Metformin sinusoidal efflux from the liver is consistent with negligible biliary excretion and absence of enterohepatic cycling

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Running Title: Low metformin biliary excretion due to sinusoidal efflux

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Abbreviations: OCT, organic cation transporter; multidrug and toxin extrusion protein, MATE
Abstract

Although metformin hepatic distribution is critical to pharmacological activity, the drug is cleared by urinary excretion. At present, metformin hepatobiliary disposition was studied in rodents representative of clinical pharmacokinetics to elucidate why metformin is not appreciably eliminated in bile. On average, 1.0 ± 0.1% of metformin oral dose was present in the liver (liver/plasma ratio = 4.5 ± 0.6) over a pharmacologically-relevant dose and time range in mice (10-300 mg/kg; 1.5-2.5 hours; Tmax = 1.4 ± 0.5; bioavailability >59%). Distribution was not markedly higher to the kidneys, which contained 0.87 ± 0.08% of oral dose (kidney/plasma ratio = 11.9 ± 1.1). However, only 0.11 ± 0.02% of intravenous and bioavailable oral dose was recovered in bile, suggesting that biliary excretion is not the only route of clearance for hepatic metformin. Consistent with negligible biliary excretion, pharmacokinetics were unaffected by bile duct-cannulation, proving the effective absence of enterohepatic cycling. In single-pass liver perfusion studies, 2.4 ± 0.3% of perfused metformin dose was distributed to the liver, which during the subsequent drug-free washout perfusion underwent >300 fold greater sinusoidal than biliary excretion (74.0 ± 39.3% vs. 0.222 ± 0.003% recovery of hepatic metformin in perfusate vs. bile, respectively). These studies demonstrated that despite similar magnitude of metformin liver and kidney distribution, metformin biliary excretion is negligible due to predominant sinusoidal efflux from the liver.
Introduction

Although the antidiabetic agent, metformin, is cleared by urinary excretion, its hepatic distribution is critical to pharmacological activity (Gong et al., 2012). Since metformin intravenous dose was completely recovered as parent in urine, and because drug-related material was not detected in feces, it has been broadly assumed that biliary excretion does not contribute to systemic clearance (Glucophage Prescribing Information, 2009; Tucker et al., 1981; Graham et al., 2011). This assumption was confirmed in biliary excretion studies, where ≤0.3% of the intravenous dose was recovered in bile (Maeda et al., 2007; Ito et al., 2010). Nonetheless, metformin distribution to the kidney is only ~3 fold higher than to the liver: kidney/plasma ratio = 6-25; liver/plasma ratio = 2-7 (Maeda et al., 2007; Ito et al., 2010; Higgins et al., 2012), which raises the question why is the drug wholly eliminated in urine with negligible biliary excretion?

Metformin has virtually no passive membrane permeability, such that cellular ingress and egress are transporter-governed processes (Graham et al., 2011). Hepatic uptake is mediated primarily by organic cation transporter (OCT) 1 and biliary excretion by multidrug and toxin extrusion protein (MATE)1 (Gong et al., 2012). Various studies established that mechanistically and kinetically metformin hepatobiliary disposition is conserved between mice/rats and humans (Wang et al., 2002; Tsuda et al., 2009; Graham et al., 2011). Notably, OCT1 and MATE1 modulation have large effects on hepatic distribution, and therefore are pharmacodynamically consequential. In Mate1-knockout mice, metformin hepatic concentrations were ~20-fold increased (Tsuda et al., 2009). In mice lacking hepatic Oct1, liver distribution was up to 30-fold reduced (Wang et al., 2002). Clinically, OCT1 and MATE1 genetic polymorphisms resulted in altered metformin pharmacodynamics, consistent with the expected changes in hepatic drug exposure (Shu et al., 2007; Stocker et al., 2013). The active secretory component of metformin
renal clearance, which accounts for ~80% of systemic clearance (glomerular filtration contributes ~20%), is mediated by OCT2 uptake into the proximal renal tubule followed by secretion into urine via MATE1 and MATE2-K (Gong et al., 2012). Kinetically, renal clearance in rodents is analogous to human, but mechanistically uptake is mediated by both Oct1 and Oct2, while secretion into urine occurs solely via Mate1 in mice and rats (Tsuda et al., 2009; Higgins et al., 2012).

So, why is metformin wholly eliminated in urine when kidney and liver distribution are similar in magnitude? One proposed answer was markedly greater renal uptake activity based on 10-100-fold greater \textit{in vitro} transport efficiency by renal OCT2 than hepatic OCT1 (Kimura et al., 2005). This hypothesis essentially stipulates that metformin OCT/MATE vectorial transport across the liver is one to two orders of magnitude less efficient than transport across the renal proximal tubule, which is consistent with markedly lower hepatic than renal extraction ratio (~2% and \leq 100%, respectively), nearly complete urinary but negligible biliary excretion, yet comparable magnitude of tissue distribution (Chou, 2000; Ito et al., 2010; Higgins et al., 2012). Considerably less efficient metformin transport across the liver versus renal proximal tubule is supported at a gross \textit{in vivo} pharmacokinetic level (Ito et al., 2010). However, other mechanistic studies showed that renal OCT2 was only ~3 fold more efficient at transporting metformin than hepatic OCT1 (Choi et al., 2007), while renal and hepatic OCT expression was insufficiently different to explain the 50-fold difference in extraction ratio (Nishimura and Naito, 2005; Tsuda et al., 2009). The hypothesis of less efficient metformin hepatobiliary transport appears to be correct overall, but its mechanistic justification may be more complex than merely less efficient hepatic OCT uptake activity.
Further complicating the understanding of metformin hepatobiliary disposition are the first-pass pharmacodynamic effect and absorption rate-limited oral pharmacokinetics (Stepensky et al., 2001; 2002). Specifically, metformin exhibits route of administration-dependent pharmacodynamics, where the glucose lowering effect increases in the following order of administration: intravenous < intra portal < oral (Stepensky et al., 2002). This first-pass pharmacodynamic effect, combined with absorption rate-limited oral pharmacokinetics (Stepensky et al., 2001), and >50% oral bioavailability (Tucker et al., 1981) has led to speculation of potentially high first-pass hepatic extraction, biliary clearance, and enterohepatic recycling during oral drug absorption. Although metformin biliary excretion is negligible following intravenous dosing (Tucker et al., 1981; Maeda et al., 2007; Ito et al., 2010), first-pass biliary clearance has not been characterized after oral administration.

The present study demonstrated that metformin biliary excretion is negligible due to predominant sinusoidal efflux from the liver, which helps explain why the liver is an organ of distribution and not elimination. The data unequivocally support the absence of enterohepatic cycling following oral drug administration, disproving this potential contributing factor to metformin absorption rate-limited oral pharmacokinetics and first-pass pharmacodynamic effects.
Materials and Methods

**Chemicals:** Metformin-HCl and D₆-metformin-HCl were purchased from Toronto Research Chemicals (North York, Ontario).

**Animals:** Wild-type FVB male mice (19-30 g) were purchased from Taconic (Hudson, NY). Male Sprague-Dawley rats (300-360g) were purchased from Harlan (Indianapolis, IN). The vendor performed rat femoral artery and vein cannulations for *in vivo* blood sampling and intravenous dosing, respectively. In biliary excretion studies, bile ducts were also cannulated; bile flow was in the normal ~10 μL/min range for rats after intravenous (10 ± 1 μL/min) and oral (7 ± 3 μL/min) drug administration, as well as during liver perfusions (7 ± 3 μL/min). All animal procedures were approved by the Institutional Animal Care and Use Committee at Covance (Greenfield, IN), Harlan, and Eli Lilly and Company (Indianapolis, IN).

**Tissue Drug Content:** Metformin (10, 30, 60, 100, 300 mg/kg; 10 mL/kg in 1% hydroxyethyl cellulose/0.25% polysorabte-80/0.05% antifoam in water) was administered by oral gavage to mice. Mice were sacrificed at 1.5, 2.0, and 2.5 hours post dose, when livers, kidneys, and plasma were collected for analysis of metformin concentrations.

**Biliary Excretion and Pharmacokinetics:** Metformin plasma concentration time course was determined over 24 hours in an IV/oral cross-over study in bile duct cannulated- and intact-rats. Metformin was administered by IV bolus injection (5 mg/kg; 2 mL/kg in isotonic phosphate buffered saline pH = 7.4) and by oral gavage (10 mg/kg; 10 mL/kg in 1% hydroxyethyl cellulose/0.25% polysorabte-80/0.05% antifoam in water). Arterial blood samples were collected at the following times post dose: 0, 0.08, 0.25, 0.5, 1, 2, 4, 8, 12, 24 hours. Additionally in bile duct-cannulated rats, bile was collected in 6-hour intervals.
Rat Liver Perfusions: Livers were prepared for perfusion by cannulation of the bile duct, portal vein (inflow), and inferior vena cava above the liver (outflow) as previously described (Zamek-Gliszczynski et al., 2011). Following acclimation, livers were perfused in a single-pass manner with Krebs-Henseleit buffer containing 5 μM taurocholate (30 mL/min; 0-30 min with 10 μM metformin and drug-free 30-60 min). Bile and perfusate samples were collected every 5 min for up to 60 min; livers were snap-frozen at either 30 or 60 min.

Bioanalysis: All samples were mixed with an organic internal standard solution to precipitate protein, centrifuged, and the resulting supernatants were directly analyzed. Metformin and its internal standard, D₆-metformin, were eluted from a C18 column [Betasil Si 2.1x50 mm, 5 μm (Thermo Fisher Scientific, Inc.; Waltham, MA)] with a mobile phase gradient [1% trifluoroacetic acid/1% 1M ammonium bicarbonate in water (A) or acetonitrile (B)] optimized for each matrix. Analytes were detected in positive ion mode using multiple reaction monitoring [Sciex API 4000 triple quadrupole mass spectrometer equipped with a TurboIonSpray interface (Applied Biosystems/MDS; Foster City, CA)]: metformin 130.1 → 71.1 and D₆-metformin 136.1 → 77.1 m/z. The dynamic range was 1-5,000 ng/mL in all matrices. Samples with analyte concentrations above the upper limit of quantification were diluted with matrix to within the assay range.

Data Analysis: Noncompartmental pharmacokinetic parameters were calculated using Watson v. 7.4 (Thermo Scientific; Waltham, MA). Excel 2007 (Microsoft, Redmond, WA) was used for all statistical analyses. The Student's two-tailed t-test was used to assess statistical significance between pharmacokinetic parameters estimated in bile duct cannulated- and intact-rats. The minimal criterion for significance was p < 0.05. Data are reported as mean ± SEM with the associated n reported in all cases.
Results

Table 1 summarizes the fraction of metformin oral dose present in the liver and kidney over a sub-efficacious-to-maximally efficacious dose range (10-300 mg/kg) and time period (1.5-2.5 hours post dose) previously demonstrated to be pharmacologically-relevant in mice (Higgins et al., 2012). This time frame spans the systemic Tmax to the time of maximal glucose-lowering effect following oral administration (Stepensky et al., 2002; Higgins et al., 2012). Overall on average, 1.0 ± 0.1% of metformin oral dose was present in the liver (liver/plasma ratio = 4.5 ± 0.6). For comparison, the kidneys on average contained 0.87 ± 0.08% of the oral dose (kidney/plasma ratio = 11.9 ± 1.1). Metformin oral bioavailability in mice was ≥59% (Higgins et al., 2012), so the low fraction of dose present in these tissues is not an artifact of poor absorption.

Metformin pharmacokinetics in bile duct-cannulated and -intact rats are presented in Figure 1 and Table 2. On average, 0.11 ± 0.02% of intravenous and bioavailable oral dose was recovered in bile over 24 hours, a period accounting for >95% of systemic drug exposure. Consistent with negligible biliary excretion, metformin intravenous and oral pharmacokinetics were identical in bile duct-cannulated rats (Figure 1, Table 2), supporting the effective absence of enterohepatic cycling.

Since 1.0 ± 0.1% of the metformin oral dose resided in the liver over a pharmacologically-relevant period, but only 0.11 ± 0.2% of the dose was ultimately recovered in bile, the potential for hepatic sinusoidal efflux was evaluated in perfusion studies (Figure 2, Table 3). During liver perfusion with 10 μM metformin (0-30 min), hepatic bioavailability was nearly complete (98.8 ± 4.7%), although it was relatively lower at the first 5-min time point (93.5 ± 5.6%). At the end of 30-min drug perfusion, livers on average contained 2.4 ± 0.3% of total perfused dose (liver/perfusate = 2.1 ± 0.3), which decreased ~50 fold to 0.041 ± 0.004% of
perfused metformin during the subsequent 30-min drug-free perfusion. During the 30-60 min
washout perfusion period, metformin present in the liver at 30 min was >300 fold preferentially
excreted into drug-free perfusate than bile (Figure 2, Table 3).
Discussion

Metformin hepatic distribution was previously believed to involve unidirectional vectorial transport from blood to bile, with hepatic uptake by OCT1 and subsequent biliary excretion via MATE1 (Graham et al., 2011; Gong et al., 2012). For the first time, the present study demonstrated that hepatic metformin is not exclusively excreted into bile, but that it is primarily cleared from the liver via sinusoidal efflux. This finding is consistent with the liver being an organ of distribution but not elimination for metformin, and explains why biliary excretion is so low with no evident enterohepatic cycling.

Previously, it has been proposed that the reason for metformin clearance by urinary and not biliary excretion is markedly higher renal OCT uptake activity (Kimura et al., 2005). Considerably less efficient vectorial transport across the liver than renal proximal tubule would be consistent with markedly lower hepatic than renal extraction ratio (~2% and ≤100%, respectively), nearly complete urinary but negligible biliary excretion, yet fairly similar magnitude of tissue distribution (Chou, 2000; Ito et al., 2010; Higgins et al., 2012), and is supported at a gross in vivo pharmacokinetic level (Ito et al., 2010). However, the difference in renal vs. hepatic OCT transport in other studies was not sufficiently large to fully explain the observed difference in extent of urinary vs. biliary excretion (Nishimura and Naito, 2005; Choi et al., 2007). Findings from the present study conceptually support inefficient transport of metformin from blood to bile; however, a major contributor to this inefficiency is extensive sinusoidal efflux, which counteracts OCT1 hepatic uptake, effectively reducing net uptake activity.

Although sinusoidal efflux is an appreciable clearance pathway for hepatic metformin, it merits noting that liver perfusions may have exaggerated its magnitude relative to biliary
Known metformin transporters are classified as secondary active transport, which relies on various electrochemical gradients: electronegative membrane potential for OCTs and proton exchange for MATEs (Giacomini et al., 2010; Zamek-Gliszczynski et al., 2012). The drug-free washout phase of liver perfusion, when sinusoidal and biliary excretion of hepatic metformin were studied, represents a physiologically unrealistic condition in which drug is present in the liver but not in circulation. In this scenario this infinite liver/perfusate concentration ratio may have enhanced the driving force for sinusoidal efflux. As such the >300 fold higher hepatic excretion into drug-free perfusate than bile, may not be physiologically relevant. Furthermore, ~5-fold greater than physiological bile flow/hepatic perfusion rate ratio (~4,300 in single-pass liver perfusion vs. ~880 in vivo (Davies and Morris, 1993)) may have further contributed to the overestimation of metformin sinusoidal excretion.

Potentially elevated driving force for sinusoidal efflux during liver perfusion with drug-free buffer may have in turn resulted in underestimation of the fraction of hepatic metformin excreted in bile (0.2% of liver drug content). This point is supported by lower in vivo metformin bile/plasma concentration ratio (1.3 ± 0.3) and bile/perfusate ratio (0.8 ± 0.1) during liver perfusion with metformin than during the washout phase of perfusion, when bile/perfusate concentration ratio was 10-16 fold higher (12.6 ± 3.2). However, even after correcting for the 10-16 fold concentration ratio difference, the fraction of hepatic metformin excreted in bile would at most be 3.2%. In vivo biliary recovery of metformin dose was only 0.11 ± 0.02%. In comparison, between the systemic Tmax and time of maximal glucose-lowering effect, on average 1.0 ± 0.1% of oral dose was present in the liver. Since biliary recovery was measured to infinity, but hepatic content was determined at individual time points, biliary excretion at most accounts for <10% of total metformin clearance from the liver in vivo. In pervious murine
intravenous infusion studies, biliary dose recovery was $\leq 0.3\%$ with $\sim 2.5\%$ of the dose in the liver at steady state (Maeda et al., 2007; Ito et al., 2010), independently confirming the fraction of hepatic metformin excreted in the bile to be at most $<10\%$ in vivo.

Notably, the current estimate of 0.2% to <10% fraction of hepatic metformin cleared by biliary excretion contradicts the indirect estimate in Mate1-knockout mice. Metformin hepatic distribution was increased 5 fold following genetic ablation of Mate 1 [20-fold increase in hepatic concentration = 4-fold increase in systemic concentration x 5-fold in increase in hepatic distribution] (Tsuda et al., 2009), which puts the metformin fraction excreted by Mate1 from the liver at a considerably higher 80% (Zamek-Gliszczynski et al., 2009). The reasons for this discrepancy remain to be elucidated, but Mate1-knockout findings indicate that the magnitude of increase in hepatic distribution is greater than can be accounted for by biliary excretion alone. Thus, sinusoidal efflux is likely to be additionally impaired due to either direct downregulation (ex., Mate1 is also present on the basolateral membrane or its knockout results in compensatory downregulation of the relevant sinusoidal transporter) or indirect inhibition (ex., absence of Mate1 results in competition for sinusoidal efflux with an accumulated endogenous Mate1 substrate or reduced driving force for sinusoidal efflux). Supporting the notion that impairment of hepatic Mate1 alters metformin hepatobiliary disposition in more ways than just biliary excretion are inhibition studies with pyrimethamine, where hepatic metformin concentrations were increased 3 fold, while biliary excretion was unchanged and insufficiently extensive to explain the magnitude of increase in liver drug concentration (Ito et al., 2010).

MATE1 is localized to the hepatic canalicular and not basolateral membrane (Otsuka et al., 2005), so the mechanism by which MATE1 affects extra-biliary clearance of hepatic metformin may be complex.
Metformin absorption rate-limited oral pharmacokinetics (Stepensky et al., 2001) and route of administration-dependent first-pass pharmacodynamics [intravenous < intra portal < oral (Stepensky et al., 2002)] have raised the possibility of high first-pass hepatic extraction, biliary clearance, and/or enterohepatic recycling during oral drug absorption. Metformin oral bioavailability is adequately high to support enterohepatic cycling (Tucker et al., 1981). Previous intravenous biliary/fecal mass balance studies contradict this first-pass hypothesis (Tucker et al., 1981; Maeda et al., 2007; Ito et al., 2010); however, the possibility was not directly tested following oral administration. The present study unequivocally proved the absence of first-pass biliary excretion, which was similarly low following oral and intravenous dosing, and consequently, enterohepatic cycling was not observed after drug administration by either route. Although in liver perfusions, the hepatic extraction ratio was initially 6.5% vs. 1.2% at steady state, biliary dose recovery was nonetheless low over the entire perfusion period.

In conclusion, the present study demonstrated that metformin biliary excretion is negligible due to predominant sinusoidal efflux from the liver, which helps explain why the liver is an organ of distribution and not elimination. The data unequivocally support the absence of enterohepatic cycling following oral drug administration, disproving this potential contributing factor to metformin absorption rate-limited oral pharmacokinetics and first-pass pharmacodynamic effects.
Authorship Contributions

Participated in research design: Zamek-Gliszczynski, Day, Bao, Higgins

Contributed new reagents: N/A

Conducted experiments: Day, Bao

Performed data analysis: Zamek-Gliszczynski, Day, Bao, Higgins

Wrote or contributed to the writing of the manuscript: Zamek-Gliszczynski, Higgins, Bao
References


Figure Legends:

Figure 1. Metformin plasma concentration-time profiles in bile duct intact- (closed circles) and cannulated-rats (open circles) following administration of metformin as (A) a 5-mg/kg IV bolus dose, or (B) a 10-mg/kg oral dose, and the corresponding biliary concentrations in bile duct cannulated-rats (open triangles); n = 5-6/group.

Figure 2. Metformin outflow perfusate concentrations (open squares), sinusoidal excretion rate during washout phase of perfusion (closed squares), biliary concentrations (open triangles), biliary excretion rate (closed triangles), and liver concentrations (stars). Livers were perfused in a single-pass manner with 10 μM metformin between 0-30 min and were perfused with drug-free buffer between 30-60 min, when sinusoidal and biliary excretion of hepatic metformin were studied; n = 6 (0-30 min), n = 3 (30-60 min).
Table 1: Percentage of metformin oral dose in liver or kidneys over a pharmacologically-relevant dose range and time frame in mice (n = 3-4 mice/time/dose).

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Time Post Dose</th>
<th>Liver</th>
<th>Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5 hr</td>
<td>2.0 hr</td>
<td>2.5 hr</td>
</tr>
<tr>
<td>10</td>
<td>1.40 ± 0.19%</td>
<td>0.81 ± 0.14%</td>
<td>0.63 ± 0.08%</td>
</tr>
<tr>
<td>30</td>
<td>1.64 ± 0.33%</td>
<td>1.12 ± 0.04%</td>
<td>1.79 ± 0.95%</td>
</tr>
<tr>
<td>60</td>
<td>0.97 ± 0.17%</td>
<td>0.89 ± 0.16%</td>
<td>0.49 ± 0.04%</td>
</tr>
<tr>
<td>100</td>
<td>2.36 ± 0.30%</td>
<td>0.77 ± 0.07%</td>
<td>1.48 ± 0.10%</td>
</tr>
<tr>
<td>300</td>
<td>0.20 ± 0.06%</td>
<td>0.19 ± 0.03%</td>
<td>0.14 ± 0.04%</td>
</tr>
</tbody>
</table>
Table 2: Metformin pharmacokinetics in bile duct intact- and cannulated-rats (n = 5-6/group).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bile Duct-Intact Rats</th>
<th>Bile Duct-Cannulated Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IV Dose (mg/kg)</strong></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AUC_{0-t} (ng*hr/mL)</td>
<td>1750 ± 71</td>
<td>1720 ± 45</td>
</tr>
<tr>
<td>AUC_{0-∞} (ng*hr/mL)</td>
<td>1790 ± 72</td>
<td>1810 ± 43</td>
</tr>
<tr>
<td>CL (mL/min/kg)</td>
<td>46.9 ± 1.8</td>
<td>46.3 ± 1.1</td>
</tr>
<tr>
<td>V_{d,ss} (mL/kg)</td>
<td>7280 ± 433</td>
<td>6230 ± 511</td>
</tr>
<tr>
<td>Biliary Recovery (% Dose)</td>
<td>N/A</td>
<td>0.16 ± 0.0</td>
</tr>
<tr>
<td><strong>PO Dose (mg/kg)</strong></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>AUC_{0-t} (ng*hr/mL)</td>
<td>2740 ± 153</td>
<td>2430 ± 404</td>
</tr>
<tr>
<td>AUC_{0-∞} (ng*hr/mL)</td>
<td>2790 ± 150</td>
<td>2480 ± 407</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>672 ± 57</td>
<td>691 ± 87</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>1.1 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>T_{1/2} (hr)</td>
<td>6.4 ± 0.4</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>F (%)</td>
<td>78 ± 4</td>
<td>71 ± 11</td>
</tr>
<tr>
<td>Biliary Recovery (% Dose)</td>
<td>N/A</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

N/A = not applicable

Significant differences are not indicated, because none were present.
Table 3: Metformin hepatobiliary disposition in single-pass rat liver perfusions. Livers were perfused with 10 μM metformin for 30 min and then with blank buffer for the following 30 min (n = 6, 0-30 min; n = 3, 30-60 min and liver values).

<table>
<thead>
<tr>
<th>Recovery of Perfused Drug During 0-30-min Metformin Perfusion</th>
<th>(% Perfused Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile</td>
<td>0.021 ± 0.003%</td>
</tr>
<tr>
<td>Liver (30 min)</td>
<td>2.4 ± 0.3%</td>
</tr>
<tr>
<td>Perfusate</td>
<td>97.6 ± 11.6%</td>
</tr>
<tr>
<td>Total</td>
<td>99.1 ± 11.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recovery of Hepatic Metformin During 30-60-min Washout Perfusion</th>
<th>(% of Hepatic Metformin Content at the end of 30-min Metformin Perfusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile</td>
<td>0.222 ± 0.003%</td>
</tr>
<tr>
<td>Liver (60 min)</td>
<td>1.9 ± 0.3%</td>
</tr>
<tr>
<td>Perfusate</td>
<td>74.0 ± 39.3%</td>
</tr>
<tr>
<td>Total</td>
<td>76.1 ± 39.5%</td>
</tr>
</tbody>
</table>
Figure 1

A.

B.
Figure 2