Thematic Review Series: Bile Acids

Bile acids: the role of peroxisomes

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Abstract   It is well established that peroxisomes play a crucial role in de novo bile acid synthesis. Studies in patients with a peroxisomal disorder have been indispensable for the elucidation of the precise role of peroxisomes. Several peroxisomal disorders are associated with distinct bile acid abnormalities and each disorder has a characteristic pattern of abnormal bile acids that accumulate, which is often used for diagnostic purposes. The patients have also been important for determining the pathophysiological consequences of defects in bile acid biosynthesis.■ In this review, we will discuss all the peroxisomal steps involved in bile acid synthesis and the bile acid abnormalities in patients with peroxisomal disorders. We will show the results of bile acid measurements in several tissues from patients, including brain, and we will discuss the toxicity and the pathological effects of the abnormal bile acids.—Ferdinandusse, S., S. Denis, P. L. Faust, and R. J. A. Wanders. **Bile acids: the role of peroxisomes.** *J. Lipid Res.* 2009. **50:** 2139–2147.

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THE BILE ACID BIOSYNTHESIS PATHWAY

The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are formed from cholesterol by a sequence of enzymatic modifications involving several enzymes, multiple subcellular compartments, and two main pathways (see Ref. 1 for a detailed review on the bile acid biosynthesis pathway). The most important difference between the classic and alternative pathway is the order of reactions and the subcellular compartment where the first reaction takes place (endoplasmic reticulum vs. mitochon-

drion, respectively). The enzymes involved in the two pathways are the same except for those responsible for the hydroxylation of the steroid nucleus at the C-7 position. In the classic pathway, this reaction is performed by cholesterol 7α-hydroxylase (CYP7A1), whereas oxysterol 7α-hydroxylase (CYP7B1) is responsible for this hydroxylation in the alternative pathway. The biosynthesis pathway basically consists of modification of the steroid nucleus, oxidation of the sterol side chain, cleavage of the side chain, and finally, conjugation with an amino acid, either taurine or glycine. Microsomal and cytosolic enzymes modify the steroid nucleus. Oxidation of the sterol side chain occurs in the mitochondrion, and finally, both the cleavage of the side chain and subsequent conjugation take place in the peroxisome.

Oxidation of the sterol side chain results in formation of the C_{27}-bile acid intermediates $3\alpha,7\alpha$-dihydroxycholestanoic acid (DHCA) and $3\alpha,7\alpha,12\alpha$-trihydroxycholestanoic acid (THCA). DHCA is the precursor of the primary C_{24}-bile acid CDCA and, when hydroxylated at C-12, the precursor of CA. THCA is the precursor of CA. The C_{24}-bile acids are formed from the C_{27}-bile acid intermediates by peroxisomal β-oxidation of the side chain (**Fig. 1**). Before this side chain can be shortened by β-oxidation, it has to be activated to a CoA-thioester and the C_{27}-bile acyl-CoA has to be transported into the peroxisome. To date, two enzymes have been identified that are capable of activating the C_{27}-bile acid intermediates. The first enzyme is the bile acyl-CoA synthetase (BACS, also called bile acid CoA ligase), which is liver-specific and located at the endoplasmic reticulum. It accepts both C_{27}- and C_{24}-bile acids as substrates (2, 3). The second enzyme

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Abbreviations: AMACR, α-methylacyl-CoA racemase; BACS, bile acyl-CoA synthetase; BAAT, bile acyl-CoA: amino acid *N*-acyltransferase; BCOX, branched-chain acyl-CoA oxidase; CA, cholic acid; CDCA, chenodeoxycholic acid; DBP, D-bifunctional protein; DCA, deoxycholic acid; DHCA, dihydroxycholestanoic acid; FXR, farnesoid X receptor; SCPx, sterol carrier protein X; THCA, trihydroxycholestanoic acid; UDCA, ursodeoxycholic acid.
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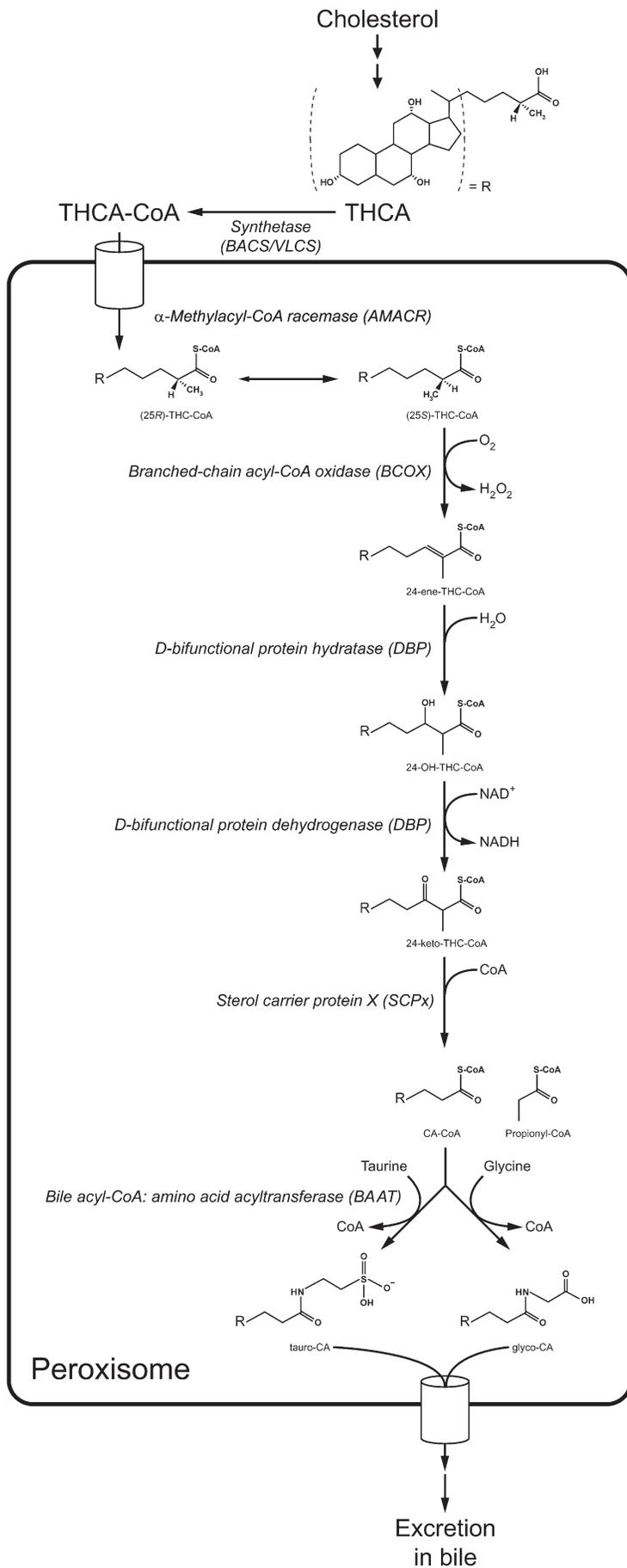
Fig. 1. Schematic representation of the peroxisomal steps involved in bile acid biosynthesis.

that has been shown to be capable of handling the C_{27}-bile acid intermediates in expression studies is the very long-chain acyl-CoA synthetase (2). It is expressed primarily in liver and kidney and is located in both the endoplasmic reticulum and in the peroxisome. In addition to the C_{27}-bile acid intermediates, the enzyme has been shown to handle C24:0, C16:0, phytanic and pristanic acid as substrates, but is not capable of activating the primary C_{24}-bile acids (2, 4). To date, formation of DHC-CoA and THC-CoA has only been demonstrated at the endoplasmic reticulum and not in peroxisomes (5, 6). The kinetic properties of the THC-CoA synthetase and choloyl-CoA synthetase activities at the endoplasmic reticulum have been shown to be different (6), which suggests that both BACS and very long-chain acyl-CoA synthetase are involved in the activation of the C_{27}- and C_{24}-bile acids at the endoplasmic reticulum. In agreement with a microsomal, and not peroxisomal, activation of the C_{27}-bile acid intermediates is the demonstration of ATP-dependent transport of THC-CoA into the peroxisome (7). THCA and THC-CoA were incubated with isolated rat liver peroxisomes and the products of peroxisomal β-oxidation were measured (7). The results of these experiments showed that THC-CoA is the preferred substrate for transport. However, it must be noted that peroxisomes isolated by differential centrifugation are leaky, which makes them not optimal for these kinds of experiments. For this reason, transport should ideally be studied by reconstitution of the transporter or peroxisomal membrane proteins in liposomes. The identity of the transporter for the C_{27}-bile acyl-CoA's is at this moment still unknown.

Once inside the peroxisome, the side chain of the C_{27}-bile acid intermediates will be shortened via β-oxidation (Fig. 1). First racemisation of the methyl-group at C-25 will occur by the enzyme α-methylacyl-CoA racemase (AMACR) (8, 9). This is necessary because the first enzyme of the β-oxidation system, branched-chain acyl-CoA oxidase (BCOX), only accepts (S)-isomers as substrate (10, 11), whereas cholesterol 27-hydroxylase (CYP27A1), which forms the carboxyl-group at the side chain in the mitochondrion, only produces the (25R)-isomers of the C_{27}-bile acid intermediates (12, 13). After racemisation, BCOX forms the 24-enoyl-CoA of THCA and DHCA (14). This is followed by a subsequent hydration and dehydrogenation reaction, both performed by D-bifunctional protein (DBP). DBP is also a stereospecific enzyme and only forms (24R)-OH (25R), -isomers, which it subsequently dehydrogenates into 24-keto-CoA-esters (15–18). Finally, sterol carrier protein X (SCPx) is responsible for the thiolytic cleavage into propionyl-CoA and CA-CoA/CDC-CoA (19–21). A detailed description of the different β-oxidation steps has been reviewed previously (22).

The final step in de novo bile acid synthesis is the conjugation/amidation of the C_{24}-bile acids to glycine or taurine. This reaction is performed by the enzyme bile acyl-CoA: amino acid N-acyltransferase (BAAT), which converts the bile acyl-CoAs into tauro- or glyco-bile acids, thereby releasing free CoA (23–25). This reaction occurs in the peroxisome, after which the conjugated C_{24}-bile ac-

ids are transported out of the peroxisome and subsequently out of the hepatocyte and into the bile. Recently, bile acid transport activity has been detected and characterized by reconstitution of peroxisomal membrane proteins into liposomes; however, the identity of the transporter remains unknown (26). Conjugation of bile acids not only involves newly synthesized bile acids but also bile acids that have been deconjugated in the intestine and recycled via enterohepatic circulation (2). The deconjugated primary and secondary bile acids are reactivated to CoA thioesters at the cytosolic side of the endoplasmic reticulum by BACS (2); however, the question remains as to where they are reconjugated. By now, the peroxisomal localization of BAAT has been firmly established (25, 27, 28) but there is a longstanding dispute in the literature about a possible dual localization of BAAT, both in the peroxisome and in the cytosol (29–32). A cytosolic localization would negate the need for recycled bile acids to be transported back into the peroxisome just to undergo conjugation. For this reason, further research is needed to firmly establish the precise localization of BAAT.

BILE ACID ABNORMALITIES IN PATIENTS WITH PEROXISOMAL DISORDERS

Investigation of the bile acid abnormalities in the various peroxisomal disorders has been of great value, not only for establishing that functional peroxisomes are necessary for bile acid biosynthesis, but also for determining which peroxisomal enzymes are involved in this pathway. Analysis of plasma from patients with a peroxisome deficiency disorder (i.e., Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease) reveals the presence of C_{27}-bile acid intermediates but also a C_{29}-dicarboxylic acid (33, 34). This dicarboxylic acid (3α,7α,12α-trihydroxy-27-carboxymethyl-5β-cholestane-26-oic acid) is thought to be formed by chain-elongation of THC-CoA (35, 36). The extent of the accumulation of bile acid intermediates has been shown to vary greatly and correlates with the extent to which peroxisomes are deficient (34). In urine of patients with a peroxisome deficiency disorder, the C_{27}-bile acid intermediates are also present, but predominantly as tetrahydroxy-5β-cholestanoic acid and sometimes even pentahydroxy-5β-cholestanoic acid (37–39). Hydroxylation of THCA (of the steroid nucleus mainly at C-1 and C-6 but also of the side chain at C-24, C-25 or C-26) is an important mechanism for increasing the polarity and thereby, the urinary excretion of the C_{27}-bile acid intermediates. In addition, bile alcohol glucuronides are present in urine, which are formed via an alternative pathway for bile acid biosynthesis not requiring the participation of peroxisomes, i.e., the microsomal 25-hydroxylase pathway. The C_{29}-dicarboxylic acid is poorly excreted and for this reason it is not always present in urine.

The most striking bile acid abnormality in AMACR deficiency is the presence of only the (25R)-isomer of the C_{27}-bile acid intermediates (40, 41). In contrast, in other

peroxisomal disorders with a defect in bile acid biosynthesis, both the (25R)- and (25S)-isomers accumulate. Just like in patients with a peroxisome deficiency disorder, many polyhydroxylated C_{27}-bile acids are present in urine (41).

To date, no patients with a deficiency of BCOX have been identified but the bile acid abnormalities will most likely resemble those of peroxisome deficiency disorders and AMACR deficiency, although the main accumulating isomer will be the (25S)-isomer in BCOX deficiency. The role of BCOX in the oxidation of bile acids has been established by in vitro studies with the purified enzyme (42, 43) and by the fact that patients with a deficiency of the other peroxisomal acyl-CoA oxidase (straight-chain acyl-CoA oxidase) do not have a bile acid biosynthesis defect (44).

The bile acid abnormalities in DBP deficiency are more complex because DBP displays two catalytic activities, resulting in multiple types of DBP deficiency. In addition, the other peroxisomal bifunctional enzyme, L-bifunctional protein, also displays activity with certain isomers of the C_{27}-bile acid intermediates. In all types of DBP deficiency, DHCA, THCA, and the direct substrate of DBP, the 24-enoyl of the C_{27}-bile acid intermediates are present in plasma (45, 46). When the hydratase activity is deficient (DBP type I and II deficiency, i.e., combined deficiency of the hydratase and dehydrogenase activity and isolated hydratase deficiency, respectively) (24S)-OH (25S), -THC-CoA is also present, being formed by L-bifunctional protein. This isomer is not a substrate for any of the two peroxisomal dehydrogenases and cannot be metabolized further. When only the dehydrogenase activity is deficient (DBP type III deficiency), (24S), -OH (25S), -THC-CoA is also present, but the main accumulating metabolite is (24R)-OH (25R), -THC-CoA, which is formed by the hydratase unit of DBP (45, 46). A small portion is converted into (24R)-OH (25S), -THC-CoA by AMACR, which cannot be metabolized further (47). In urine, the presence of the taurine conjugate of hydroxylated 24-ene-THCA and dihydroxylated 24-ene-THCA is characteristic for patients with DBP deficiency (22). As for peroxisome deficiency disorders, the extent of the accumulation of the C_{27}-bile acid intermediates varies between patients and this seems to correlate with the extent of the deficiency of DBP. Bile acid abnormalities are not found in plasma in 26% of DBP-deficient patients (48).

In plasma of the only SCPx-deficient patient identified to date, only trace amounts of DHCA and THCA were present (49). However, large amounts of bile alcohol glucuronides were identified in urine. In addition, products formed by hydroxylation and decarboxylation of the SCPx substrate $3\alpha,7\alpha,12\alpha$-trihydroxy-24-keto-cholestanoyl-CoA were present, namely pentahydroxy-27-nor-5β-cholestane-24-one (m/z 611) and hexahydroxy-27-nor-5β-cholestane-24-one glucuronides (m/z 627).

Although the bile acid abnormalities in plasma and urine of patients with a peroxisomal fatty acid oxidation disorder have been well characterized and can be used as diagnostic markers, bile acids in tissues of these patients have not been well studied. It is important to gain insight into the accumulation of bile acids and their intermediates in tissues of patients because bile acid toxicity is postulated to play an important role in the underlying pathological mechanisms of these disorders, not only in liver but also in other organs such as the brain. For this reason, we analyzed bile acids by HPLC-negative ion electrospray tandem mass spectrometry (50, 51) in several tissues of patients with different peroxisomal disorders. The results of these analyses have not been published previously. We studied liver, brain, kidney, heart, spleen, lung, pancreas, and bile of patients with a peroxisome deficiency disorder (including patients with the severe Zellweger phenotype and patients with the milder neonatal adrenoleukodystrophy phenotype), patients with a deficiency of DBP (including patients with a DBP deficiency type I and type III) and one patient with an AMACR deficiency. The results of the bile acid measurements in liver are presented in **Table 1** and **Fig. 2A**. The main accumulating bile acids in the liver of patients with a peroxisome deficiency disorder were t-THCA and THCA. Total C_{27}-bile acid intermediates were significantly increased and total C_{24}-bile acids significantly decreased compared with control livers. This resulted in clearly elevated C_{27}/C_{24}-bile acid ratios in all patient livers. The percentage of conjugated C_{27}-bile acid intermediates was only 51.5% compared with 96.4% for the C_{24}-bile acids. This shows that C_{27}-bile acid intermediates are poor substrates for the conjugating enzyme BAAT. The fact that the C_{24}-bile acids were almost fully conjugated (96.4% compared with 99% in control livers) suggests that BAAT could very well have a dual localization, not only in peroxisomes but also in the cytosol, or at least that BAAT is normally active and stable in the cytosol when peroxisomes are deficient. Both in the livers with a peroxisome deficiency and in the AMACR-deficient liver, the C_{29}-dicarboxylic acid ($3\alpha,7\alpha,12\alpha$-trihydroxy-27-carboxymethyl-5β-cholestane-26-oic acid) was present, whereas it was not detected in DBP-deficient livers. In livers of patients with a DBP deficiency, the main accumulating C_{27}-bile acid intermediates were OH-THCA, t-THCA:1, and THCA, whereas those in the liver from the AMACR-deficient patient were t-THCA and THCA. The bile acid measurements showed that two DBP-deficient livers and the AMACR-deficient liver were cholestatic but this did not change the fact that the C_{27}/C_{24}-bile acid ratios were elevated in all patient livers. As in the peroxisome-deficient livers, the C_{27}-bile acid intermediates were partly conjugated, confirming that BAAT is poorly active with bile acids with an increased side chain length compared with C_{24}-bile acids (29, 52).

Bile of one patient with a peroxisome deficiency and the AMACR-deficient patient was analyzed in addition to the liver sample. For both patients, the C_{27}/C_{24}-bile acid ratio was higher in liver than in bile (2.58 vs. 1.66 in the peroxisome-deficient patient) and the percentage of conjugated C_{27}-bile acid intermediates was higher in bile than in liver (81% vs. 66% in the same patient). This suggests that C_{27}-bile acid intermediates are less efficiently transported across the canalicular membrane and that conju-

TABLE 1. Bile acids in liver of control subjects, patients with a peroxisome deficiency disorder (PDD), and patients with a D-bifunctional protein (DBP) deficiency

		Controls		PDD		DBP		AMACR
		n = 16		n = 11		n = 5		n = 1
	m/z	Mean	Range	Mean	Range	Mean	Range	
C29DCA	507	0.00	0.00-0.02	1.71**	0.37-4.66	0.00	0.00-0.00	0.19
t-OH-THCA	572	0.03	0.00-0.14	2.99**	0.54-9.78	1.48*	0.00-5.35	1.28
t-OH-THCA:1	570	0.03	0.00-0.53	0.06	0.00-0.29	3.45**	0.02-8.78	0.37
t-THCA	556	0.36	0.05-2.26	26.51**	4.54-95.43	4.80*	0.38-19.61	74.14
t-THCA:1	554	0.01	0.00-0.11	0.74**	0.06-2.04	10.85**	0.82-32.68	13.85
t-DHCA	540	0.02	0.00-0.08	3.40**	0.39-8.87	0.53*	0.05-2.22	4.66
t-DHCA:1	538	0.01	0.00-0.17	0.10*	0.00-0.47	1.32**	0.19-4.56	0.13
t-CA	514	9.97	1.66-33.00	4.89	0.41-9.30	37.38*	0.91-100.18	21.05
t-UDCA	498	0.30	0.00-1.17	0.04*	0.00-0.15	0.92*	0.00-1.90	1.90
t- CDCA/DCA	498	11.64	3.16-24.41	3.26**	0.94-4.98	14.23	1.27-30.81	44.43
g-THCA	506	0.15	0.00-0.33	0.80**	0.04-2.45	0.47*	0.02-1.42	9.93
g-CA	464	10.08	1.45-41.30	3.67	0.39-10.01	37.08*	2.31-96.25	52.96
g-UDCA	448	0.65	0.00-2.23	0.14*	0.00-0.81	1.40	0.02-3.77	6.34
g-CDCA/DCA	448	18.47	2.00-48.50	4.26**	0.86-18.07	20.62	1.26-48.68	74.16
OH-THCA	465	0.31	0.00-1.89	6.99**	1.20-19.73	35.06*	8.41-126.43	68.41
OH-THCA:1	463	0.49	0.14-1.71	1.30**	0.60-2.63	5.58*	2.13-16.01	22.24
THCA	449	0.09	0.00-0.95	14.56**	5.69-37.11	9.00**	5.13-12.27	88.82
THCA:1	447	0.36	0.00-0.62	1.76**	0.70-4.04	6.13**	2.76-13.87	10.54
DHCA	433	0.03	0.00-0.12	3.74**	0.46-8.15	3.15**	1.25-7.66	0.43
DHCA:1	431	0.15	0.01-0.55	0.57**	0.15-1.16	0.54*	0.21-1.29	0.06
CA	407	0.41	0.07-2.22	0.29	0.14-0.75	1.99	0.31-7.78	4.89
UDCA	391	0.06	0.01-0.20	0.07	0.00-0.31	0.36	0.00-1.74	0.00
CDCA/DCA	391	0.07	0.01-0.21	0.12	0.05-0.30	0.93	0.03-4.09	9.07
Total C27-BA		2.04	0.53-7.63	63.52**	18.54-178.36	82.36**	32.37-252.15	294.86
Total C24-BA		51.97	15.46-80.62	16.74**	6.38-34.40	121.66*	9.89-317.55	216.46
Total BA		54.01	15.98-88.30	80.25**	24.91-190.01	204.02**	42.97-444.28	444.28
C27/C24-BA ratio		0.04	0.01-0.1	4.69**	1.57-15.31	1.45**	0.16-3.35	1.36

Bile acids are expressed as nmol/g wet weight. BA, bile acids. CDCA and DCA cannot be distinguished with the method used for bile acid measurement. * $P < 0.05$; ** $P < 0.005$ (t-test).

gated C_{27}-bile acid intermediates are the preferred substrates for this transport. At the moment, it is not known which transporters in the canalicular membrane are specifically involved in this process.

In the analyzed peripheral tissues (kidney, heart, spleen, lung, pancreas) of the different peroxisomal patients, the C_{27}-bile acid intermediates clearly accumulated and the C_{27}/C_{24}-bile acid ratio was comparable to the one found in liver (Fig. 2B). The pattern of accumulating metabolites was also comparable to liver except that the main accumulating C_{27}-bile acid intermediates were mostly unconjugated (resulting in a percentage of conjugated C_{27}-bile acid intermediates of around 20-25% in the different tissues), which again suggests that the conjugated C_{27}-bile acid intermediates are more efficiently excreted into the bile.

In brains of patients with a peroxisome deficiency disorder, there were clear bile acid abnormalities (**Table 2** and Fig. 2C). C_{27}-bile acid intermediates were increased compared with control brains and C_{24}-bile acids were decreased, resulting in a significantly increased C_{27}/C_{24}-bile acid ratio. The accumulating C_{27}-bile acid intermediates were THCA, DHCA, and OH-THCA, all unconjugated, consistent with a less efficient passage of conjugated C_{27}-bile acid intermediates of the blood-brain barrier. The C_{29}-dicarboxylic acid was also detectable in brain. The brains of DBP-deficient patients were much harder to distinguish from control brains based on the bile acid measurements. There were no significant differences for total C_{27}-bile acid

intermediates or C_{24}-bile acids levels. The only bile acids that were elevated were OH-THCA and DHCA. For two patients with a peroxisome deficiency disorder, two DBP-deficient patients, and four control subjects, tissue from different brain areas was analyzed, including frontal white and gray matter, parietal white and gray matter, and extra frontal and parietal cortex. No significant difference between the bile acid content in the different brain areas was detected.

TOXICITY OF C_{27}-BILE ACID INTERMEDIATES AND THE PATHOLOGICAL CONSEQUENCES OF A BILE ACID BIOSYNTHESIS DEFECT AT THE LEVEL OF THE PEROXISOME

The bile acid abnormalities in patients with a peroxisomal disorder have been shown to contribute to the liver disease associated with these disorders (53, 54) and also have been hypothesized to have an effect on the developing nervous system (55). The C_{27}-bile acid intermediates are more hydrophobic than their C_{24}-products, and as shown by the bile acid measurements in tissues of patients described above, they are less efficiently conjugated (augmenting to their hydrophobicity) and excreted into the bile. These factors will contribute to the retention of these intermediates in the liver, cholestasis, and, consequently, to hepatic injury in these patients. In addition, the primary bile acid deficiency decreases the intestinal dietary lipid
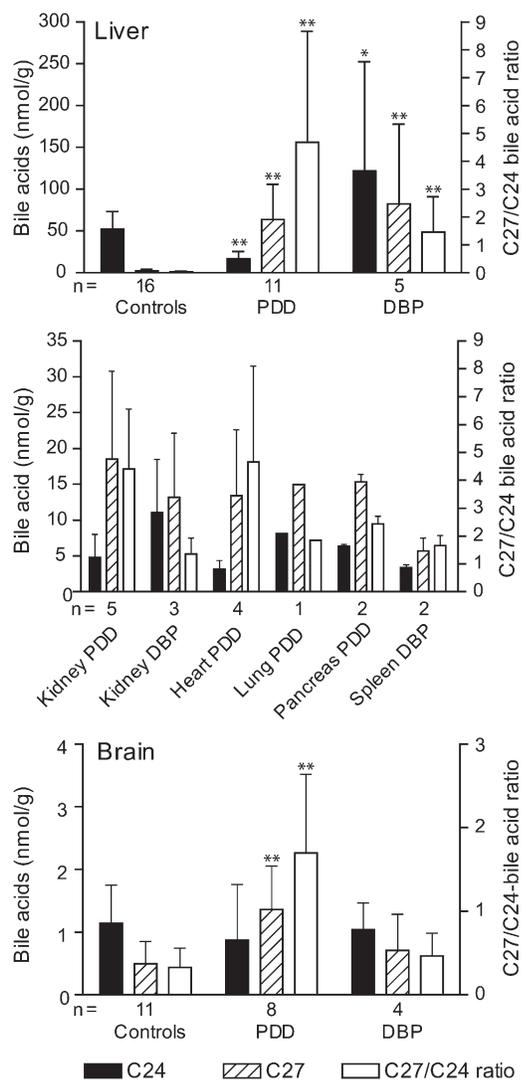
Fig. 2. Bile acids measured in tissues of control subjects, patients with a peroxisome deficiency disorder (PDD), and patients with a D-bifunctional protein (DBP) deficiency. C_{24}-bile acids are the sum of conjugated and unconjugated CA, CDCA, ursodeoxycholic acid, and deoxycholic acid. C_{27}-bile acids are the sum of conjugated and unconjugated THCA, THCA:1, OH-THCA, OH-THCA:1, DHCA, DHCA:1, and C29DCA. Data represent the mean ± SD, all measurements were performed in duplicate. The number (n) of patients analyzed per tissue is indicated. *, $P < 0.05$; ** $P < 0.005$ (t-test).

and lipid soluble vitamin absorption. Furthermore, we have shown that in brains of peroxisome deficiency patients, C_{27}-bile acid intermediates accumulate and that these were almost exclusively unconjugated, increasing their potential toxic effect. However, because peroxisomes are involved in multiple metabolic pathways, interpretation of the pathological consequences of the bile acid synthesis defect in patients is complex. For this reason, we have recently performed an in vitro study on the toxicity of the conjugated and unconjugated C_{27}-bile acid intermediates. The effects on cell viability, mitochondrial respiratory chain function, and the production of oxygen radicals were examined in a rat hepatoma cell line and compared with the effect on these parameters of the mature C_{24}-bile acids (56). These studies showed that C_{27}-bile acids intermedi-

ates are more cytotoxic than mature C_{24}-bile acids, with DHCA being clearly the most cytotoxic bile acid. In addition to the membrane-disruptive effects of bile acids due to their detergent properties, other mechanisms, like the induction of mitochondrial dysfunction, have been shown to be involved in the toxicity of bile acids (57). For this reason, the effect on the mitochondrial respiratory chain function was studied (56). It was shown that the different bile acids caused a dose-dependent decrease in ATP synthesis by isolated mitochondria oxidizing malate and glutamate (the order of potency of inhibition was: DHCA>THCA>CDCA>g-THCA>g-CDCA>t-THCA>CA). The unconjugated bile acids acted predominantly as uncouplers of the oxidative phophorylation whereas the conjugated bile acids (t-THCA, g-THCA and g-CDCA) primarily inhibited the respiratory chain. By inhibiting the respiratory chain, the conjugated bile acids t-THCA, g-THCA, and g-CDCA enhanced reactive oxygen species (ROS) production at the level of complex III. Increased ROS generation leads to depletion of the antioxidant defenses and can eventually lead to cell death. Interestingly, in the very first report describing Zellweger syndrome, brain and liver mitochondria from a patient showed strongly reduced respiration with malate and succinate as substrates (58). In addition, in a mouse model with hepatocyte-selective elimination of peroxisomes, severely reduced activities of complex I, III, and V were measured (59) and in a Pex5 knockout mouse, a model for Zellweger syndrome, reduction in the activities of complex I and V were observed (60), supporting the physiological relevance of the in vitro findings. The reduction of the activities of the respiratory chain complexes was accompanied by alterations of the mitochondrial inner membrane structure, which has also been observed in Zellweger patients (58, 61, 62).

Because the C_{27}-bile acid intermediates are especially cytotoxic, reducing their levels could be beneficial not only for the liver disease in peroxisomal patients but also to prevent potential injury to other organs by circulating C_{27}-bile acid intermediates. C_{27}-bile acid levels can be reduced by treatment with C_{24}-bile acids, which downregulate bile acid biosynthesis via activation of the nuclear receptor farnesoid X receptor (FXR), resulting in reduced transcriptional activation of CYP7A1, the rate-limiting enzyme in bile acid biosynthesis. In addition, bile acid therapy will increase bile flow by replenishing the decreased levels of C_{24}-bile acids and it will increase the intraluminal bile acid concentration, thereby facilitating the absorption of fats and fat soluble vitamins. Oral bile acid therapy has been tried with variable success in peroxisomal disorders (41, 63–65). The first report of such a bile acid treatment was in a 6-month-old Zellweger patient who showed significant improvement of biochemical indices of liver function and a histological improvement in the extent of inflammation and bile duct proliferation and disappearance of canalicular plugs (64). Steatorrhea improved and was accompanied by an improvement in growth. Plasma and urinary C_{27}-bile acid intermediates decreased with treatment. Despite the observed improvements, the patient died at one year of age. Also, in subsequent studies it has been

TABLE 2. Bile acids in brain of control subjects, patients with a peroxisome deficiency disorder (PDD) and patients with a D-bifunctional protein (DBP) deficiency

	m/z	Controls n = 11		PBD n = 8		DBP n = 4	
		Mean	Range	Mean	Range	Mean	Range
C29DCA	507	0.01	0.00-0.03	0.18**	0.01-0.38	0.00	0.00-0.00
t-CA	514	0.25	0.07-1.18	0.22	0.05-1.04	0.15	0.08-0.19
t- CDCA/DCA	498	0.18	0.07-0.50	0.11	0.02-0.21	0.15	0.08-0.19
g-CA	464	0.16	0.03-0.36	0.18	0.00-0.83	0.22	0.09-0.37
g-CDCA/DCA	448	0.26	0.04-0.43	0.14*	0.00-0.18	0.26	0.10-0.39
OH-THCA	465	0.05	0.00-0.22	0.24**	0.11-0.43	0.13	0.06-0.22
OH-THCA:1	463	0.15	0.00-0.40	0.14	0.03-0.38	0.15	0.00-0.38
THCA	449	0.05	0.00-0.23	0.50**	0.07-1.29	0.06	0.00-0.16
DHCA	433	0.02	0.00-0.10	0.20*	0.00-0.53	0.06*	0.04-0.09
CA	407	0.12	0.06-0.25	0.10	0.05-0.27	0.11	0.08-0.18
CDCA/DCA	391	0.14	0.03-0.28	0.04**	0.01-0.09	0.09	0.05-0.14
Total C27-BA		0.51	0.04-0.98	1.36**	0.40-2.49	0.71	0.12-1.40
Total C24-BA		1.20	0.39-2.77	0.87	0.34-3.06	1.04	0.49-1.51
Total BA		1.71	0.78-3.75	2.23	1.35-4.67	1.75	0.61-3.02
C27/C24-BA ratio		0.44	0.05-1.00	2.27**	0.47-3.83	0.62	0.27-0.94

Bile acids are expressed as nmol/g wet weight. BA, bile acids. CDCA and DCA cannot be distinguished with the method used for bile acid measurement. * $P < 0.05$; ** $P < 0.005$ (t-test).

shown that patients with Zellweger syndrome are too severely affected by the multiple metabolic derangements for bile acid therapy to have a positive effect on the outcome of the disease (63, 65). However, in the *Pex*2 knockout mouse, another model for Zellweger syndrome, positive effects of bile acid therapy were observed (55, 66). *Pex*2 knockout mice were fed a mixture of CA and ursodeoxycholic acid (UDCA), which resulted in a significant improvement in postnatal survival. Increased body fat deposition was observed and improvement of the intestinal malabsorption. Compared with untreated mutant mice, bile acid treated *Pex2* knockout mice were more active and less hypotonic but problems with balance and spasticity persisted. Interestingly, there was a significant improvement in the extent of dendritic arborization of cerebellar Purkinje cells (55). Thus, bile acid therapy may improve some aspects of central nervous system development. In the liver, bile acid treatment alleviated intrahepatic cholestasis and prevented the development of severe steatohepatitis. However, the therapy exacerbated the degree of hepatic steatosis and worsened the already severe mitochondrial and cellular damage in the liver (66). These studies showed that peroxisome-deficient hepatocytes are particularly sensitive to bile acid toxicity, indicating that bile acid therapy will be more beneficial in patients with a relatively mild peroxisome biogenesis disorder (neonatal adrenoleukodystrophy or infantile Refsum disease) or patients with a single enzyme defect (AMACR, DBP, SCPx deficiency). Indeed, a child with AMACR deficiency has been reported to respond well to oral bile acid therapy for 7 years (41). On treatment, the C_{27}-bile acids intermediates are virtually absent in urine, her liver enzymes have remained normal, and her fat-soluble vitamins have remained normal without supplementation. As an alternative to oral bile acid therapy, treatment with an artificial FXR ligand should be considered. These artificial FXR ligands are not harmful for the hepatocyte but they will downregu-

late the synthesis of the toxic C_{27}-bile acid intermediates (67).

CONCLUDING REMARKS

The importance of proper peroxisomal function for the biosynthesis of bile acids has been firmly established. The peroxisomal enzymes involved in bile acid biosynthesis have all been identified. Only the transporters required for the transport of the C_{27}-bile acid intermediates into the peroxisome and for the conjugated C_{24}-bile acids out of the peroxisome remain unidentified. In addition, it is not clear yet whether deconjugated and recycled C_{24}-bile acids need to reenter the peroxisome for reconjugation or whether this occurs in the cytosol. The bile acid abnormalities in patients with peroxisomal disorders have been well characterized and evidently play a role in the pathology of these disorders. The C_{27}-bile acid intermediates have been shown to be more cytotoxic than the C_{24}-bile acids, and the finding of primarily unconjugated C_{27}-bile acid intermediates in brains from children with Zellweger syndrome suggests that the bile acid synthesis defect in these patients could also play a role in central nervous system pathology. Unfortunately, the clinical presentation of children with classical Zellweger syndrome is too severe due to the multiple metabolic derangements for a true positive effect of oral bile acid therapy in these patients but patients with a mild presentation (i.e., infantile Refsum disease) or a single enzyme deficiency could very well benefit from such a therapy or treatment with an FXR ligand to reduce the toxic C_{27}-bile acid intermediates.

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