.[9]

An emerging novel concept is that steatotic, hypoxic hepatocytes (mainly in the centrilobular zone) secrete less water in bile as a consequence of HIF1A-mediated suppression of AQPs (specifically AQP8), a family of integral membrane proteins that facilitate osmotically induced water transport through cell membranes.[14] Bile secretion is an osmotically driven secretory process resulting from the flow of water into the canalicular lumen in response to osmotic gradients created by active solute excretion.[14] The current findings, therefore, open new scenarios in different and unexplored areas. How do water movement and cell volume regulatory mechanisms change in hepatocytes accumulating lipid droplets or in cells where lipid and glucose metabolism is changed? What are the key regulators of these adaptive mechanisms? The answers could lie within the HIF family of proteins, where HIF1A is the most well-established member.[15] HIFs act as key mediators of cellular adaptation to changes in oxygen tension.[15] Under hypoxic conditions, HIF1A up-regulates a series of genes, which enables cells to adapt to reduced oxygen availability; it is estimated that >800 genes are

direct HIF targets, including heme oxygenase-1, vascular endothelial growth factor, glucose transporters, and glycolytic enzymes.[15] Indeed, increased glycolysis during hypoxia is a crucial step needed to meet modified cell energy demands.[15] It is now known that HIF1A can be induced and activated also at physiological oxygen tensions in a mitogen-activated protein kinase-dependent manner.[15] Asai et al[10] suggest that HIF1A activation is a defensive and survival pathway for hypoxic hepatocytes. However, HIFs can be activated by proinflammatory cytokines, extracellular hyperosmolarity owing to high sodium intake, and also merely as a consequence of metabolic cellular changes.[15] It remains unclear how oxygen cell tension changes during the different facets of NAFLD and whether hypoxic hepatocytes reflect only certain degrees of oxidative stress, damage, and hepatic microcirculation impairment, but not simple steatosis; these are key areas of future research. However, independent of cell damage or hypoxia, HIF1A could be activated to modulate, as consequence of lipid accumulation or fatty liver–associated dysmetabolism, ion transport mechanisms involved in the regulation of cell size/volume and intracellular pH (pHi; Figure 1).[15–18] Indeed, in the first signs of hypoxia, the rapid intracellular accumulation of lactate and H^+ implies activation of pHi and cell volume regulatory ion transport mechanisms, including Na^+/H^+ exchange, that are vital for maintaining cell viability and represent steps where HIF1A could play a role.[15,16] Notably, the accumulation of lactate and H^+, mainly owing to increased whole body rates of nonoxidative glycolysis, may occur in insulin-resistant cells independent of hypoxia.[19] Indeed, hyperlactemia induced by insulin

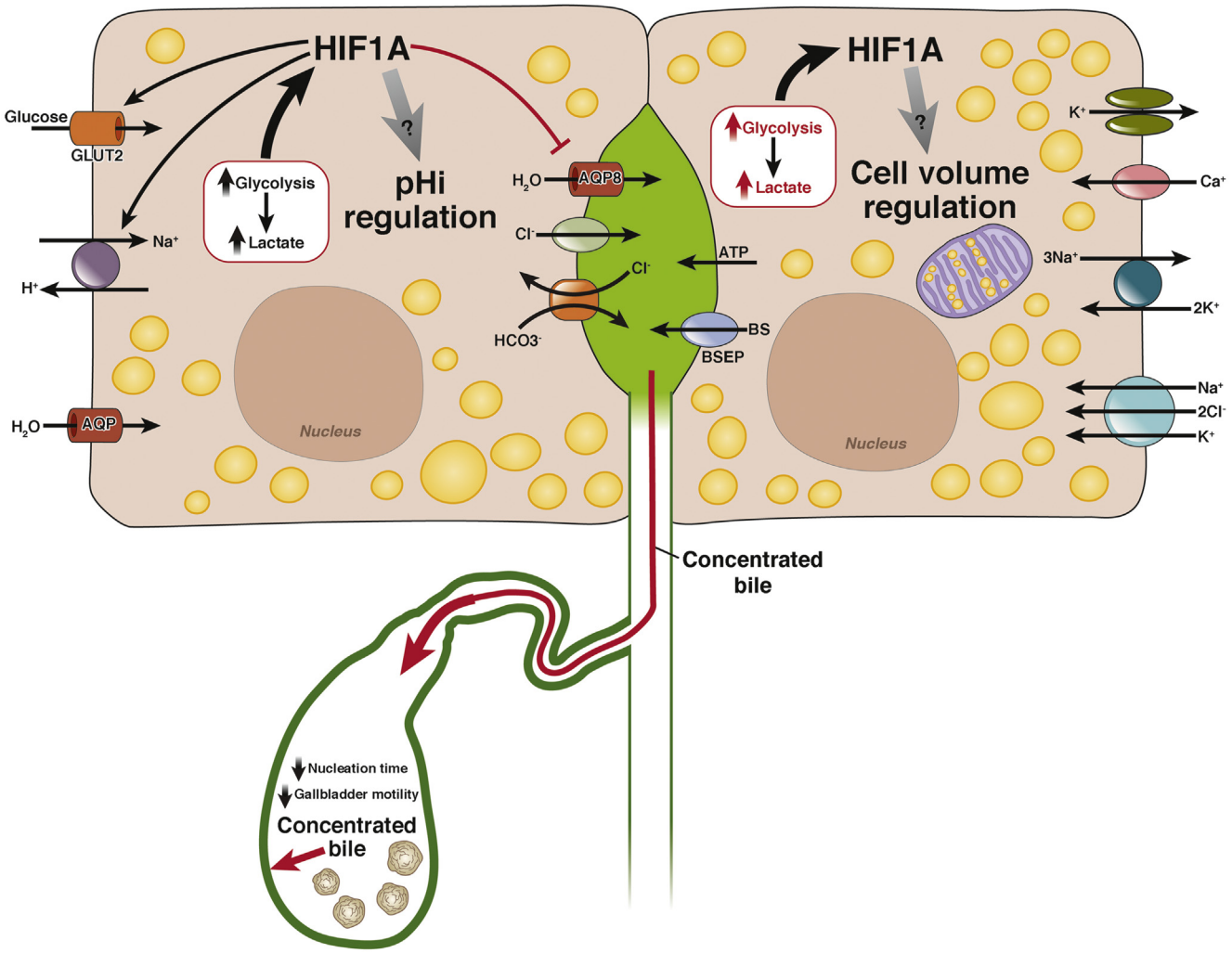

Figure 1. Steatotic hypoxic hepatocytes secrete more concentrated bile primarily owing to suppressed water secretion, where the inhibitory effects of hypoxia-inducible factor 1 alpha subunit (HIF1A) on aquaporin 8 (AQP8) play a role. This significantly affects gallbladder wall integrity and function, thus favoring gallstone formation. As an alternative mechanism, secretion of concentrated bile may simply reflect the dysmetabolic changes occurring in insulin-resistant hepatocytes, where water consumption is enhanced by metabolic needs and water exchange with extracellular spaces is adapted to intracellular osmolarity; at low oxygen tension, all these mechanisms could be amplified. HIF1A could also modulate ion transport mechanisms involved in the regulation of intracellular pH (pHi) and of cell volume, which are activated by intracellular hyperlactemia.

resistance may modulate HIF1A activity[20] being associated with profound changes in intracellular glucose metabolism, including decreased glycogen synthesis, increased nonoxidative glycolysis, and impaired glucose oxidative metabolism.[19] The hypothesis that increased levels of lactate would be able to activate pHi and cell volume regulatory ion transport mechanisms, in addition and irrespective of hypoxia, and the exact involvement of HIFs deserves special attention.

In summary, secretion of concentrated bile may simply reflect dysmetabolic changes occurring in steatotic hepatocytes where water consumption is enhanced by metabolic needs and water exchange with extracellular spaces is adapted to the intracellular osmolarity. At low oxygen tension, all these mechanisms could be amplified.

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Acknowledgments
The author thanks Prof. Raffaella Buzzetti (Chief of Diabetes Center, Sapienza University of Rome, Italy) and Prof. Stefano Ginanni-Corradini (Gastroenterology Division, Sapienza University of Rome, Italy) for the critical discussion on the content of the present document.

Conflicts of interest
The author discloses no conflicts.

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0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2017.03.020