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Relative contributions of L-FABP, SCP-2/SCP-x, or both to hepatic biliary phenotype of female mice

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ABSTRACT

Both sterol carrier protein-2/sterol carrier protein-x (SCP-2/SCP-x) and liver fatty acid binding protein (L-FABP) have been proposed to function in hepatobiliary bile acid metabolism/accumulation. To begin to address this issue, the impact of ablating L-FABP (LKO) or SCP-2/SCP-x (DKO) individually or both together (TKO) was examined in female mice. Biliary bile acid levels were decreased in LKO, DKO, and TKO mice; however, hepatic bile acid concentration was decreased in LKO mice only. In contrast, biliary phospholipid level was decreased only in TKO mice, while biliary cholesterol levels were unaltered regardless of phenotype. The loss of either or both genes increased hepatic expression of the major bile acid synthetic enzymes (CYP7A1 and/or CYP27A1). Loss of L-FABP and/or SCP-2/SCP-x genes significantly altered the molecular composition of biliary bile acids, but not the proportion of conjugated/unconjugated bile acids or overall bile acid hydrophobicity index. These data suggested that L-FABP was more important in hepatic retention of bile acids, while SCP-2/SCP-x more broadly affected biliary bile acid and phospholipid levels.

1. Introduction

Bile acids are not only biological detergents facilitating intestinal fat and fat-soluble vitamin absorption, but are also key metabolic regulators of glucose, lipid, and energy homeostasis [1]. Yet, little is known about how these relatively hydrophobic molecules are transported between the various subcellular compartments involved in their synthesis (peroxisomes, endoplasmic reticulum, mitochondria) and secretion (bile canaliculus) [2–7]. In fact, unlike non-mammalian vertebrates the mammalian liver does not contain the liver bile acid binding protein (L-BABP), which directly facilitates uptake and intracellular bile acid transport [3,4]. Instead, studies performed *in vitro*, with cultured hepatocytes, and with gene targeted mice suggest potential roles for three other genes encoding cytosolic proteins that bind bile acids in the mammalian liver: liver fatty acid binding protein (L-FABP), glutathione *S*-transferase (GST), and 3α-hydroxysteroid dehydrogenase (3α–HSD) [8,9]. Although a functional role for GST and 3α-HSD in intracellular transport of bile acids remains to be demonstrated, both ligand binding *in vitro* and studies *in vivo* with L-FABP null mice suggest potential roles for L-FABP.

L-FABP is a universal bile acid binding protein characterized by a single bile acid binding site with affinities in the 0.6–7 μM range, with highest affinities for specific bile acids with high and low, but less so intermediate, hydrophobicity indices [10–17]. L-FABP is present at very high concentration in murine (2–6% of cytosolic protein; 200–400 μM) and human (7–10% of cytosolic protein; 700–1000 μM) liver [11,18]. In addition to acting as a cytosolic bile acid transporter, L-FABP binding bile acids such as glycolithocholic acid inhibits liver microsomal sulfation of glycolithocholic acid *in vitro*-suggesting potential impact of L-FABP on the activities of other cytosol exposed membrane enzymes in bile acid metabolism [19].

Studies *in vivo* with cultured primary hepatocytes and L-FABP gene-ablated mice further support a role for L-FABP in bile acid metabolism. By binding bile acids, L-FABP reduces the toxicity of

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Abbreviations

ABCG5 or G8	ATP-binding cassette transporter G5 or G8
ACAT-2	acyl-CoA cholesterol acyltransferase-2
B; BA	bile acid
C	cholesterol
CA	cholic acid
CE	cholesteryl ester
CSI	cholesterol saturation index
CYP7A1	cholesterol 7α-hydroxylase
CYP27A1	sterol 27-hydroxylase
DKO	SCP-2/SCP-x double null mouse
FXR	farnesoid × receptor
G-CA	glyco-cholic acid
HDL	high density lipoprotein
HI	hydrophobicity index
3α-HSD	3α-hydroxysteroid dehydrogenase
LCFA	long chain fatty acid
LDL	low density lipoprotein
L-FABP	liver fatty acid binding protein or FABP1
LKO	L-FABP null mouse
LXRα	liver × receptor α
α-MCA	α-muricholic acid
β-MCA	β-muricholic acid
MDR3	multidrug-resistance-3 P-glycoprotein
OATP1 or 2	organic anion transporting polypeptide 1 or 2
PL	phospholipid
PPARα, -β/δ, or -γ	peroxisome proliferator-activated receptor alpha, beta/delta, or gamma
QrtPCR	quantitative real-time polymerase chain reaction
SCP-2	sterol carrier protein-2
SCP-x	sterol carrier protein-x/peroxisomal thiolase 2
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SHP	short heterodimer partner
T-CA	tauro-cholic acid
T-CDCA	tauro-chenodeoxycholic acid
T-DCA	tauro-deoxycholic acid
TG	triglyceride
TKO	L-FABP/SCP-2/SCP-x triple null mouse
T-LCA	tauro-lithocholic acid
T-MCA	tauro-muricholic acid
T-UDCA	tauro-ursodeoxycholic acid
WT	wild-type C57BL/6NCr mouse

these potent detergent-like molecules [10,11,15,20]. In addition, L-FABP is thought to bind/transport bound bile acids or bile acid intermediates between sites of synthesis (peroxisomes, mitochondria, endoplasmic reticulum) and to the bile canaliculus for secretion [10,11,15,20]. Indeed, photoaffinity cross-linking studies show that L-FABP is essential for bile acid uptake and intracellular transport in rat hepatocytes [15]. Ablation of the L-FABP gene has been shown to significantly alter bile acid metabolism in male mice [21].

Finally, L-FABP, as well as the two products (SCP-2 and SCP-x, coded through alternate transcription sites) of the sterol carrier protein-2/sterol carrier protein-x gene (SCP-2/SCP-x), impact intracellular transport/targeting of cholesterol, the immediate precursor of bile acid synthesis. L-FABP [22,23] and SCP-2 [24–27] bind cholesterol with high affinity. SCP-2 stimulates liver microsomal cholesterol 7α-hydroxylase (rate limiting enzyme in hepatic bile acid synthesis) *in vitro* [28]. Likewise, SCP-x is the only known peroxisomal enzyme for cleaving the branched side chain of cholesterol, another key step in bile acid synthesis [28–32]. SCP-2 overexpression increases bile acid synthesis and biliary secretion in mice and in isolated rat and human hepatocytes [33,34]. Ablation of the SCP-2/SCP-x gene decreases bile acid synthesis and biliary bile acid secretion in mice [35–38]. SCP-2 and less so L-FABP also enhance intracellular transport of cholesterol to endoplasmic reticulum [39–42] and stimulate acyl-CoA cholesterol acyltransferse (ACAT) therein to form cholesteryl esters for storage/secretion [43–46]. The net effect of these opposing influences, i.e. facilitating bile acid synthesis vs potentially diverting cholesterol to storage as cholesteryl esters, on biliary bile acid levels is not clear.

Despite these advances, interpretation of studies with SCP-2/SCP-x gene-ablated mice has been complicated by concomitant upregulation [36,37,47] or downregulation [48] of liver fatty acid binding protein (L-FABP) as well as sex-differences in response. For example, mice exhibit sex-related differences in metabolism of branched-chain lipids [49], in hepatic regulation of cholesterol metabolism [50], in the hepatic lipid accumulation in mice lacking the L-FABP gene product only [22], as well as the response to a high-cholesterol diet in L-FABP gene-ablated mice [20,51] To better resolve the impact of these proteins on hepatobiliary bile acid metabolism in female mice, studies were undertaken comparing female mice singly ablated in L-FABP (LKO), singly ablated in SCP-2/SCP-x (DKO), or ablated in both L-FABP and SCP-2/SCP-x (TKO). The data herein demonstrate that L-FABP had a much greater impact on hepatic retention of bile acids while SCP-2/SCP-x more broadly affected biliary bile acid and phospholipid levels.

2. Experimental procedures

2.1. Materials

Liver homogenate protein concentration was determined using the Protein Assay Kit I (Cat # 500-0001, bovine gamma globulin) from Bio-Rad (Hercules, CA). Free cholesterol E (free cholesterol, C) and phospholipids (PL) were determined with diagnostic kits from Wako Diagnostics (Richmond, VA). Total bile acid (BA) was determined by a kit purchased from Diazyme Labs (Poway, CA). Bile acid standards (cholic acid, α-muricholic acid, β-muricholic acid, tauro-β-muricholic acid, tauro-lithocholic acid, tauro-ursodeoxycholic acid, tauro-cholic acid, tauro-chenodeoxycholic acid, and tauro-deoxycholic acid) were from Steraloids (Newport, RI). TaqMan® One-Step PCR Master Mix reagent kit and gene-specific assays for *Abcg5* (Mm01226965_m1), *Abcg8* (Mm00445977_m1), *Cyp7a1* (Mm00484152_m1), *Slc10a1* (NTCP, Mm01302718_m1), *Slco1a1* (OATP1A1, Mm01267414_m1), and *Slc22a7* (OATP2, Mm00460672_m1) were purchased from Applied Biosystems (Foster City, CA). Rabbit or goat polyclonal antibodies to mouse β-actin (sc-47778), BSEP (sc-17294), CYP27A1 (sc-14835), FXR (sc-13063), LXRα (sc-1201), and SHP (sc-15283) were from Santa Cruz Biotechnology (Dallas, TX). Rabbit polyclonal anti-mouse antibodies for ACAT-2 (ab66259), COX4 (ab16056), or MDR3 (ab71792) were purchased from Abcam (Cambridge, MA). Rabbit polyclonal antibody to mouse PPARα (PA1-822A) was from Pierce Antibody (Rockford, IL). Mouse monoclonal antibody against mouse GAPDH (MAB374) was from Millipore (Billerica, MA). Rabbit polyclonal antibody to 3α-HSD was purchased from US Biological (Peabody, MA). Alkaline phosphatase-conjugated goat polyclonal antibody to rabbit IgG (product # A3687) and rabbit polyclonal antibody to goat IgG (product # A4187) were from Sigma–Aldrich (St. Louis, MO).