Review

Microbial Hydroxysteroid Dehydrogenases: From Alpha to Omega

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Abstract: Bile acids (BAs) and glucocorticoids are steroid hormones derived from cholesterol that are important signaling molecules in humans and other vertebrates. Hydroxysteroid dehydrogenases (HSDHs) are encoded both by the host and by their resident gut microbiota, and they reversibly convert steroid hydroxyl groups to keto groups. Pairs of HSDHs can reversibly epimerize steroids from α-hydroxy conformations to β-hydroxy, or β-hydroxy to ω-hydroxy in the case of ω-muricholic acid. These reactions often result in products with drastically different physicochemical properties than their precursors, which can result in steroids being activators or inhibitors of host receptors, can affect solubility in fecal water, and can modulate toxicity. Microbial HSDHs modulate sterols associated with diseases such as colorectal cancer, liver cancer, prostate cancer, and polycystic ovary syndrome. Although the role of microbial HSDHs is not yet fully elucidated, they may have therapeutic potential as steroid pool modulators or druggable targets in the future. In this review, we explore metabolism of BAs and glucocorticoids with a focus on biotransformation by microbial HSDHs.

Keywords: hydroxysteroid dehydrogenase; sterolbiome; cholesterol; bile acid; cortisol; androgen; deoxycholic acid

1. Introduction

Steroid hormones are signaling molecules derived from cholesterol that include glucocorticoids, mineralocorticoids, androgens, estrogens, progestogens, and bile acids (BAs) [1]. Steroid hormones are essential for the regulation of various physiological processes, such as metabolism, salt and water balance, reproduction, inflammation, and stress response [2]. These cholesterol-derived molecules are synthesized in the human adrenal glands, gonads, placenta, and liver [3,4]. All steroids have a cyclopentanoperhydrophenanthrene ring structure, composed of three six-carbon rings denoted A, B, and C along with a five-carbon D ring (Figure 1), with differing hydroxyl groups and side-chains [1]. Hydroxysteroid dehydrogenases (HSDH) are an important class of enzyme expressed by both host tissues and host-associated microbiota that modify the hydroxyl groups on steroids. These small modifications to steroids greatly impact their physicochemical properties and can change the steroid solubility, toxicity, host receptor affinity, and ability to activate or inhibit host receptors [5–8]. The current review focuses on the importance of gut microbial HSDHs in cholesterol, BA, and glucocorticoid metabolism.
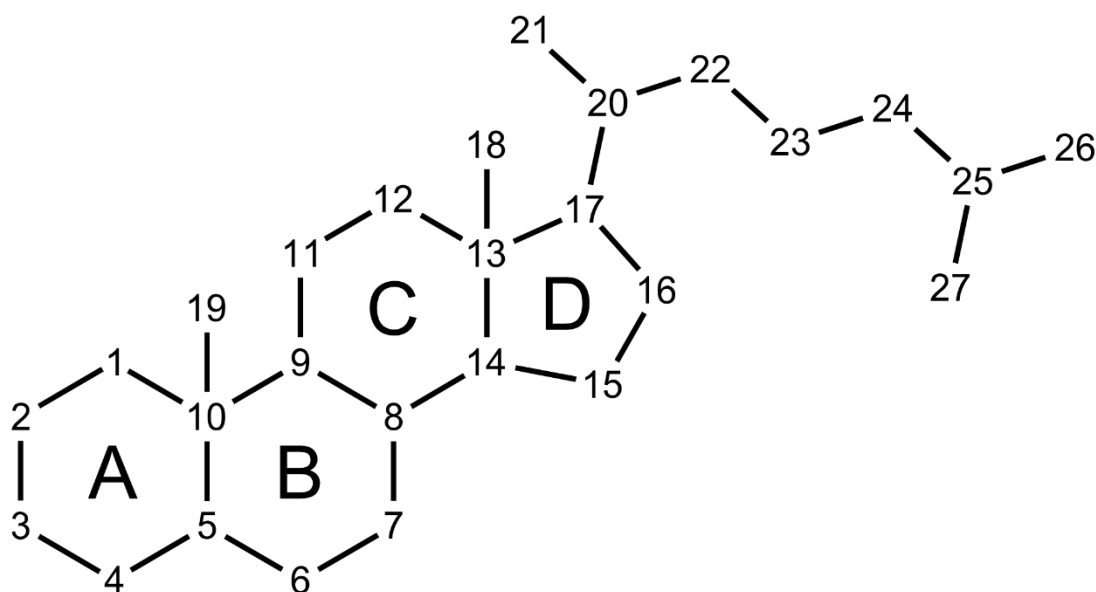
Figure 1. Steroid structure. Steroids have a cyclopentanoperhydrophenanthrene ring structure. Cholesterol, the precursor to human steroid hormones, contains 27 carbons, while the major classes of steroid hormones contain the following: C_{24} bile acids, C_{19} androgens, C_{18} estrogens, and C_{21} glucocorticoids, mineralocorticoids, and progestogens.

2. Hydroxysteroid Dehydrogenases

2.1. Hydroxysteroid Dehydrogenase Function

Hydroxysteroid dehydrogenases are nicotinamide adenine dinucleotide (phosphate) (NAD(P)(H))-dependent oxidoreductases that catalyze the reversible conversion of hydroxyl groups to keto groups on steroids [9]. HSDHs are regio- and stereospecific, meaning they are specific for the hydroxyl position on the steroid (C-3 vs. C-7) and for the orientation (α vs. β) of the hydroxyl group, respectively [5]. Pairs of HSDHs can convert steroids from the α-orientation, through an oxo-intermediate, to the epimerized β-orientation and vice versa.

Hydroxysteroid dehydrogenases are found in both host and microbial genomes, although more is known about the physiological function of host hydroxysteroid dehydrogenases, which are typically abbreviated HSDs in literature. In this review, host hydroxysteroid dehydrogenases are denoted "HSD" while bacterial enzymes are denoted "HSDH". Host HSDs are key enzymes in the biosynthesis of steroids in steroidogenic tissues [10]. They also function to activate or inactivate steroids in peripheral tissues, thus regulating local concentrations of steroid hormones [5]. Even though host HSDs catalyze reversible reactions in vitro, they typically function primarily in one direction in vivo on the basis of cofactor balance: either as dehydrogenases or as reductases [11].

Host HSDs are druggable targets important in the treatment of endocrine-dependent disorders, including cancers [12]. Host-associated microbial HSDHs may also serve as pharmacological targets or, alternatively, may be enriched in the host through engineering and delivering probiotic bacteria with rational sterolbiome phenotypes. One recent example involves identification of a cholesterol 3β-HSDH involved in conversion of cholesterol to coprostanol, the enrichment of which may be important as a probiotic approach to reducing serum cholesterol [13].

2.2. Structural Biology of Hydroxysteroid Dehydrogenases

Hydroxysteroid dehydrogenases belong to one of the following three large and diverse protein superfamilies: short-chain dehydrogenase/reductase (SDR), medium-chain dehydrogenase/reductase (MDR), or aldo-keto reductase (AKR) [5,14]. Many SDR and MDR family hydroxysteroid dehydrogenases have been identified in the gut microbiome [14–17].

HSDHs in the AKR superfamily are generally found within mammals [12], although microbial AKR family HSDHs have been reported [18].

The SDR superfamily is one of the largest, containing proteins spanning all three domains of life [19]. SDR proteins have highly diverse substrate specificities, ranging from sugars to dyes to steroids [20]. Members of this superfamily are non-metalloenzymes and typically 250 amino acids in length [5]. Due to the dependence of dehydrogenase/reductase enzymes on NAD(P)(H) to carry out redox reactions, SDR proteins contain a Rossmann fold domain for binding cofactors. This domain consists of 6–7 β-strands with 3–4 peripheral α-helices on either side [21,22]. Typically, the Rossmann fold domain is located near the N-terminus of SDR proteins, while the C-terminus binds substrates [20]. Most SDR members have a conserved Tyr, Ser, and Lys at the catalytic site. The overall folding pattern is closely conserved across the superfamily, while amino-acid sequence varies greatly [22]. This causes great difficulty in predicting substrate specificities by amino-acid homology search alone. HSDHs within the SDR superfamily include but are not limited to host 11β-HSD and 17β-HSD [5], and various microbial BA 12α-HSDHs [23], 12β-HSDH [24], 3α/β-HSDHs [17], and glucocorticoid 20β-HSDH [15].

The MDR family is similar to the SDR family both in number of members and in function, although their structures have marked differences. MDR proteins contain Rossmann fold domains for NAD(P)(H) binding like SDRs, but they are ~350 residues long and many are metal-dependent [25]. They are typically dimeric or tetrameric and many contain a catalytic zinc ion, sometimes along with a structural zinc ion, while others are non-zinc-containing [26]. The zinc-containing MDRs share a strictly conserved Gly, His, and Glu for zinc binding [27]. MDR family HSDHs include host BA 3β-HSD [26] and microbial glucocorticoid 20α-HSDH [14,16].

AKRs are NAD(P)(H)-dependent oxidoreductases acting on carbonyl groups or double bonds and are ~320 amino acids long. They are monomeric with diverse substrate recognition, including steroids, monosaccharides, and isoflavonoids. An ordered bi–bi kinetic mechanism has been shown for multiple AKR family members, where the cofactor is first to bind and last to leave [28]. Most have a conserved active site with residues Asp, Lys, Tyr, and His. Examples of members of this superfamily involved in steroid metabolism are human 3α-HSD [29], human 20α-HSD [30], and bacterial BA 3β-HSDH [18].

3. Bile Acid Metabolism

3.1. Host Bile Acid Synthesis and Signaling

Bile acids are amphipathic C_{24} steroids that play an important role in host nutrition [31]. They are essential for solubilization and later absorption of cholesterol, dietary fatty acids, triglycerides, and lipid-soluble vitamins A, D, E, and K. Bile acids assemble into mixed micelles, forming a hydrocarbon interior in order to solubilize these molecules [31,32].

Bile acid biosynthesis occurs in the liver and begins with the rate-limiting step of cholesterol 7α-hydroxylation by cytochrome P450 7α-hydroxylase (CYP7A1) in hepatocytes (Figure 2) [31,33]. While other carbon positions on cholesterol can be hydroxylated first (C-24, C-25, C-26, C-27), the classical pathway initiates through C-7 hydroxylation catalyzed by CYP7A1 [34,35]. The next step alters the ring structure through conversion to 3-oxo-Δ^4 by 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7) [34,36,37]. After HSD3B7 action, the intermediate is converted by 12α-hydroxylase (CYP8B1) if the final product contains a 12α-hydroxyl group. Ensuing steps involve additional modification to the ring structure by AKR1D1 and AKR1C1 [37]. Then, mitochondrial sterol 27-hydroxylase (CYP27A1) oxidizes the side-chain, followed by removal of three carbon atoms beginning with activation of the sterol by BA coenzyme A (CoA) synthase [34,38,39]. Subsequent reactions are catalyzed by 2-methylacyl-CoA racemase, branched-chain acyl-CoA oxidase, D-bifunctional protein, and peroxisomal thiolase 2, which cleaves the C-24–C-25 bond [34,37]. The final step in BA biosynthesis is conjugation of the BA-CoA intermediate to either glycine or taurine, catalyzed by BA CoA:amino acid *N*-acyltransferase [34,40].
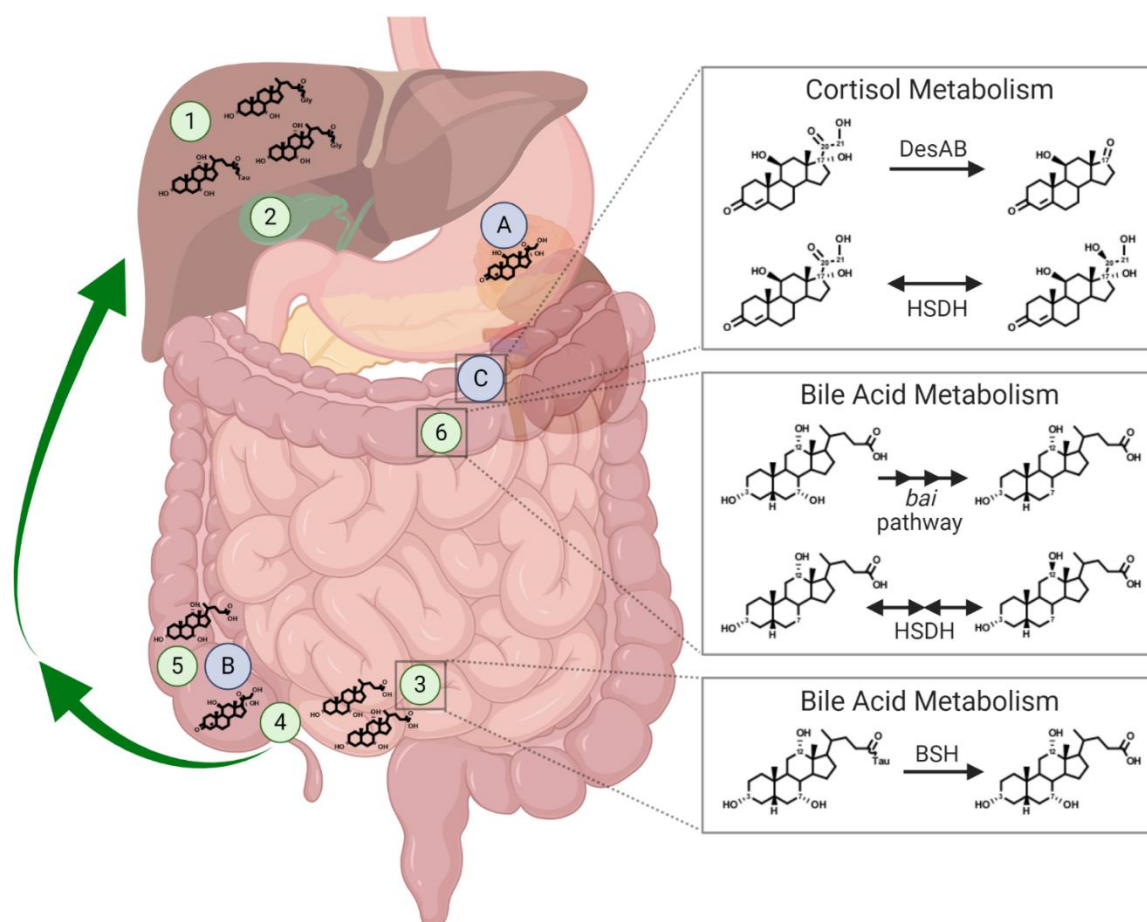
Figure 2. Synthesis and microbial metabolism of bile acids and cortisol. (1) The bile acids (BAs) cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized and conjugated to glycine (Gly) or taurine (Tau) in the liver. (2) They are then stored in the gallbladder until they are released in response to a meal. (3) Microbial deconjugation of amino acids, catalyzed by bile salt hydrolase (BSH), primarily occurs in the small intestine. (4) BAs are taken up in the terminal ileum and undergo enterohepatic circulation back to the liver indicated by green arrows. (5) About 5% of BAs are not recycled and proceed to the colon. (6) Gut microbiota residing in the colon can 7α-dehydroxylate CA or CDCA to secondary BAs in a pathway encoded by the BA-inducible (*bai*) operon. Microbial hydroxysteroid dehydrogenases (HSDHs) interconvert BA hydroxyl groups between the α- and β-conformations through an oxo-intermediate. (A) Cortisol is synthesized in the adrenal glands. (B) Cortisol and its derivatives are principally excreted in urine; however, low levels are secreted in bile and enter the gut. (C) In the gut, cortisol can be side-chain cleaved by microbiota encoding steroid-17,20-desmolase (DesAB) or reduced to 20α- or 20β-dihydrocortisol by HSDHs.

Conjugated BAs, called "bile salts" due to their ionized state at physiological pH, have increased solubility and greater amphipathicity. The biosynthetic pathway results in the formation of conjugated cholic acid (CA; 3α,7α,12α-hydroxy) or chenodeoxycholic acid (CDCA; 3α,7α-hydroxy) with their relative proportions determined by levels of 12α-hydroxylase in the liver [33,34]. The ratio of taurine- to glycine-conjugated BAs is dependent on diet in humans. A high-protein diet results in greater taurine conjugation, while vegetarian diets lead to more glycine conjugation [33]. CA and CDCA are the primary BAs produced in humans, whereas other vertebrates produce bile salts that differ in ring hydroxylation pattern, as well as side-chain length and functional groups. The main classes are C_{24} BAs, C_{27} BAs, and C_{27} bile alcohols [41]. C_{24} BAs are common in all vertebrates, but with differing hydroxylation patterns. For example, mice produce CA and convert CDCA to muricholic acids (3,6,7-hydroxy) via hydroxylation and epimerization at C-6. C_{27} bile alcohols are typically synthesized in fish [42] and amphibians, while C_{27} BAs are present in reptiles and birds [41].

Once synthesized, conjugated BAs are actively transported out of hepatocytes into the bile duct. Conjugated BAs are stored in the gallbladder until the gallbladder is emptied into the duodenum in response to a meal [43]. Conjugated bile salts form mixed micelles with cholesterol, lipid-soluble vitamins, and dietary lipids throughout the small intestine. In the ileum, a sodium-dependent transporter (IBAT) takes up BAs into ileocytes [44]. From ileocytes, they are exported by organic solute transporter OSTα/β [45,46] into the portal vein, where they circulate back to the liver in a process known as enterohepatic circulation [47]. However, ~500 mg of BAs each day are not taken up in the ileum and progress to the colon where they encounter gut microbiota [37]. Microbial metabolites of BAs can be passively absorbed in the colon, travel through the portal vein, and join the recycled host-derived BAs in the liver. Thus, the biliary pool consists of both host- and microbiota-derived BAs that are re-conjugated and, in some species, 7-hydroxylated, as they return to the liver [48].

In addition to the digestive function of BAs, they are now known to act as hormone signaling molecules. BAs are involved in regulation of their own biosynthesis, as well as energy, glucose, and lipid metabolism [43]. Farnesoid X receptor (FXR, NR1H4) is a BA-activated nuclear receptor expressed in tissues such as liver, intestine, and kidney [49,50]. FXR regulates BA biosynthesis and enterohepatic circulation through many mechanisms. The FXR/SHP (small heterodimer partner) pathway of regulation involves the inhibition of *CYP7A1*, the rate-limiting step in BA formation. FXR induces the nuclear receptor, SHP, which inhibits liver-related homolog-1 (LRH-1) and hepatocyte nuclear factor 4α (HNF4α), both leading to inhibition of *CYP7A1* transcription [51–53]. Another pathway involves FXR, fibroblast growth factor 19 (FGF19), and FGF receptor 4 (FGFR4), which also results in inhibition of *CYP7A1.* Before recirculation back to the liver, BAs stimulate intestinal FXR, which induces FGF19 synthesis in ileocytes [54]. FGF19 is transported to the liver, where it binds FGFR4 and activates the *c-jun* N-terminal kinase (JNK) 1/2 signaling cascade, leading to downregulation of *CYP7A1* [33,55].

Pregnane X receptor (PXR) and vitamin D receptor (VDR) are both nuclear receptors activated by microbial-derived BAs that also lead the binding of *CYP7A1* promoter and repression of CYP7A1 [8,56–58]. Takeda G-protein receptor 5 (TGR5) is a G-protein-coupled receptor for BAs that is expressed in intestinal and biliary epithelial cells among other cell types [59,60]. TGR5 has widespread effects throughout the body, including regulation of intestinal motility [61]. Taurine-conjugated BAs activate TGR5 more effectively than unconjugated or glycine-conjugated BAs [62]. TGR5 signaling can activate epidermal growth factor receptor (EGFR) [63]. EGFR is also a BA receptor that, once bound, initiates a signaling pathway ending in inhibition of CYP7A1 [43,64]. In the gut, primary bile salts can be microbially biotransformed to dozens of metabolites whose concentrations and affinities can impact host physiological response in the intestine.

3.2. Microbial Bile Acid Metabolism

Bile acids that enter the colon are metabolized by gut microbiota through a combination of de(re)conjugation, 7α/β-dehydroxylation, and epimerization (Figure 2). The first step of microbial BA metabolism, known as deconjugation, mainly occurs in the small intestine and involves the hydrolysis of the C-24 *N*-acyl bond linking the conjugated amino acid to the BA. This reaction is catalyzed by bile salt hydrolase (BSH) encoded by diverse microbiota, including *Clostridium* [65,66], *Bacteroides* [67,68], Lactobacillaceae [69], *Bifidobacterium* [70,71], *Enterococcus* [72], and archaea [73]. BSHs have differing substrate specificity and subunit size, but often have conserved active site Cys, Arg, Asp, Asn, and another Arg [74]. BSHs have a pH optimum of 5–6 and are typically intracellular [65,70], although activity has been reported extracellularly in some cases [66]. Interestingly, re-conjugation of BAs by gut microbiota has recently been observed with unique amino acids: Phe, Tyr, and Leu [75].

There are multiple hypotheses on the evolutionary role of BSH in microbial fitness: interspecies competition, detoxification, and release of an energy source. Deconjugated

BAs are more toxic than conjugated bile salts to some bacterial species; thus, deconjugation may serve a competitive function to inhibit other bacteria [4]. However, the reverse may also be true. Some bacteria are more sensitive to conjugated BAs and, thus, BSH may help them detoxify their environment [76]. Amino acids released from deconjugation could be an important energy source for certain microbiota, such as *Clostridium* that can utilize amino acids through Stickland fermentation [77].

Deconjugated primary BAs can be 7-dehydroxylated by a select few species within the gut, including *Clostridium scindens*, *C. hylemonae*, and *C. hiranonis* (now reclassified as *Peptacetobacter hiranonis*) [4,78–80]. Through this process, the primary BAs CA and CDCA are converted to "secondary" deoxycholic acid (DCA; 3α,12α-hydroxy) and lithocholic acid (LCA; 3α-hydroxy), respectively. Although so few species encode the 7α-dehydroxylation pathway, secondary BAs make up the majority of excreted BAs [74,81,82], meaning these microbiota have extensive dehydroxylation capacity.

The 7-dehydroxylation pathway is encoded by the polycistronic BA-inducible (*baiABCDEFGHI*) operon [4,83,84]. The first step is the import of unconjugated primary BAs by a BA transporter BaiG [85]. Next, ligation of CoA to the unconjugated BA is catalyzed by BA CoA ligase encoded by *baiB*, requiring ATP and Mg^{2+} [86]. Then, the 3α-hydroxyl group is oxidized by BaiA [87]. Three *baiA* genes from *C. scindens* have been reported in *C. scindens* VPI 12708, although completion of the *C. scindens* American Type Culture Collection (ATCC) 35704 genome revealed the presence of only two, with *baiA2* located in the *bai* operon [88–91]. These enzymes are NAD(H)-dependent BA 3α-HSDHs that are specific for BA-CoA conjugates [87]. BaiCD is an NADH:flavin-dependent oxidoreductase that creates a C-4=C-5 double bond on 7α-hydroxy BA intermediates, while BaiH has the same function on 7β-hydroxy BAs [92]. CoA is then hydrolyzed by BaiF or BaiK and transferred without requirement of ATP to an incoming primary BA [93]. Subsequent 7α-dehydration is the rate-limiting step in the pathway, catalyzed by the *baiE* product [94]. 7β-Dehydration is predicted to be carried out by BaiI [95]. Recently, a recombinant flavoprotein encoded by *baiN*, which is not a part of the *bai* operon, was shown to convert 3-dehydro-DCA to a product 4 amu less than the substrate [96]. Further characterization is necessary, but this suggests that *baiN* may catalyze reduction of both Δ^4 and Δ^6-intermediates following 7-dehydration [96]. Alternatively, BaiCD and BaiH were reported to be sufficient for C-4=C-5 and C-6=C-7 metabolism in the oxidative and reductive arms of the pathway [97]. The final step in the pathway, converting the 3-oxo intermediate to a secondary BA, is likely to be carried out by the products of one or both copies of *baiA* [98]. The BA exporter is not yet known [4]. However, two genes co-localized with *baiN* have been proposed, but not yet confirmed, to catalyze the final reaction and BA export, named BaiO and BaiP, respectively [99]. Several additional candidate export proteins were identified through transcriptomic analysis of *C. scindens* ATCC 35704 after BA induction [91].

The $7\alpha/\beta$-dehydroxylation pathway results in a net two-electron reduction, meaning a net of one NAD^+ is produced when a primary BA is used as an electron acceptor [74]. The $7\alpha/\beta$-dehydroxylation pathway is likely coupled to glucose metabolism, benefitting $7\alpha/\beta$-dehydroxylating bacteria [91]. The pathway may serve another function in producing secondary BAs, which are more hydrophobic and toxic to gut bacteria, to regulate the growth of competing gut microbiota [7,100]. For example, DCA has a minimum inhibitory concentration tenfold lower than CA against many *Lactobacillus* and *Bifidobacterium* species [100].

Both primary and secondary BAs can be oxidized and epimerized at position C-3, C-7, and/or C-12 reversibly from the α-orientation to an oxo-intermediate and further to the β-orientation by microbial HSDHs. Epimerized BAs have specific nomenclature: those containing 3β-hydroxyl groups are iso-BAs, while 7β- and 12β-BAs are recommended to be denoted epi-BAs preceded by the hydroxyl position, according to Hofmann et al. (1992) [101]. However, 7β-BAs are generally accepted to be named urso-BAs. For simplicity in this review, each prefix refers to only one of the β-hydroxyl positions: iso for 3β-, urso for 7β-, and epi for 12β-hydroxyl (Figure 3). Similarly to humans, mouse α-muricholic

acid (3α,6β,7α-hydroxy) and β-muricholic acid (3α,6β,7β-hydroxy) can be oxidized and epimerized to ω-muricholic acid (3α,6α,7β-hydroxy) via a 6-oxo-intermediate [102]. Numerous microbiota are capable of oxidoreduction of BAs, including *Eggerthella lenta* [103], *C. scindens* [23,87], *C. hiranonis* [23], *C. hylemonae* [23], *Escherichia coli* [104], and *Bacteroides fragilis* [105].
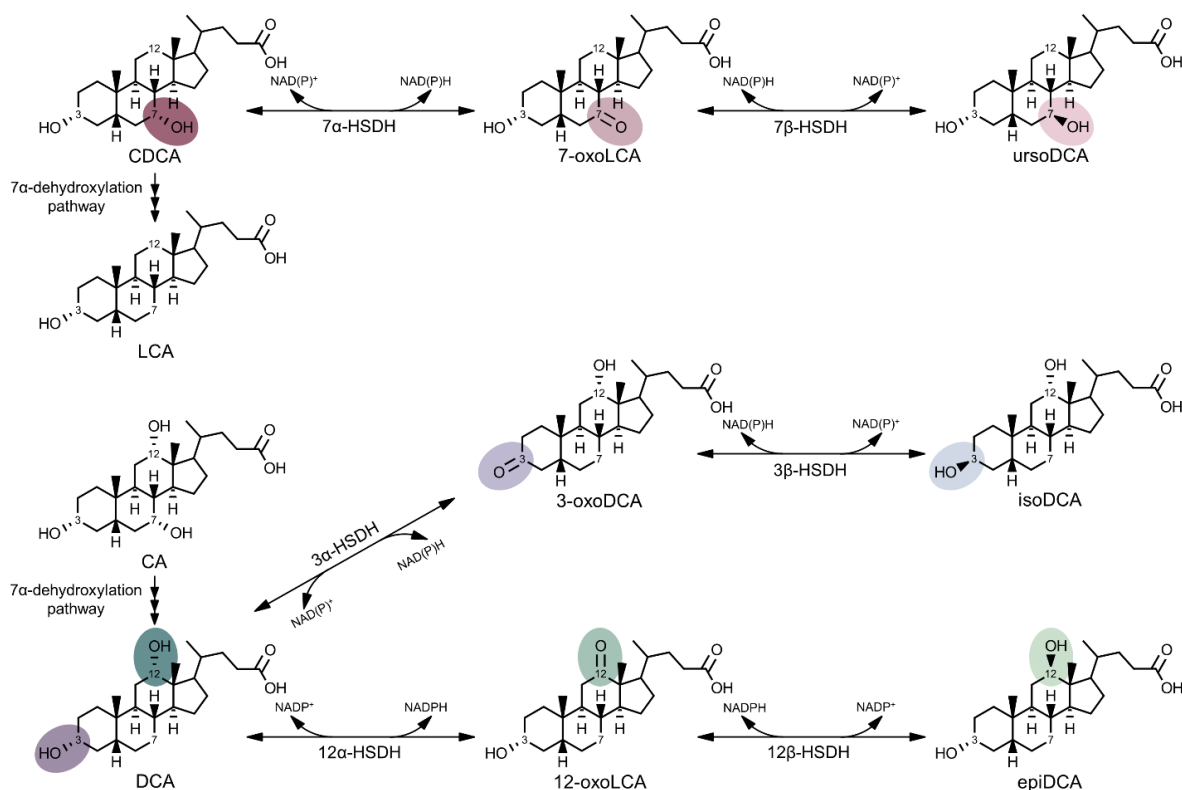

Figure 3. Microbial bile acid hydroxysteroid dehydrogenase metabolism. After deconjugation by bile salt hydrolase, the primary bile acids (BAs) chenodeoxycholic acid (CDCA) and cholic acid (CA) can be 7α-dehydroxylated or reversibly biotransformed by NAD(P)(H)-dependent hydroxysteroid dehydrogenases (HSDHs). CDCA is converted to the oxo-intermediate, 7-oxolithocholic acid (7-oxoLCA), and further to ursoDCA (UDCA) in the urso-BA pathway catalyzed by 7α- and 7β-HSDH. The secondary BAs lithocholic acid (LCA) and deoxycholic acid (DCA) are produced through the multi-step 7α-dehydroxylation of CDCA and CA, respectively. 3α-HSDH biotransforms DCA into 3-oxoDCA, and 3β-HSDH converts 3-oxoDCA to isoDCA in the iso-BA pathway. DCA is converted to 12-oxoLCA by 12α-HSDH and from 12-oxoLCA to epiDCA by 12β-HSDH. HSDHs can recognize other BAs with the correct hydroxyl group position and orientation beyond those depicted.

3.3. Microbial Bile Acid Hydroxysteroid Dehydrogenases

Microbial HSDHs catalyze the NAD(P)(H)-dependent oxidation and reduction of hydroxyl groups on BAs in the gut (Figure 3). Human interest in ursodeoxycholic acid (UDCA; 3α,7β-hydroxy) has a long and fascinating history. Asiatic black bear bile has been used in traditional Chinese medicine to treat disease for over 1000 years [106]. In the early 1900s, a BA was isolated from polar bear bile and, later, the same BA was crystallized from the American black bear. This BA was named ursodeoxycholic acid after the Latin name *ursus* [107]. UDCA makes up about 3–4% of the human BA pool but, in contrast to bear bile, is a secondary BA in humans [108,109]. UDCA and other urso-BAs are produced by combined microbial 7α-HSDH and 7β-HSDH activity in the human gut. Both microbial 7α- and 7β-HSDHs are typically NADP(H)-dependent, and they frequently exhibit specificity for dihydroxy-BAs (e.g., CDCA and UDCA) over trihydroxy-BAs (e.g., CA and UCA) [104,105,110–114], although exceptions have been reported [115,116].

Urso-BAs are more hydrophilic and less toxic both to microbiota and to the host than DCA or LCA [7]. Indeed, DCA and LCA are involved in various diseases, such as cancers of the colon and liver [117–120]. UDCA is currently approved for treatment of biliary disorders [121], is being studied for both chemoprevention and chemotherapy of various cancers [108,122], and is undergoing clinical trials as part of a combination chemotherapy for colorectal cancer (clinicaltrials.gov identifier: NCT00873275). Its mechanism of action likely involves the displacement of more toxic BAs in the BA pool and its choleretic effect of inducing secretion of BAs from the liver [123]. However, UDCA can be 7β-dehydroxylated by certain gut microbiota or isomerized back to 7α-hydroxy prior to 7α-dehydroxylation [124,125]. 7β-Dehydroxylation of UDCA forms LCA, which may explain various toxicities associated with UDCA treatment [126].

The iso-BA pathway is catalyzed by the paired action of BA 3α- and BA 3β-HSDH. Generally, 3α-HSDHs utilize NAD(H), whereas 3β-HSDHs require NADP(H). They also usually prefer dihydroxy-BAs (derivatives of DCA or CDCA) over trihydroxy-BAs (derivatives of CA) [17,18,112,127]. BA 7α-dehydroxylating bacteria express a 3α-HSDH (BaiA) that differs greatly in substrate specificity as it reacts with CoA conjugates, not free BAs [87]. Iso-BAs are present ranging from 0% to about 20% of the total BA pool in the gut [109]. Iso-BAs have greatly decreased detergent nature and are thus less cytotoxic to gut microbiota, as well as the host, than DCA or LCA [6,17]. 3α/β-HSDHs may be of pharmaceutical use with respect to modulating the BA pool in favor of less toxic iso-BAs. Iso-BAs are intrinsically poor detergents and impede nutrient absorption. The liver epimerizes iso-BAs back to the 3α-hydroxyl form via a cytosolic 3β-HSDH [128]. Further studies are needed to determine the viability of developing strategies to favor iso-BAs.

Compared to the iso- and urso-BA pathways, the least is known about the epi-BA pathway. While multiple 12α-HSDHs have been characterized [18,23,103,116,129,130], BA 12β-HSDH was only studied in cell extracts until the discovery of the first gene encoding this activity by our lab [24,131,132]. 12-Oxolithocholic acid (12-oxoLCA; 3α-hydroxy,12-oxo), the product of 12α-HSDH oxidation of DCA, is often one of the most abundant oxo-BAs found in human feces, at concentrations of about one half DCA in some studies [81,133,134]. Of note, levels of 12-oxoLCA were increased in rats with high incidence of tumors after being fed a diet high in corn oil or safflower oil [135]. Measurement of epi-BAs is rare in the literature. EpiDCA (3α,12β-hydroxy) was first identified in human feces by Eneroth et al. (1966) [136]. Recently, Franco et al. (2019) measured 3-oxo-12β-hydroxy-CDCA in humans, but little is known about concentrations of epiDCA or epiCA (3α,7α,12β-hydroxy) in feces [81]. EpiDCA has also been identified in the biliary bile of angelfish; hence, 12β-HSDH activity is likely present within the microbiome of diverse vertebrates [41].

Many gut microbial 12α-HSDHs have NADP(H) specificity [18,23,129,130], while others are NAD(H)-specific [116]. 12α-HSDHs generally have higher activity with free and dihydroxy-BAs than conjugated or trihydroxy-BAs [18,23,129]. The only gut microbial BA 12β-HSDH characterized to date, from *Clostridium paraputrificum* ATCC 25780, has affinity for NADP(H) and greater activity with dihydroxy-BAs [24,132]. Two additional 12β-HSDHs have been shown to react with 12-oxoLCA and epiDCA with NADP(H) as co-substrate, although their substrate specificities have not been fully characterized [24]. Interestingly, 12β-HSDH activity recognizing side-chain cleaved steroids derived from BAs has been observed in multiple environmental microorganisms. This activity is displayed by *Comamonas testosteroni* TA441 [137] and *Pseudomonas* sp. strain Chol1 [138] as they convert a 12-oxo-intermediate into 7α,12β-dihydroxy-androsta-1,4-diene-3,17-dione (12β-DHADD) in a cholic acid degradation pathway.

Epi-BAs are understudied compared to urso- and iso-BAs. Thus, their toxicity relative to secondary BAs is untested, although epiDCA and 12-oxoLCA are less hydrophobic than DCA according to LC–MS [24]. It is possible that isomerization of primary BAs to iso- or epi-BAs may impede formation of secondary BAs if they cannot be recognized

by 7-dehydroxylation pathway enzymes. This could be of therapeutic importance because secondary BAs DCA and LCA are not only toxic to gut microbiota, but also to the human host.

Our knowledge of microbial HSDHs is largely limited to studies in humans and rodents. Notable recent studies extend to black bears in the search for HSDHs capable of forming UDCA [139]. There is a rich diversity of bile salts produced in vertebrates, such as pythocholic acid (16α-hydroxycholic acid; 3α,12α,16α-trihydroxy-5β-cholan-24-oic acid) found in snakes, which is a 16α-hydroxylated derivative of DCA [106,140]. Avicholic acid (3α,7α,16α-trihydroxy-5β-cholan-24-oic acid), found in birds, was identified in a drug screen as a TGR5 agonist [141]. An NAD(P)-dependent 16α-HSD was purified and characterized from rat kidney [142]; however, to our knowledge microbial 16α-HSDH activity has not yet been reported in snake or bird gastrointestinal content.

3.4. Physiological Roles of Microbial Bile Acid Hydroxysteroid Dehydrogenases

The physiological function of many microbial BA HSDHs remains unclear, although species and strain context seem likely to be important. In all cases, these redox reactions affect NAD(P)/NAD(P)H ratios, and BA oxo-groups provide substrates for disposal of excess reducing equivalents or acquisition of hydrides in order to detoxify molecular oxygen close to the gut mucosa. Oxidation and epimerization of BA α-hydroxyl groups to β-hydroxyl groups is also thought to function in detoxification by converting hydrophobic BAs to hydrophilic BAs that are less damaging to biological membranes [7,17]. For example, isoDCA has a minimum inhibitory concentration of more than double that of DCA against various Gram-negative *Bacteroides* and Gram-positive species [17]. In contrast, some HSDHs seem to favorably produce DCA from oxo-derivatives, suggesting they may function to maintain high concentrations of DCA in the environment [23].

Culture-based studies indicate that the oxidation and epimerization of primary BAs affects the extent of BA 7α-dehydroxylation [143]. There are several hypotheses that could explain this observation. First, there is currently a paucity of knowledge relating to substrate specificity of the BA transporter, BaiG, and whether oxo- and iso-BAs are efficiently imported. Our recent study indicates that 3,7-dioxoLCA is converted to CDCA and low levels of LCA by *C. scindens* [143], albeit to lower levels than CDCA addition, suggesting import is occurring. Second, BA 7α-dehydroxylating bacteria appear to lack significant 3β-HSDH activity and, as a result, iso-primary BAs (3β-hydroxy) are not substrates for the BA 7α-dehydroxylation pathway [143]. As noted above, the first oxidation step and the last reductive step in the BA 7α-dehydroxylation pathway are catalyzed by 3α-HSDH (BaiA). A 3β-hydroxyl group, thus, prevents key oxidation steps that lead to 7α-dehydration. Indeed, LCA was not observed in cultures of *C. scindens* VPI 12708 induced with CA (resulting in upregulation of Bai enzymes) and then incubated with isoCDCA [143]. While trace levels of isoLCA (<1%) have been reported in vitro during BA metabolism by *C. scindens* ATCC 35704 [144], this may be due to the minor promiscuity known for some bacterial HSDHs [96]. Iso-secondary BAs (e.g., isoDCA and isoLCA) are second only to DCA and LCA in abundance in stool [109] and are less toxic than LCA and DCA to intestinal bacteria [17]. Iso-BA epimerizing HSDHs also show substrate specificity preference toward secondary BAs [18]. It is, therefore, hypothesized that isoLCA and isoDCA are generated from LCA and DCA, respectively, in the gastrointestinal tract. A third point is that enrichment of primary oxo- and β-hydroxy-BAs comes at the expense of primary BAs such as CA and CDCA, which induce expression of the *bai* operon [145]. Indeed, culture-based studies indicate that *C. scindens* VPI 12708 is capable of converting 3,7-dioxocholanoic acid and 7-oxoLCA to LCA only if the cells were preincubated with CA [143].

Numerous gut bacteria, including *Bacteroides* spp. and *E. coli*, encode 7α-HSDH and produce 7-oxo-BAs that are released into the lumen [105,146,147]. The formation of 7-oxo-primary BAs precludes 7α/β-dehydration by the *bai* pathway and must be reduced to proceed. It is, therefore, not surprising that BA 7α-dehydroxylating bacteria express NADP-dependent 7α-HSDH [114]. The BA 7α-HSDH is predicted to be important both in

regulating the NAD(H)-dependent BA 7α-dehydroxylating pathway intracellularly and in reducing 7-oxo-BAs imported from the environment.

BA 7α-dehydroxylating bacteria also encode BA 12α-HSDH [23,148]. The formation of 12-oxo-BAs reduces toxicity of BAs toward gut bacteria [7], which is likely why a wide diversity of gut bacteria encode 12α-HSDH [18,23,143,149]. However, substrate specificity of 12α-HSDHs in 7α-dehydroxylating bacteria favors the reductive direction, converting 12-oxoLCA to DCA [23]. We, therefore, hypothesize that BA 7α-dehydroxylating bacteria express BA 12α-HSDH principally to "retoxify" 12-oxoLCA that was generated by bacteria less resistant to DCA.

We recently demonstrated extensive oxidation of BAs by *Eggerthella lenta* [143]. Indeed, *E. lenta* strains C592 and DSM 2243 encode 3α-, 3β-, 7α-, and 12α-HSDHs capable of converting CA to trioxo-cholanoic acid under a nitrogen or carbon dioxide atmosphere. However, BA oxidation was inhibited under a hydrogen gas atmosphere (Figure 4). Genomic analysis revealed genes encoding energy conserving hydrogenase (*echABCDEF*) and Rnf complex (*rnfABCDEG*), as well as a complete Wood–Ljungdahl pathway, suggesting that *E. lenta* is an acetogen [143,150]. The classical acetogen fixes CO_2 or CO in the presence of H_2 [151]; however, acetogens are known to utilize a wide range of electron donors. Under this scheme, *E. lenta* HSDH enzymes are hypothesized to generate NADH by oxidizing BAs, which provides reducing equivalents to fix CO_2. In the presence of H_2, *E. lenta* hydrogenases reduce NAD^+ via molecular hydrogen, and BA oxidation is prevented. Additional studies will be needed to confirm this hypothesis linking BA metabolism and H_2 partial pressure in a novel acetogen.
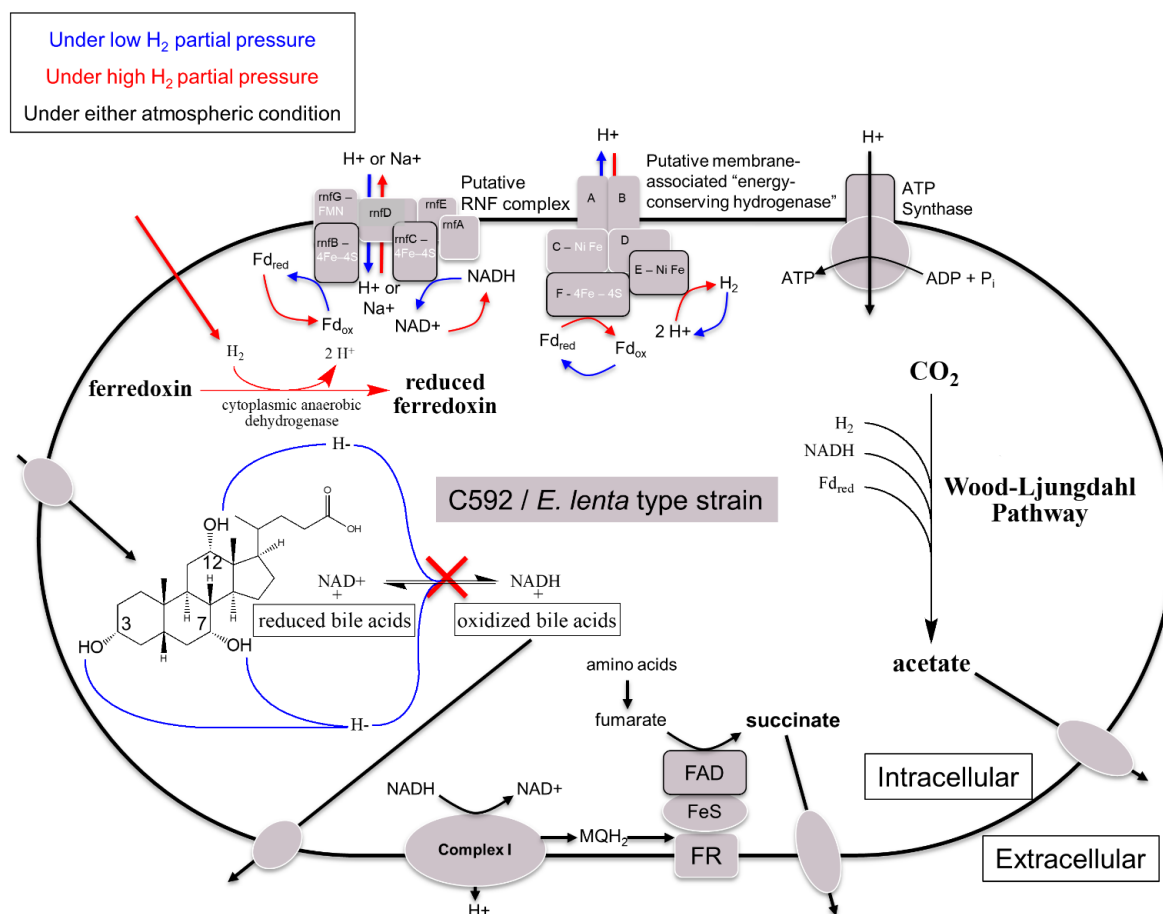

Figure 4. Proposed model for the role of *Eggerthella lenta* hydroxysteroid dehydrogenases: bile acid oxidation provides reductant for the Wood–Ljundahl Pathway (WLP) of acetogenesis. This model is based on biochemical and genomic data demonstrating that *E. lenta* strains contain complete WLP genes, and that bile acid oxidation is inhibited by a hydrogen gas atmosphere.

The role of microbial BA HSDHs in host physiology is also relatively unclear. While the involvement of oxo- and β-BAs in host signaling pathways has not been fully explored, there is evidence that products in the iso-BA pathway activate various host receptors. For example, along with LCA, 3-oxoLCA has been shown to activate the BA receptors FXR, VDR, and PXR [8,56]. In contrast, 12-oxoLCA, 7-oxoLCA, and UDCA did not efficiently activate either FXR or VDR [8,50]. Recently, 3-oxoLCA and a planar iso-BA, isoalloLCA, were shown to be regulators of interleukin (IL)-17a expressing T helper cells (T$_H$17) and regulatory T cells (T$_{reg}$) in mice [152]. Determining the full spectrum of both primary and secondary oxo- and β-derivatives against BA-responsive nuclear and G protein-coupled receptors will be important future work.

4. Glucocorticoid Metabolism

4.1. Host Glucocorticoid Synthesis

Glucocorticoids are involved in diverse essential physiological processes throughout the body [153]. Cortisol and corticosterone are the primary C$_{21}$ glucocorticoids present in humans. However, cortisol concentrations are about 10 times greater than corticosterone [154]. Cortisol plays a major role in the stress response and maintenance of blood glucose concentration, as well as in inhibition of protein synthesis in muscle, of lipogenesis in fat cells, and of the immune system [155].

Cortisol is synthesized in the adrenal gland from cholesterol and involves the action of both cytochrome P450 enzymes and hydroxysteroid dehydrogenases, much like BA biosynthesis (Figure 2). The first step is catalyzed by CYP11A1, which side-chain cleaves cholesterol and results in pregnenolone [9]. This is the rate-limiting step and precursor to many other steroid hormones, including progesterone, corticosterone, aldosterone, testosterone, and estradiol [156]. 17α-Hydroxyprogesterone is then produced by CYP17A1 (17-hydroxylase/17,20 lyase) and HSD3B2 (3β-HSD/Δ$^{5/4}$-isomerase type 2). CYP21A2 converts 17α-hydroxyprogesterone to 11-deoxycortisol. The last reaction results in the formation of cortisol through the action of CYP11B1 [9,10]. Cortisol circulates in serum at concentrations between 100 and 600 nM [9]. Cortisol then acts in peripheral tissues by binding to the nuclear glucocorticoid receptor, resulting in regulation of numerous genes, including those involved in inflammation, immune function, and gluconeogenesis. Cortisol can also bind to mineralocorticoid receptor, which regulates electrolyte balance [157,158]. Cortisol concentrations are tightly regulated by 11β-HSD isoforms 1 and 2. 11β-HSD1/2 interconvert cortisol (C-11 hydroxyl) to its inactive form, cortisone (C-11 ketone), which cannot bind the glucocorticoid receptor or mineralocorticoid receptor. 11β-HSD1 functions primarily as a reductase to activate cortisol in the liver, muscle, and bone. In contrast, 11β-HSD2 acts as a dehydrogenase, inactivating cortisol to cortisone in the kidney, colon, and salivary glands [9].

Human tissues metabolize cortisol in various ways, leading to its excretion primarily in urine. However, low levels of cortisol and its derivatives are secreted in bile and enter the gut [159]. Cortisol undergoes 5α- or 5β-reduction in the liver, while cortisone is only 5β-reduced [160]. After 3α-reduction, 5α/β-tetrahydrocortisol and tetrahydrocortisone are produced, which are the main metabolites of cortisol and cortisone in urine, respectively [9]. Cortisol can also be metabolized by 20α- and 20β-HSDs, yielding either 20α- or 20β-dihydrocortisol [161]. Carbonyl reductase-1 (CBR1) has 20β-HSD activity producing 20β-dihydrocortisol, while a host 20α-HSD has been observed with specificity for progesterone, but not cortisol [9,162]. 20α/β-Reduction of tetrahydrocortisol and tetrahydrocortisone results in α/β-cortols or α/β-cortolones [163].

4.2. Host Androgen Synthesis

Androgens are important for metabolic homeostasis and reproductive function in men, as well as women. Androgens are C$_{19}$ steroids that are synthesized in the Leydig cells of the testes or adrenal glands [164]. The primary active androgens in circulation are testosterone and dihydrotestosterone, although, in the adrenal glands, the major products are the

androgen precursors dehydroepiandrosterone (and its sulfate ester), androstenedione, and 11β-hydroxyandrostenedione (11β-OHAD) [165].

Androgen biosynthesis in the adrenal cortex begins with side-chain cleavage of cholesterol to pregnenolone by CYP11A1. Then, CYP17A1 hydroxylase and 17,20-lyase activities produce dehydroepiandrosterone (DHEA). HSD3B2 (3β-HSD/$\Delta^{5/4}$-isomerase type 2) converts DHEA to androstenedione. Alternatively, AKR1C3 (17β-HSD) can produce androstenediol from DHEA, and HSD3B2 then yields testosterone. Androstenedione can be further converted to 11β-OHAD by adrenal-specific CYP11B1 (11β-hydroxylase) [166].

Even though 11β-OHAD makes up a large proportion of adrenal steroidogenesis, it has historically largely been ignored (except in fishes) due to its low androgenic activity [167]. Storbeck et al. (2013) reported that 11β-OHAD leads to the formation of 11-ketotestosterone (11KT) [168], a potent 11-oxygenated C_{19} androgen involved in castration-resistant prostate cancer [169,170] and polycystic ovary syndrome [170,171]. This is important because, although 11β-OHAD is primarily produced in the adrenal glands by CYP11B1, peripheral side-chain cleavage of cortisol to 11β-OHAD also occurs [172]. Peripheral 11β-OHAD is not formed by CYP17A1 [173]. Thus, the enzyme responsible for cortisol-derived 11β-OHAD may be an unknown host enzyme and/or of microbial origin. Intriguingly, 11β-OHAD has been shown to be produced from side-chain cleavage of cortisol by human gut microbiota [14,174–176].

Androgens signal throughout the body by binding to androgen receptor (AR) expressed in various cell types, including B cells, T cells, neutrophils, and macrophages [177], as well as colon cancer cell lines [178]. Nuclear AR is a ligand-dependent transcription factor that, when activated by an androgen, regulates expression of cell growth, differentiation, and even carcinogenesis in some cases [179]. Intestinal cells express both nuclear AR and membrane AR [178–180]. Importantly, the gut microbiome has evolved enzymes that catalyze many of the same reactions described for host glucocorticoid and androgen metabolism. This indicates that the host endocrine system has interkingdom components in need of further exploration.

4.3. Microbial Cortisol Metabolism

The earliest evidence of microbial biotransformation of cortisol was observed when rectal infusion of cortisol in ulcerative colitis patients led to an increase in urinary excretion of 17-ketosteroids [181]. This increase in urinary steroids was not detected when cortisol treatment coincided with oral neomycin [182], suggesting microbial biotransformation of cortisol. Thereafter, side-chain cleavage of cortisol or steroid-17,20-desmolase activity was observed when human fecal samples produced C_{19} steroids after incubation with cortisol [176].

In 1984, a bacterium was isolated from human fecal material exhibiting steroid-17,20-desmolase activity producing 11β-OHAD from cortisol (Figure 2) [174,175]. This organism was named *Clostridium scindens*, formerly *Clostridium* strain 19, which also has BA 7α-dehydroxylation activity [4]. Additional organisms with steroid-17,20-desmolase activity were then isolated: *Butyricicoccus desmolans* ATCC 43058 (formerly *Eubacterium desmolans*), *C. cadaveris* AGR2141 [183], and the urinary microbe *Propionimicrobium lymphophilum* ACS-093-V-SCH5 [184,185]. The operon encoding this activity (*desABCD*) has since been identified by performing RNA-Seq after inducing *C. scindens* ATCC 35704 with cortisol [14]. The inducible *desABCD* operon consists of steroid-17,20-desmolase (DesAB) encoded by *desAB*, a 20α-HSDH (DesC), and a putative transporter (DesD) (Figure 5) [14,186]. *C. scindens* ATCC 35704 DesAB was determined to be a heterotetramer and recognized both cortisol and 11-deoxycortisol, which only differs from cortisol in the absence of an 11β-hydroxyl group [186].
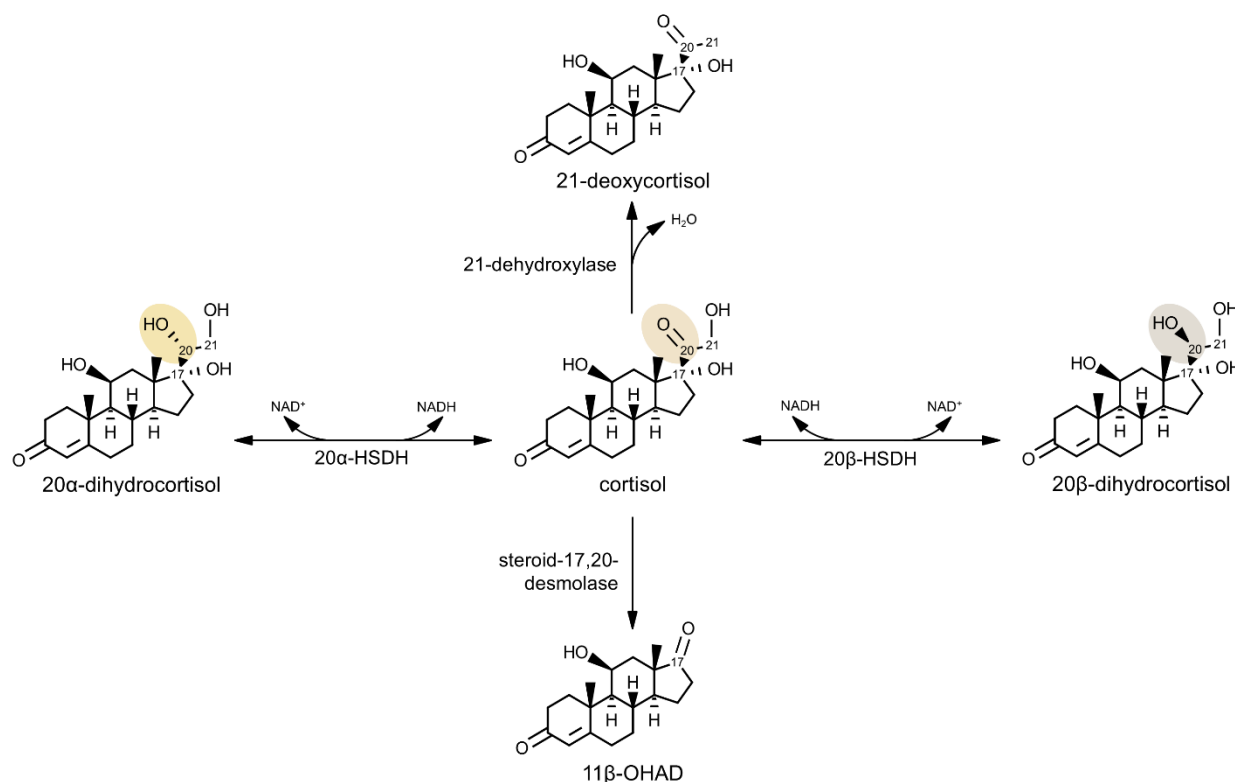
Figure 5. Microbial cortisol hydroxysteroid dehydrogenase metabolism. Cortisol can be reversibly biotransformed by 20β-hydroxysteroid dehydrogenase (20β-HSDH; DesE) to 20β-dihydrocortisol, or by 20α-HSDH (DesC) to 20α-dihydrocortisol. Steroid-17,20-desmolase (DesAB) converts cortisol to 11β-hydroxyandrostenedione (11β-OHAD). 21-Dehydroxylase catalyzes conversion of cortisol to 21-deoxycortisol.

C-20 reduced metabolites of cortisol have been observed in human urine, likely attributable to host enzymes that produce 20α- or 20β-dihydrocortisol and their derivatives [163,187]. However, Winter et al. (1982) showed that gut microbiota can reduce cortisol to 20β-dihydrocortisol, exhibiting 20β-HSDH (DesE) activity [188]. *B. desmolans* and *C cadaveris* express 20β-HSDH [183], along with *Bifidobacterium adolescentis* [188]. Additionally, the gut microbe *Clostridium scindens* ATCC 35704 can convert cortisol to 20α-dihydrocortisol [174]. Thus, gut microbiota encode 20α- and 20β-HSDHs that biotransform cortisol (Figure 5).

Human gut microbiota are also capable of 21-dehydroxylation of corticosteroids. 21-Dehydroxylase activity was first detected in *Eggerthella lenta* (formerly *Eubacterium lentum*) [189,190]. *E. lenta* 21-dehydroxylase has substrate specificity for 11-deoxycorticosterone, deoxycortisol, dehydrocorticosterone, and corticosterone [191,192]. The enzyme requires NAD(P)H and flavin or only reduced flavin mononucleotide for activity [192]. Although this enzyme seems to be specific for corticosterone, 21-dehydroxylation of cortisol to 21-deoxycortisol also occurs [176]. Interestingly, 21-deoxycortisol is a substrate for 11β-HSD2 [193] while the 21-dehydroxylation product of corticosterone is a potent inhibitor [194].

4.4. Microbial Cortisol Hydroxysteroid Dehydrogenases

Host hydroxysteroid dehydrogenases have been established as important for biosynthesis and modulation of steroid hormones such as androgens, estrogens, and glucocorticoids for years [5]. Since the discovery of steroid hormone-converting HSDHs in the human gut microbiome, gut bacteria have been proposed to play an important role beyond that of the host in modification of steroids [14]. Within the steroid-17,20-desmolase pathway, two HSDHs have been identified that convert cortisol to 20α- or 20β-dihydrocortisol and may act as enzymatic switches to control formation of 11β-OHAD (Figure 5).

20β-Dihydrocortisol is excreted in urine at rates comparable to that of free cortisol in healthy individuals [161,187]. Urinary excretion of 20α-dihydrocortisol occurs at rates of about 1.5 times the excretion of cortisol [161,187]. Although the physiologic role of 20α- and 20β-dihydrocortisol is not extensively studied, they are elevated in patients with Cushing's syndrome [187], as well as in patients with hypertension [195].

One of the first organisms studied expressing 20β-HSDH activity was the soil microbe *Streptomyces hydrogenans* [196]. This enzyme reacted with not only cortisol, but also cortisone, cortexolone (lacks C-11 oxygen group), and their 21-aldehydes [196]. More recently, the genes encoding 20β-HSDH in *B. desmolans* and *C. cadaveris*, organisms that were previously shown to have this activity in culture, have been identified [183,184]. The gene is denoted *desE* due to its involvement in the DesAB pathway and because it forms an operon with the *desAB* genes [14,184]. Both *B. desmolans* and *C. cadaveris* are capable of cortisol side-chain cleavage, as well as 20β-oxidoreduction [183,184]. 20β-HSDH has been characterized in detail from *B. desmolans* ATCC 43058, which exhibits specificity for cortisol as substrate and is NAD(H)-dependent [184]. *Bifidobacterium scardovii* ATCC BAA-773 and the urinary tract microbe *Propionimicrobium lymphophilum* ACS-093-V-SCH5 also express 20β-HSDH according to HPLC [184], and *P. lymphophilum* has also been shown to encode *desAB* [184,185]. Additionally, the SDR family NAD(H)-dependent 20β-HSDH product of *desE* in *B. adolescentis* strain L2-32 has been characterized. It is specific for cortisol and was crystallized in both the apo-form without any binding and the binary form with NADH bound at 2.2 and 2.0 Å, respectively [15].

Thus far, 20α-HSDH activity seems to be significantly less widespread than 20β-HSDH, with only one organism shown to exhibit the activity [14,197]. Reduction of cortisol at the C-20 position to 20α-dihydrocortisol was observed in pure cultures of *C. scindens* along with steroid-17,20-desmolase activity [175]. 20α-HSDH from *C. scindens* ATCC 35704 was initially characterized from cell extracts and shown to be NAD(H)-dependent [198]. The gene for 20α-HSDH was identified in 2013 after RNA-Seq analysis revealed a cortisol-inducible operon including *desAB* and *desC*, encoding steroid-17,20-desmolase and 20α-HSDH, respectively [14]. Recently, the *C. scindens* ATCC 35704 20α-HSDH was crystallized for further characterization of the enzymatic mechanism. Hybrid quantum mechanical molecular modeling simulations revealed a reaction mechanism involving a multistep proton relay, which was validated by site-directed mutagenesis experiments of active site and substrate binding residues [16]. An amino-acid homology search based on *C. scindens* ATCC 35704 20α-HSDH within the National Center for Biotechnology Information (NCBI) database uncovered two additional organisms, *Denitratisoma oestradiolicum* DSM 16959 and *Intestinibacillus* sp. Marseille-P4005, which may express 20α-HSDH, although activity has not yet been confirmed [24].

Microbial 20α- and 20β-HSDH may be important regulators of the steroid-17,20-desmolase/DesAB pathway. By competing for cortisol as substrate with DesAB, they would decrease the potential for 11β-OHAD formation. Microbial steroid-17,20-desmolase activity may be one of the important missing enzymes contributing to peripheral 11β-OHAD production in the body [199]. Recent work showed that *Clostridium scindens* ATCC 35704 and the urinary microbe *Propionimicrobium lymphophilum* ACS-093-V-SCH5 can side-chain cleave both cortisol and glucocorticoid drugs [185], suggesting microbial production of 11β-OHAD may occur in both the gut and urinary tract. As mentioned above, 11β-OHAD can be further converted to highly androgenic 11KT [168]. This has compelling implications for androgen-dependent diseases, such as castration-resistant prostate cancer, or diseases defined by androgen excess, such as polycystic ovary syndrome [170]. Further studies are necessary to assess the efficacy of utilizing 20α- and/or 20β-HSDH to mediate 11β-OHAD formation in vivo.

5. Conclusions

Overall, both host and microbial HSDHs play pivotal roles in BA and glucocorticoid metabolism. Research on the importance of HSDH-derived BAs on host physiology is in

its infancy. However, the immense diversity of these BA metabolites, due to combinations of HSDH activity, means that the gut harbors a multitude of potential candidates for host receptor signaling. Gut microbial cortisol HSDHs are likely important regulators of steroid-17,20-desmolase activity, although additional research is needed to ascertain the physiological significance of 20α- and 20β-HSDH products. New microbial HSDHs are continually being discovered and characterized, which will allow mechanistic study of their impacts in disease models.

Microbial HSDHs may have potential as therapeutic modulators in diseases such as colorectal cancer, liver cancer, castration-resistant prostate cancer, and polycystic ovary syndrome. However, to work toward therapeutics, we must first connect HSDH function to host phenotypes through mechanistic experiments, such as gnotobiotic animal studies [200,201]. Such avenues include developing genetic knockouts of HSDHs in microbes naturally encoding them or, when genetic systems are unavailable, engineering genetically tractable microbes to encode HSDHs. Furthermore, crystal structures of microbial HSDHs will aid in any necessary mutagenesis to rationally design substrate specificity for these enzymes. Integrating functional studies, genetic manipulation, structural biology, and gnotobiotic animal experiments will be imperative to reach a clearer picture of microbial steroid metabolism in the future.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Bile acid (BA), hydroxysteroid dehydrogenase (HSDH), nicotinamide adenine dinucleotide (phosphate) (NAD(P)(H)), short-chain dehydrogenase/reductase (SDR), medium-chain dehydrogenase/reductase (MDR), aldo-keto reductase (AKR), cytochrome P450 7α-hydroxylase (CYP7A1), 3β-hydroxy-Δ5-C27-steroid oxidoreductase (C27 3β-HSD), 12α-hydroxylase (CYP8B1), 27-hydroxylase (CYP27A1), coenzyme A (CoA), cholic acid (CA), chenodeoxycholic acid (CDCA), farnesoid X receptor (FXR), small heterodimer partner (SHP), liver-related homolog-1 (LRH-1), hepatocyte nuclear factor 4α (HNF4α), fibroblast growth factor 19 (FGF19), fibroblast growth factor receptor 4 (FGFR4), c-jun N-terminal kinase (JNK), pregnane X receptor (PXR), vitamin D receptor (VDR), Takeda G-protein receptor 5 (TGR5), epidermal growth factor receptor (EGFR), bile salt hydrolase (BSH), deoxycholic acid (DCA), lithocholic acid (LCA), BA-inducible (bai), American Type Culture Collection (ATCC), ursodeoxycholic acid (UDCA), 7α,12β-dihydroxy-androsta-1,4-diene-3,17-dione (12β-DHADD), interleukin (IL)-17a-expressing T helper cells (TH17), regulatory T cells (Treg), 17-hydroxylase/17,20 lyase (CYP17A1), 3β-HSD/Δ5/4-isomerase type 2 (HSD3B2), carbonyl reductase 1 (CBR1), 11β-hydroxyandrostenedione (11β-OHAD), dehydroepiandrosterone (DHEA), 11β-hydroxylase (CYP11B1), 11-ketotestosterone (11KT), androgen receptor (AR), steroid-17,20-desmolase (DesAB), 20α-HSDH (DesC), putative cortisol transporter (DesD), 20β-HSDH (DesE), National Center for Biotechnology Information (NCBI).

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