

## ARTICLE

# Differential effects of metformin-mediated BSEP repression on pravastatin and bile acid pharmacokinetics in humans: A randomized controlled trial

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**Abstract**

Metformin has been shown to repress transcription of the bile salt export pump (BSEP) in human primary hepatocytes. The primary objective of this study was to assess the effect of oral metformin on the human pharmacokinetics (PKs) of two BSEP probe substrates: pravastatin and chenodeoxycholic acid (CDCA; also known as chenodiol). Endogenous bile acid levels were assessed as a secondary measure of metformin impact. An open-label, randomized, single-dose, placebo-controlled, fasted, crossover PK study was conducted in 12 healthy adult volunteers. Metformin (500 mg b.i.d.) or placebo (b.i.d.) was administered orally for 6 days. On day 7, a single dose of the BSEP substrates pravastatin (80 mg) and CDCA (250 mg) were administered orally. Plasma samples were quantified for pravastatin, CDCA, and endogenous bile acids. Compared to placebo, metformin increased pravastatin plasma exposure, did not impact CDCA plasma exposure, and reduced conjugated primary bile acid levels in the blood. These results are consistent with metformin repressing BSEP expression. This differential effect reflects the degree of enterohepatic recirculation of victim substrates.

**Study Highlights****WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

The bile salt export pump (BSEP), mainly expressed on the canalicular membrane of hepatocytes, contributes to drug elimination but plays a critical role in the efficient re-absorption of conjugated primary bile acids via enterohepatic recirculation. Metformin repressed BSEP expression in human primary hepatocytes and is hypothesized here to repress BSEP in vivo to reduce pravastatin body clearance and the re-absorption of conjugated primary bile acids. It was also hypothesized that BSEP repression does not impact oral chenodeoxycholic acid (CDCA), an unconjugated primary bile acid, because oral CDCA itself is not highly systemically available and is practically not secreted by BSEP in its unchanged form.

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**WHAT QUESTION DID THIS STUDY ADDRESS?**

Does in vivo pretreatment with metformin (500 mg b.i.d. for 6 days) affect the plasma exposure of pravastatin, CDCA, or conjugated primary bile acids?

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

Compared to placebo, metformin pretreatment increased pravastatin plasma exposure, reduced conjugated primary bile acid plasma exposure, and did not impact CDCA plasma exposure.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

Metformin appears to repress BSEP in humans and exhibit differential effects on BSEP substrates depending upon their degree of enterohepatic recirculation.

## INTRODUCTION

We recently showed that metformin represses the expression of the bile salt export pump (BSEP) in cultured human primary hepatocytes at high concentrations (>500  $\mu$ M), although it did not directly inhibit BSEP-mediated efflux of taurocholic acid (TCA).<sup>1</sup> Given the pivotal roles of BSEP in hepatic bile secretion and the biliary secretion of some drugs, BSEP dysfunction may lead to transporter-mediated drug–drug interactions (DDIs) with metformin. Some studies have shown transporter-mediated DDIs that affect metformin's pharmacodynamics but not its pharmacokinetics (PKs; e.g., maximum plasma concentration [ $C_{\max}$ ], and area under the curve [AUC]).<sup>2,3</sup>

Pravastatin is currently the only known non-bile acid BSEP substrate.<sup>4</sup> Its oral absorption is 34% and absolute bioavailability is 17%, indicating low and incomplete intestinal absorption and high first-pass effect.<sup>5</sup> About 70% of a radiolabeled oral dose is excreted in the feces.<sup>5</sup> Pravastatin undergoes extensive hepatic extraction (extraction ratio 0.66),<sup>6</sup> does not undergo metabolism by cytochrome P450 enzymatic pathways, and is excreted largely in its unchanged form. Pravastatin is also excreted by the kidneys.<sup>7</sup> Although biliary excretion of pravastatin via multidrug resistance protein 2 (MRP2, ABCC2) has been shown in rats, the relative contribution of MRP2 and BSEP to the overall biliary excretion of pravastatin is unknown in humans.<sup>4</sup>

BSEP is the major transporter responsible for the biliary secretion of bile acids, which are synthesized exclusively in the liver.<sup>8</sup> Bile acid synthesis mainly (90%) occurs through the classic pathway initiated by cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) to produce the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA). Bile acids are secreted into bile almost completely (>98%) as glycine and taurine conjugates (3:1).<sup>8</sup> Despite efficient transport of both conjugated and unconjugated bile acids via BSEP-transfected mammalian cells, unconjugated bile acids are minimally secreted by the liver.<sup>8</sup> In the intestines, bile salt hydrolase from bacteria deconjugates bile acids

and 7 $\alpha$ -dehydroxylase removes the 7 $\alpha$ -HO-group from CA and CDCA to form the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. CDCA also forms the secondary bile acid ursodeoxycholic acid (UDCA) in the distal small intestine and colon by bacterial 7 $\beta$ -hydroxyl epimerization.<sup>9</sup>

A whole-body bile acid pool of ~2–4 g consisting of ~40% CA, ~40% CDCA, ~20% DCA, and trace amounts of LCA and UDCA, is recycled 4–12 times each day within the enterohepatic circulation. The majority of bile acids (95%) are re-absorbed in the ileum, mainly by apical sodium-dependent bile salt transporter (ASBT, SLC10A2) in the terminal ileum. Bile acids lost in the feces (~0.5 g/day) are replenished by de novo synthesis in the liver to maintain a constant bile acid pool. Fibroblast growth factor 19 (FGF19) secretion from the ileum regulates this synthesis as an enterohepatic feedback mechanism.<sup>10</sup>

In this study, CDCA was administered with pravastatin as a BSEP probe substrate as it is an endogenous unconjugated primary bile acid that can be safely taken orally.<sup>11</sup> Intravenously administered CDCA has an apparent half-life of  $1.7 \pm 0.1$  h.<sup>12</sup> CDCA shows 60–80% first-pass hepatic extraction.<sup>11,13</sup> Unlike conjugated primary bile acids, BSEP does not function as the initial step toward its re-absorption. Rather, CDCA is conjugated in the liver prior to BSEP-mediated secretion. Of the only 20–40% of the administered CDCA that becomes systemically available, little will be recirculated even once back into the plasma.

The primary objective of this study was to assess the effect of oral metformin on the human PKs of two BSEP probe substrates: pravastatin and CDCA. Endogenous bile acid levels were assessed as a secondary measure of metformin impact. In this PK study which focused on plasma levels, uptake into the liver was considered clearance from the plasma. Hence, secretion by BSEP was considered a potential step toward re-absorption, such that BSEP repression could increase, decrease, or have no effect on plasma exposure, depending upon extent of enterohepatic recirculation.

We hypothesized that metformin represses BSEP, a contributor to pravastatin body elimination, resulting in *increased* pravastatin plasma exposure, as pravastatin is poorly recycled by enterohepatic circulation (Figure 1a). We also hypothesized that metformin represses BSEP, a major contributor to the efficient recirculation of conjugated bile acids, resulting in *reduced* conjugated bile acid plasma exposure, as conjugated bile acids are highly recycled by enterohepatic circulation (Figure 1b). In particular, after bile acids have been hepatically cleared from plasma, BSEP functions as the initial step toward conjugated bile acid re-absorption. We also hypothesized that metformin repression of BSEP has no effect on CDCA plasma exposure after CDCA oral administration, as the disposition of CDCA itself does not rely on BSEP.

## METHODS

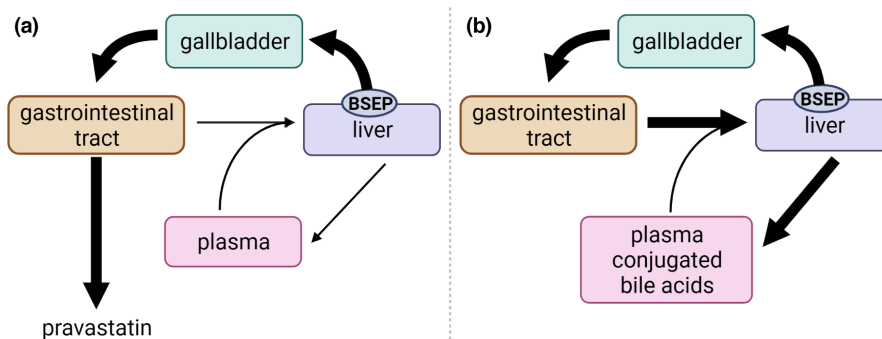
### Materials

CDCA 250 mg tablets (Chenodal; lot #004949) were kindly supplied by Travers Therapeutics (San Diego, CA). Pravastatin 80 mg tablets were obtained from Accord Healthcare (Durham, NC). Metformin 500 mg tablets were obtained from Granules Pharmaceuticals (Chantilly, VA). Placebo tablets were manufactured at the University of Maryland Good Manufacturing Practice facility using PROSOLV EASYtab SP (JRS PHARMA, Weissenborn, Germany). The FGF19 human enzyme-linked immunosorbent assay (ELISA) kit was obtained from Invitrogen (Waltham, MA). Liquid-chromatography tandem mass spectrometry (LC-MS/MS) grade solvents were purchased from Fisher Scientific (Pittsburgh, PA). Pravastatin was obtained from United States Pharmacopeia (Rockville, MD). Pravastatin- $d_3$  was obtained from Toronto Research Chemicals (North York, ON, Canada). Bile acid standards and stable isotope labeled bile acid standards

were purchased from Sigma Aldrich (St. Louis, MO), Toronto Research Chemicals (North York, ON, Canada), Steraloids (Newport, RI), Cambridge Isotope Laboratories (Tewksbury, MA), or CDN Isotopes (Pointe-Claire, QC, Canada). ISOLUTE PLD+ phospholipid depletion columns were purchased from Biotage (Uppsala, Sweden).

### Clinical study

An open-label, randomized, single-dose, placebo-controlled, fasted, crossover PK study was conducted in 12 healthy adult volunteers (ClinicalTrials.gov identifier: NCT04640571). The study was approved by the Institutional Review Board at University of Maryland, Baltimore, and was conducted at the General Clinical Research Center at the University of Maryland. Informed consent was obtained from all participants in the study. There were no important protocol changes. The trial spanned from April 26, 2021, to November 4, 2021. Table 1 describes participant demographics. All volunteers received metformin and placebo with a minimum wash out period of 10 days and a maximum wash out period of 28 days. The wash out period varied within a range to accommodate clinic availability. Metformin or placebo was administered b.i.d. orally for 6 days. Metformin was administered at a typical dose of 500 mg b.i.d. and a dosing schedule of 6 consecutive days to allow for potential BSEP repression, as alteration of protein expression typically takes 48–72 h.<sup>14</sup> On day 7, after a minimum 10 h overnight fast, a single dose of the BSEP substrates pravastatin (80 mg) and CDCA (250 mg) were administered orally with 240 ml water. Enalaprilat (20 mg) and valacyclovir (500 mg) were also administered concomitantly in a cocktail approach for a corresponding substudy with a shared placebo arm. The four probes of the cocktail (i.e., pravastatin, CDCA, enalaprilat, and valacyclovir) were not expected to modulate one another.<sup>5,7,11,15–17</sup> Nonetheless,



**FIGURE 1** Differential function of BSEP in pravastatin and conjugated bile acid disposition. BSEP functions to eliminate pravastatin from the body (a) and re-absorb conjugated bile acids within the enterohepatic circulation (b). BSEP repression by metformin increased pravastatin exposure and reduced conjugated primary bile acid plasma exposure. BSEP, bile salt export pump.

**TABLE 1** Demographics of age, sex, and race of 12 participants

| Characteristics                           | Number of participants | Percent of participants |
|---|------------------------|-------------------------|
| Age, years                                |                        |                         |
| ≤30                                       | 9                      | 75%                     |
| >30                                       | 3                      | 25%                     |
| Sex                                       |                        |                         |
| Male                                      | 5                      | 41.7%                   |
| Female                                    | 7                      | 58.3%                   |
| Race                                      |                        |                         |
| White                                     | 4                      | 33.3%                   |
| Black/African American                    | 6                      | 50%                     |
| American Indian or Alaska Native          | 0                      | 0%                      |
| Native Hawaiian or Other Pacific Islander | 0                      | 0%                      |
| Asian                                     | 2                      | 16.7%                   |
| Other                                     | 0                      | 0%                      |

Note: All participants were not Hispanic/Latino.

the cocktail was administered orally in the same fashion across all three arms to minimize the impact of potential interactions among the four probes.

Water was not allowed 1 h before and 1 h after administration of the probe cocktail. Participants were provided standardized lunch and a snack 4 and 7.5 h after cocktail administration, respectively. Blood samples (~5 cc, heparinized tubes) for PK analysis were drawn prior to cocktail administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 h postdose. The blood samples were centrifuged at >1430 g at 4°C for 10 min within 15 min of collection to produce plasma. Harvested plasma aliquots were stored at -80°C until assayed. Plasma FGF19 was quantified by ELISA. Hepatic function testing (i.e., aspartate aminotransferase, alanine aminotransferase [ALT], and total bilirubin) was performed from a blood sample taken at the participant's last sampling of the metformin arm to evaluate potential hepatotoxicity.

### Quantification of pravastatin, CDCA, endogenous bile acids, and FGF19

Pravastatin, CDCA, and endogenous bile acids were measured using LC-MS/MS. Bile acids were quantified using a method previously described with minor adjustments.<sup>18</sup> Batches were considered acceptable if standards and quality controls met the acceptance criteria, as detailed by the US Food and Drug Administration (FDA) Guidance for Bioanalytical Method Validation.<sup>19</sup> FGF19 was measured from plasma samples (0–10 h) by ELISA. See [Supporting Information](#).

## Statistical analysis

All numerical results were expressed as mean ± SEM. Differences were determined to be statistically significant using Student's *t*-test.

Noncompartmental analysis was used to calculate the PK parameters  $C_{max}$  and AUC for drugs within the cocktail (i.e., pravastatin, baseline-corrected CDCA, valacyclovir's active moiety acyclovir, and enalaprilat) and endogenous plasma bile acids. Differences between metformin and placebo arms were considered statistically significant when  $p < 0.05$  for pravastatin, CDCA, and endogenous bile acids using an unpaired one-tailed Student's *t*-test.  $p < 0.003$  was also considered for endogenous bile acids. Differences in  $C_{max}$  and AUC point estimate ratios (i.e., means of the ratios of metformin/placebo) were considered statistically significant from a value of one when  $p < 0.05$  for pravastatin, CDCA, and endogenous bile acids using a one sample, unpaired one-tailed Student's *t*-test.  $p < 0.003$  was also considered for endogenous bile acids. Use of  $p < 0.003$  reflects effort to address multiple comparisons, as there were multiple endogenous bile acids assessed.

Student's unpaired, two-sided *t*-test was used to evaluate average FGF19 concentration differences between metformin and placebo arm among participants at each timepoint and was considered significant when  $p < 0.05$ .

## RESULTS

### Participant adverse events

Metformin was well-tolerated with the most common adverse event being gastrointestinal disturbances. All adverse events were expected, not serious, and occurred in seven out of the 12 completed participants. Two participants decided to drop out and did not complete the study. Per protocol, dropouts were replaced. Adverse events included nausea, stomach discomfort/tightness and uneasiness, easy bowel movements, diarrhea, mildly elevated ALT (i.e., 69 units/L, relative to reference range of 0–49 units/L), and headache. One participant experienced diarrhea on placebo. Two participants exhibited common adverse events due to intravenous needle insertion during their placebo round (i.e., lightheadedness, vision tunneling, and vasovagal syncope).

### Pravastatin

Mean profiles of pravastatin in the metformin and placebo arms are shown in [Figure 2](#). Metformin increased pravastatin plasma exposure, as hypothesized.

Mean PK parameters of pravastatin after metformin and placebo treatment are shown in [Table 2](#). Metformin



increased pravastatin  $C_{\max}$  by 38% and AUC by 55%. Pravastatin AUC, but not  $C_{\max}$ , was statistically increased with metformin compared to placebo ( $p = 0.02$ ). Pravastatin  $C_{\max}$  and AUC ratios for metformin versus placebo were 1.62 and 1.75, respectively, and statistically different than unity ( $p = 0.02$  and  $0.01$ , respectively; [Table 2](#)).

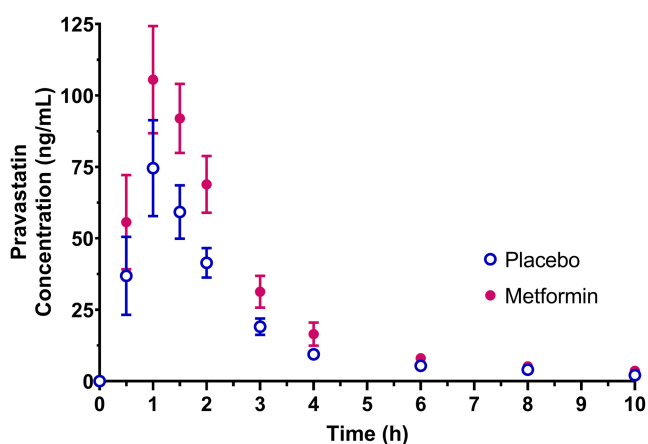
## Chenodeoxycholic acid

Mean profiles of CDCA in the metformin and placebo arms are shown in [Figure 3](#). Metformin did not alter CDCA plasma exposure. Mean PK parameters of baseline-corrected CDCA after placebo and metformin treatment are shown in [Table 2](#). Metformin had no effect on  $C_{\max}$  or AUC, or their respective metformin versus placebo ratios.

## Endogenous bile acids: Primary bile acids

In [Figure S1](#) (see Supporting Information), metformin generally reduced the plasma concentrations of conjugated primary bile acids and had no effect on the PK parameters of all other endogenous bile acids, as hypothesized. Mean profiles of conjugated bile acids (i.e., glycocholic acid [GCA], TCA, glycochenodeoxycholic acid [GCDCA], and taurochenodeoxycholic acid [TCDCA]) in the metformin and placebo arms are shown in [Figure 4](#). Mean PK parameters of each conjugated primary bile acid is summarized in [Table 3](#).

Metformin decreased TCA  $C_{\max}$  by 58% and AUC by 49% ( $p = 0.01$  and  $p = 0.01$ , respectively). TCA  $C_{\max}$



**FIGURE 2** Mean concentration versus time profile of pravastatin in 12 participants in the metformin and placebo arms. Participants took 500 mg metformin or placebo b.i.d. for 6 days. On day 7, participants were administered the cocktail, which included 80 mg pravastatin and 250 mg CDCA. Metformin increased the plasma concentrations of pravastatin. Data are expressed as mean  $\pm$  SEM. CDCA, chenodeoxycholic acid.

and AUC ratios for metformin versus placebo were 0.51 and 0.59 ( $p = 0.002$  and  $p = 0.01$ , respectively). TCA  $C_{\max}$  and AUC were statistically reduced. Metformin decreased GCA  $C_{\max}$  by 43% and AUC by 36% ( $p = 0.003$  and  $p = 0.06$ , respectively). GCA  $C_{\max}$  and AUC ratios for metformin versus placebo were 0.63 and 0.76 ( $p = 0.01$  and  $p = 0.09$ , respectively). GCA  $C_{\max}$  was statistically reduced whereas AUC was not, although supportive of a reduction. Unconjugated CA was not impacted by metformin.

Metformin decreased TCDCA  $C_{\max}$  by 45% and AUC by 37% ( $p = 0.03$  and  $p = 0.02$ , respectively). TCDCA  $C_{\max}$  and AUC ratios for metformin versus placebo were 0.78 and 0.72 ( $p = 0.18$  and  $p = 0.02$ , respectively). GCDCA was not impacted by metformin.

## Endogenous bile acids: secondary bile acids

Profiles of secondary bile acids in the placebo and metformin arms are shown in [Figure S1](#). Unlike conjugated primary bile acids, secondary bile acids were virtually unchanged with metformin ([Table 3](#)).

## FGF19

Plasma FGF19 was measured at every time point between 0 and 10 h. There was no apparent or statistical difference at any timepoint between the metformin and placebo arm (data not shown). In addition, there was no apparent or statistical difference in  $C_{\max}$  and AUC of FGF19 between the metformin and placebo arm (data not shown).

## DISCUSSION

This study assessed the effect of oral metformin on the human PKs of two BSEP probe substrates: pravastatin and CDCA. Endogenous bile acid levels were assessed as a secondary measure of metformin impact. Results showed a slight increase in pravastatin plasma exposure and no impact on CDCA plasma exposure. In contrast to secondary endogenous bile acids, conjugated primary bile acid plasma exposure generally decreased. FGF19 was not impacted by metformin.

## Transporter considerations

As is often the case, the probe compounds are substrates for multiple transporters, such that substrate specificity is a consideration. [Figure 5a](#) illustrates transporters involved with metformin. Metformin does not directly inhibit BSEP.<sup>1,20</sup> We understand metformin would not affect pravastatin or

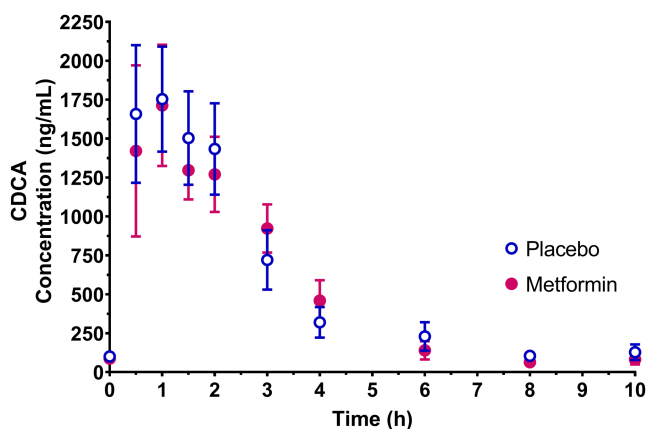
**TABLE 2** PK parameters of pravastatin and baseline-corrected CDCA in 12 participants on day 7 after metformin and placebo treatment for 6 days

| Parameter          | Pravastatin                  |               |                            | Baseline-corrected CDCA |            |             |
|--------------------|------------------------------|---------------|----------------------------|-------------------------|------------|-------------|
|                    | Metformin                    | Placebo       | Ratio                      | Metformin               | Placebo    | Ratio       |
| $C_{\max}$ (ng/ml) | 119.3 ± 17.7                 | 86.43 ± 15.86 | 1.62 ± 0.26*<br>$p = 0.02$ | 2695 ± 453              | 2881 ± 351 | 1.08 ± 0.23 |
| AUC (ng/ml × h)    | 264.2 ± 34.1**<br>$p = 0.02$ | 170.5 ± 24.7  | 1.75 ± 0.27*<br>$p = 0.01$ | 4646 ± 352              | 4665 ± 370 | 1.02 ± 0.07 |

Note: Participants took 500 mg metformin or placebo b.i.d. for 6 days. On day 7, participants were administered the cocktail, which included 80 mg pravastatin and 250 mg CDCA. Pravastatin and CDCA were measured 0–10 h postdose. Metformin had an effect on the PK parameters of pravastatin, but not baseline-corrected CDCA. Data are expressed as mean ± SEM.

Abbreviations: AUC, area under the curve; CDCA, chenodeoxycholic acid;  $C_{\max}$ , maximum plasma concentration; PK, pharmacokinetic.

\* $p < 0.05$  (compared to one), one-tail; \*\* $p < 0.05$  (compared to placebo), one-tail.



**FIGURE 3** Mean concentration versus time profile of CDCA in 12 participants in the metformin and placebo arms. Participants took 500 mg metformin or placebo b.i.d. for 6 days. On day 7, participants were administered the cocktail, which included 80 mg pravastatin and 250 mg CDCA. Time zero was approximately at 8 a.m. Metformin had no effect on the plasma concentrations of CDCA. Data are not baseline corrected. Data are expressed as mean ± SEM. CDCA, chenodeoxycholic acid.

bile acid hepatic uptake. Figure 5b illustrates transporters involved with pravastatin. Pravastatin is a substrate of canalicular MRP2 and BSEP for biliary excretion. However, the in vivo significance of MRP2 for pravastatin is unknown. Notably, a recent study has shown that pravastatin may in fact not be a MRP2 substrate, but rather a substrate of MRP3, a sinusoidal hepatic efflux transporter.<sup>21</sup> Figure 5c illustrates transporters involved with conjugated and unconjugated bile acids.

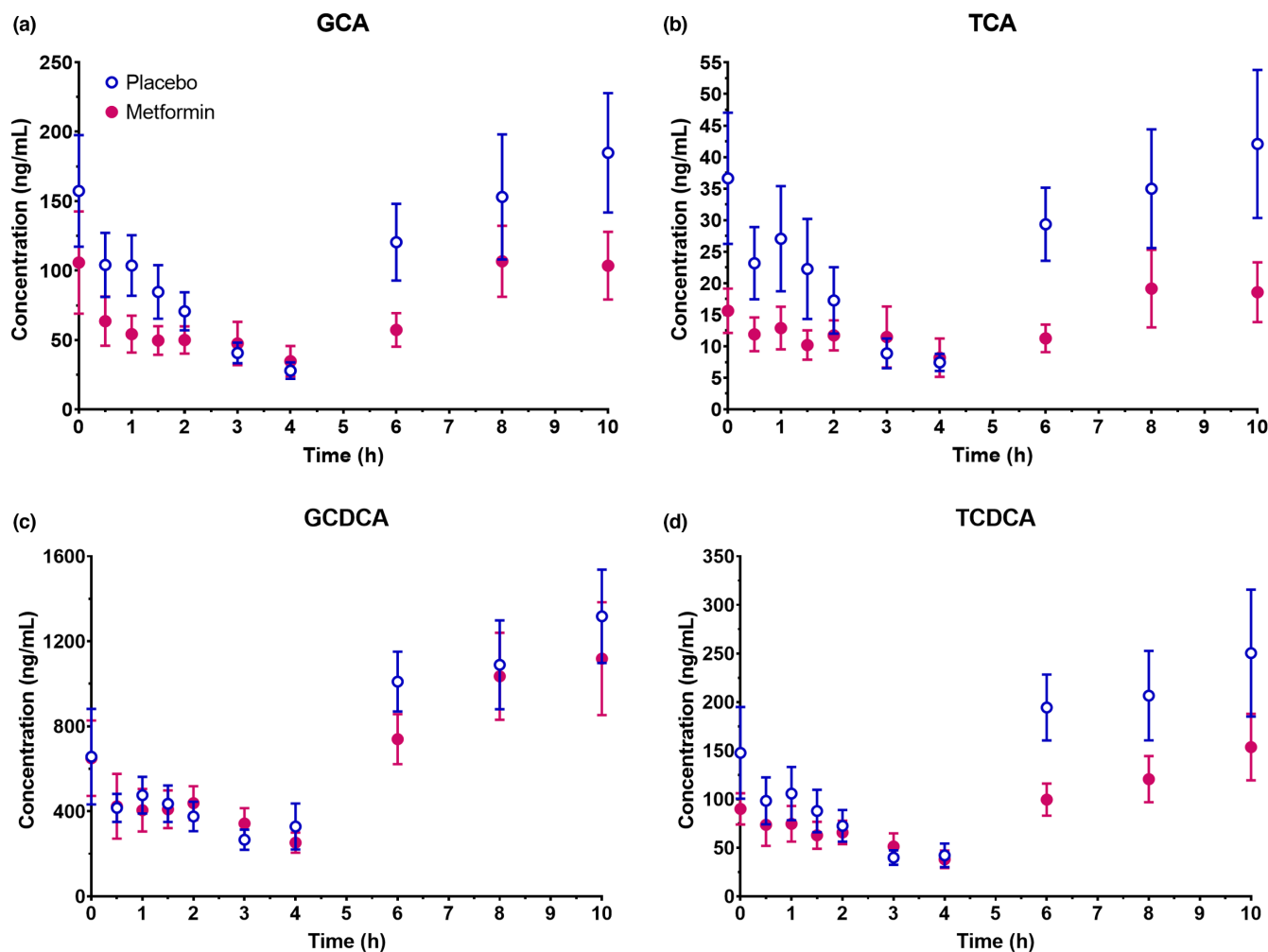
### Differential impact of metformin on pravastatin and conjugated primary bile acids

Interestingly, pravastatin and conjugated primary bile acids qualitatively differed in response to metformin.

Pravastatin plasma exposure slightly increased. For three of the four conjugated primary bile acids, their plasma exposure decreased with metformin. This differential impact of metformin-mediated BSEP repression was likely due to the contrasting roles of BSEP in pravastatin and conjugated primary bile acid PKs. For pravastatin, BSEP is a significant contributor for its elimination. The liver functions to eliminate pravastatin, although some pravastatin in the bile is re-absorbed by the intestines.<sup>6</sup> BSEP repression reduced biliary excretion to cause a slight increase in pravastatin plasma concentration, which may have been attenuated likely due to a compensatory hepatic efflux of pravastatin by MRP3 or organic anion transporter 7 (OAT7).<sup>21,22</sup> Similarly, MRP2 may provide a pathway for biliary excretion of pravastatin due to BSEP repression.<sup>23</sup>

For conjugated primary bile acids, which are efficiently extrahepatically recycled, BSEP plays a critical role in their bile acid homeostasis.<sup>8</sup> Bile acids must be excreted from the liver to allow for their re-absorption and the maintenance of the bile acid pool.<sup>8</sup> Bile acids are about 99% re-absorbed per cycle. A bile acid pool that recycles about six times per day loses a total of only 0.5 g per day.<sup>8</sup> In light of this efficient recycling, BSEP is essentially a significant contributor to conjugated primary bile acid re-absorption. BSEP allows bile acids in the liver to be secreted into the bile and hence be subsequently re-absorbed by ASBT. This high enterohepatic recycling of bile acids contrasts with the low enterohepatic recycling of pravastatin.

Three of the four conjugated primary bile acids showed reduced plasma exposure with metformin. Specifically, GCA, TCA, and TCDCA were reduced with metformin, but GCDCA was not. The reduced exposure is notable in two ways. First, virtually no other bile acid or cocktail drug (i.e., pravastatin, acyclovir, or enalaprilat) exhibited reduced exposure. Second, these conjugated primary bile acids are directly synthesized and secreted by the liver.<sup>8</sup> This concurrence that the conjugated primary bile acids were the only BSEP substrates to show reduced exposure



**FIGURE 4** Mean concentration vs time profile of primary conjugated bile acids in 12 participants in the metformin and placebo arms. GCA (a), TCA (b), GCDCA (c), and TCDCA (d) were measured from 0 to 10 h postdose. Time zero was approximately at 8 a.m. Metformin reduced plasma concentrations of primary conjugated bile acids GCA, TCA, and TCDCA. Data are expressed as mean  $\pm$  SEM. GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid.

supports a metformin effect, including the hypothesis that metformin repressed BSEP.

Although the conjugated primary bile acids exhibited reduced exposure, pravastatin was unique in exhibiting increased exposure with metformin. No other cocktail drug or bile acid exhibited increased exposure. For example, the exposures of acyclovir and enalaprilat were not modulated by metformin (Table S2, Figure S2, and Figure S3).

The oral administration of 250 mg CDCA is only about 7–13% of the bile acid pool. Hence, oral administration of a single 250 mg dose of CDCA was not expected to significantly alter the bile acid pool over the 10 h PK window, other than for CDCA itself. Unlike pravastatin and conjugated primary bile acids, no change was observed in baseline-corrected CDCA  $C_{max}$ , AUC, or their respective metformin/placebo ratios. This result for CDCA is consistent with the hypothesis that metformin repressed BSEP, as only taurine and glycine-conjugated bile acids are secreted into bile by BSEP. This result is consistent with the pattern that, among

the bile acids, only conjugated bile acids were impacted by metformin. CA was also not impacted by metformin.

The synthesis of de novo bile acids, as measured by FGF19 in plasma, was unaffected. This lack of impact is consistent with the majority of bile acids (i.e., all but conjugated primary bile acids) not being impacted by metformin, as assessed by bile acid plasma levels. Collective results point toward no metformin-mediated change in bile acid synthesis, but lower plasma exposure of conjugated primary bile acids.

### Study limitations and distinction between conjugated primary bile acids and conjugated secondary bile acids

This study in healthy volunteers has several limitations. As a first human study to examine PK effects of BSEP-mediated repression, additional studies of this incompletely

**TABLE 3**  $C_{max}$ ,  $C_{max}$  ratio, AUC, and AUC ratio of endogenous plasma bile acids in 12 participants on day 7, after metformin and placebo treatment for 6 days

| Bile Acid         | $C_{max}$ (ng/ml)                |               |                                   | AUC (ng/ml × h)                   |               |                                 |
|-------------------|----------------------------------|---------------|-----------------------------------|-----------------------------------|---------------|---------------------------------|
|                   | Metformin                        | Placebo       | Ratio                             | Metformin                         | Placebo       | Ratio                           |
| CA                | 109.6 ± 40.5                     | 123.8 ± 51.0  | 2.51 ± 1.10                       | 212.0 ± 46.3                      | 259.7 ± 64.6  | 1.29 ± 0.33                     |
| GCA               | 149.8 ± 37.5*<br><i>p</i> = 0.03 | 263.9 ± 44.8  | 0.63 ± 0.14*<br><i>p</i> = 0.01   | 678.5 ± 131.4                     | 1053 ± 187    | 0.76 ± 0.17                     |
| TCA               | 28.58 ± 6.20*<br><i>p</i> = 0.01 | 67.64 ± 12.96 | 0.51 ± 0.12**<br><i>p</i> = 0.002 | 126.5 ± 30.2*<br><i>p</i> = 0.01  | 248.1 ± 39.5  | 0.59 ± 0.16*<br><i>p</i> = 0.01 |
| DCA               | 234.0 ± 44.9                     | 199.7 ± 28.6  | 1.27 ± 0.21                       | 1366 ± 314                        | 1222 ± 158    | 1.17 ± 0.22                     |
| GDCA              | 338.1 ± 61.1                     | 375.7 ± 50.9  | 1.03 ± 0.26                       | 1618 ± 269                        | 1507 ± 200    | 1.11 ± 0.18                     |
| TDCA              | 49.66 ± 8.07*<br><i>p</i> = 0.03 | 86.14 ± 16.50 | 0.90 ± 0.31                       | 233.3 ± 36.9                      | 329.3 ± 53.1  | 0.88 ± 0.19                     |
| CDCA <sup>a</sup> | 2782 ± 451                       | 2982 ± 359    | 1.08 ± 0.22                       | 5290 ± 353                        | 5552 ± 471    | 0.98 ± 0.05                     |
| GCDCA             | 1338 ± 267                       | 1667 ± 234    | 0.90 ± 0.19                       | 6499 ± 1122                       | 7386 ± 847    | 0.87 ± 0.10                     |
| TCDCa             | 192.9 ± 32.9*<br><i>p</i> = 0.03 | 349.8 ± 69.7  | 0.78 ± 0.23                       | 879.8 ± 132.5*<br><i>p</i> = 0.02 | 1393 ± 195    | 0.72 ± 0.12*<br><i>p</i> = 0.02 |
| LCA               | 10.88 ± 1.62                     | 9.400 ± 2.040 | 1.58 ± 0.33                       | 70.10 ± 10.74                     | 67.64 ± 14.64 | 1.39 ± 0.30                     |
| GLCA              | 36.20 ± 6.84                     | 43.31 ± 12.76 | 1.43 ± 0.53                       | 164.3 ± 30.3                      | 192.1 ± 57.8  | 1.49 ± 0.53                     |
| TLCA              | 4.020 ± 0.610                    | 6.150 ± 1.800 | 0.93 ± 0.18                       | 17.39 ± 3.00                      | 21.02 ± 4.55  | 1.30 ± 0.37                     |
| UDCA              | 64.54 ± 39.40                    | 41.60 ± 24.98 | 1.87 ± 0.45*<br><i>p</i> = 0.04   | 283.5 ± 168.9                     | 243.7 ± 159.7 | 1.91 ± 0.64                     |
| GUDCA             | 121.5 ± 42.6                     | 118.0 ± 38.3  | 1.11 ± 0.21                       | 625.9 ± 220.8                     | 542.1 ± 181.9 | 1.21 ± 0.15                     |
| TUDCA             | 3.350 ± 0.420                    | 5.010 ± 0.930 | 0.96 ± 0.28                       | 15.03 ± 3.17                      | 17.07 ± 3.33  | 3.60 ± 2.64                     |

Note: Endogenous bile acids were measured 0–10 h postdose. Metformin reduced plasma concentrations of primary conjugated bile acids GCA, TCA, and TCDCa. Data are expressed as mean ± SEM.

Abbreviations: AUC, area under the curve; CA, cholic acid; CDCA, chenodeoxycholic acid;  $C_{max}$ , maximum plasma concentration; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glyoursodeoxycholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCa, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, tauroolithocholic acid; TUDCA, taoursodeoxycholic acid; UDCA, ursodeoxycholic acid.

<sup>a</sup>Data includes administered CDCA.

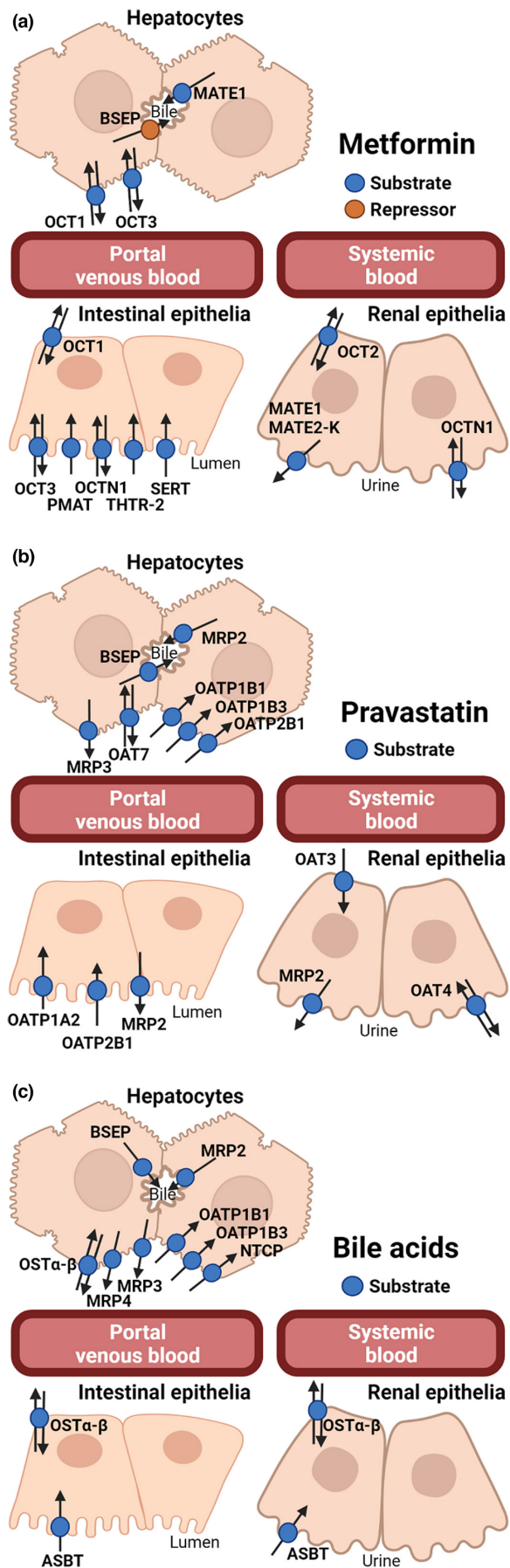
\**p* < 0.05 (metformin compared to placebo; ratio compared to one), one tail; \*\**p* < 0.003 (compared to one), one tail.

examined and challenging topic are merited. The study can be viewed as a DDI study and used a routine sample size of 12. Relatedly, the study examines several probes and involves multiple PK profile comparisons of metformin versus placebo. Statistical methods addressed this familiar multiple comparison issue, although we understand no one approach to consider multiple comparisons is the best approach. Another limitation is the unavailability of BSEP-only substrate probes and follows from general challenges of *in vivo* studies of transporter phenomena.

The study differentiated between conjugated primary bile acids and the orally administered unconjugated primary bile acid CDCA. Study results differed between conjugated primary bile acids and conjugated secondary bile acids, where conjugated primary bile acids generally showed reduced exposure with metformin, whereas conjugated secondary bile acids did not. Given that conjugated

secondary bile acids are efficiently re-absorbed too, we cannot explain why conjugated secondary bile acids did not show reduced plasma exposure. One potential explanation is that BSEP repression did reduce conjugated secondary bile acids and plasma exposure, but this repression effect was compensated by an enhanced conversion of primary bile acids to secondary bile acids. With respect to bile acid type, 80% of the bile pool are primary bile acids, and only 20% is secondary bile acids. The vast majority of the secondary bile acid pool is the more soluble DCA form (from the tri-hydroxylated CA, rather than LCA from the di-hydroxylated CDCA), as LCA is poorly soluble. Hence, only a small fraction of the primary bile acid pool would need to be converted to secondary bile acids to compensate for any enhanced conversion due to BSEP-mediated repression. This explanation is speculative, and no single mechanism is immediately likely.





**FIGURE 5** Schematic of hepatic, intestinal, and renal transport of metformin, pravastatin, and bile acids. ASBT, apical sodium-bile acid transporter; BSEP, bile salt export pump; MATE1, multidrug and toxin extrusion transporter 1; MATE2-K, multidrug and toxin extrusion transporter 2-K; MRP2, multidrug resistance protein 2; MRP3, multidrug resistance protein 3; MRP4, multidrug resistance protein 4; NTCP, Na<sup>+</sup>-taurocholate cotransporting polypeptide; OAT3, organic anion transporter 3; OAT4, organic anion transporter 4; OAT7, organic anion transporter 7; OATP1A2, organic anion transporting polypeptide 1A2; OATP1B1, organic anion transporting polypeptide 1B1; OATP1B3, organic anion transporting polypeptide 1B3; OATP2B1, organic anion transporting polypeptide 2B1; OCT1, organic cation transporter 1; OCT2, organic cation transporter 2; OCT3, organic cation transporter 3; OCTN1, organic cation/carnitine transporter 1; OSTα-β, organic solute transporter alpha and beta; PMAT, plasma membrane monoamine transporter; SERT, serotonin transporter; THTR-2, thiamine transporter 2.

### Possible next steps to investigate BSEP repression

Metformin is generally viewed to have an excellent safety profile. Results here indicate BSEP repression in healthy volunteers had a modest effect on pravastatin exposure and conjugated primary bile acid exposure. Many research groups have speculated that BSEP inhibition causes drug-induced liver injury (DILI) due to intrahepatic bile acid accumulation, but this association is questionable.<sup>24</sup> Future studies should aim to characterize the frequency and extent that metformin or other drugs repress BSEP in patients, using standard approaches for these complex interactions.<sup>25</sup> Such studies should also aim to predict the direction and extent of interactions with other drugs and with conjugated and unconjugated bile acids (e.g., increase or decrease in plasma and/or liver exposure). Such studies should also aim to predict scenarios when such interactions cause adverse events, such as DILI, as metformin has caused DILI on rare occasions.<sup>26,27</sup>

Overall, BSEP is a significant contributor to pravastatin elimination and is a significant contributor to conjugated primary bile acid re-absorption via enterohepatic recirculation. Metformin repressed BSEP in vivo to reduce pravastatin body clearance and the re-absorption of conjugated primary bile acids. BSEP repression did not impact oral CDCA, an unconjugated primary bile acid, because oral CDCA itself is not highly systemically available and is practically not secreted by BSEP in its unchanged form. In conclusion, the differential effect of BSEP repression is reflected by the degree of enterohepatic recirculation of the victim substrates.

## AUTHOR CONTRIBUTIONS

M.M., M.A.K., Y.S., H.W., and J.E. wrote the manuscript. M.M., H.W., and J.E. designed the research. M.M., J.C.F., and J.E.P. performed the research. M.M., H.W., Y.S., and J.E.P. analyzed the data. M.M., S.A.K., V.K.K., and M.A.K. contributed new reagents/analytical tools.

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## CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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## REFERENCES

- Garzel B, Hu T, Li L, et al. Metformin disrupts bile acid efflux by repressing bile salt export pump expression. *Pharm Res*. 2020;37(2):26. doi:10.1007/s11095-019-2753-x
- Cho SK, Kim CO, Park ES, Chung JY. Verapamil decreases the glucose-lowering effect of metformin in healthy volunteers. *Br J Clin Pharmacol*. 2014;78(6):1426-1432. doi:10.1111/bcp.12476
- Hibma JE, Zur AA, Castro RA, et al. The effect of famotidine, a MATE1-selective inhibitor, on the pharmacokinetics and pharmacodynamics of metformin. *Clin Pharmacokinet*. 2016;55(6):711-721. doi:10.1007/s40262-015-0346-3
- Hirano M, Maeda K, Hayashi H, Kusuhara H, Sugiyama Y. Bile salt export pump (BSEP/ABCB11) can transport a nonbile acid substrate, pravastatin. *J Pharmacol Exp Ther*. 2005;314(2):876-882.
- Pravachol. Package insert. Bristol-Myers Squibb; 2016.
- Hatanaka T. Clinical pharmacokinetics of Pravastatin. *Clin Pharmacokinet*. 2000;39(6):397-412. doi:10.2165/00003088-200039060-00002
- Neuvonen PJ, Backman JT, Niemi M. Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. *Clin Pharmacokinet*. 2008;47(7):463-474. doi:10.2165/00003088-200847070-00003
- Dawson PA. Chapter 12 – bile acid metabolism. In: Ridgway ND, McLeod RS, eds. *Biochemistry of Lipids, Lipoproteins and Membranes*. Sixth ed. Elsevier; 2016:359-389.
- Hofmann AF. The continuing importance of bile acids in liver and intestinal disease. *Arch Intern Med*. 1999;159(22):2647-2658. doi:10.1001/archinte.159.22.2647
- Chiang JYL, Ferrell JM. Bile acid biology, pathophysiology, and therapeutics. *Clinical Liver Disease*. 2020;15(3):91-94. doi:10.1002/cld.861
- Chenodal. Package insert. Retrophin, Inc. 2020.
- Pentikäinen PJ, Neuvonen PJ, Penttilä A. Pharmacokinetics of Metformin after Intravenous and Oral Administration to Man. *Eur J Clin Pharmacol*. 1979;16:195-202.
- Sundelin E, Gormsen LC, Jensen JB, et al. Genetic polymorphisms in organic cation transporter 1 attenuates hepatic metformin exposure in humans. *Clin Pharmacol Ther*. 2017;102(5):841-848. doi:10.1002/cpt.701
- Food and Drug Administration (FDA). *Guidance for Industry: In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*. Center for Drug Evaluation and Research (CDER); 2020.
- Enalaprilat Injection. Package insert. Hospira, Inc. 2018.
- IBM Watson Health. Drug interactions: Pravastatin, Chenodiol, Enalaprilat, & Valacyclovir. IBM Micromedex: Drug Interaction Checking [Electronic version]; 2022. Accessed March 8, 2022. <https://www.micromedexsolutions.com/>.
- Pravastatin, Chenodiol, Enalaprilat, & Valacyclovir. In *Lexicomp: Interactions [Electronic version]*. Wolters Kluwer UpToDate, Inc.; 2022. Accessed March 8, 2022. <http://online.lexi.com>.
- Shiffka SJ, Jones JW, Li L, et al. Quantification of common and planar bile acids in tissues and cultured cells. *J Lipid Res*. 2020;61(11):1524-1535. doi:10.1194/jlr.D120000726
- U.S. Food and Drug Administration. *Bioanalytical method validation. Guidance for Industry*; 2018.
- Köck K, Ferslew BC, Netterberg I, et al. Risk factors for development of cholestatic drug-induced liver injury: inhibition of hepatic basolateral bile acid transporters multidrug resistance-associated proteins 3 and 4. *Drug Metab Dispos*. 2014;42(4):665-674.
- Deng F, Tuomi S-K, Neuvonen M, et al. Comparative hepatic and intestinal efflux transport of statins. *Drug Metab Dispos*. 2021;49(9):750-759.
- Emami Riedmaier A, Burk O, van Eijck BA, et al. Variability in hepatic expression of organic anion transporter 7/SLC22A9, a novel pravastatin uptake transporter: impact of genetic and regulatory factors. *Pharmacogenomics J*. 2016;16(4):341-351. doi:10.1038/tpj.2015.55
- Holtzman CW, Wiggins BS, Spinler SA. Role of P-glycoprotein in statin drug interactions. *Pharmacotherapy*. 2006;26(11):1601-1607. doi:10.1592/phco.26.11.1601
- Chan R, Benet LZ. Measures of BSEP inhibition in vitro are not useful predictors of DILI. *Toxicol Sci*. 2018;162(2):499-508. doi:10.1093/toxsci/kfx284
- Nguyen JT, Tian DD, Tanna RS, et al. Assessing transporter-mediated natural product–drug interactions via in vitro–in vivo extrapolation: clinical evaluation with a probe cocktail. *Clin Pharmacol Ther*. 2021;109(5):1342-1352. doi:10.1002/cpt.2107
- Zheng L. Metformin as a rare cause of drug-induced liver injury, a case report and literature review. *Am J Ther*. 2016;23(1):e315-e317. doi:10.1097/mjt.000000000000007

27. Hashmi T. Probable hepatotoxicity associated with the use of metformin in type 2 diabetes. *BMJ Case Rep.* 2011; 2011:bcr0420114092. doi:[10.1136/bcr.04.2011.4092](https://doi.org/10.1136/bcr.04.2011.4092)

### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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