The clinically-relevant drug-drug interaction (DDI) between the dyslipidemia drug gemfibrozil and the insulin secretagogue repaglinide is well-documented throughout the literature. Co-administration of repaglinide with gemfibrozil causes a DDI associated with an up to 8-fold increase in repaglinide plasma concentration (Kass et al., 2012; Säll et al., 2012). In that study, negligible effects on drug metabolizing liver enzyme activity were observed in vivo (Bidstrup et al., 2003). In vitro, hepatocytes were exposed to gemfibrozil glucuronide pretreatment for two days followed by administration of repaglinide alone or co-dosed with gemfibrozil glucuronide. The observed plasma clearance and volume of distribution were both approximately 200-fold higher in the presence of gemfibrozil glucuronide, a decrease in repaglinide uptake was observed at 30 min at 0.5 \( µM \) repaglinide, and 10.2% and 11.5% repaglinide uptake inhibition at 3 and 10 min, respectively, was observed in rat hepatocytes co-dosed with repaglinide and gemfibrozil compared to vehicle-pretreated hepatocytes alone. Similar gemfibrozil glucuronide pretreatment (6). At 0.5 \( µM \) repaglinide, following 2 days of hepatocyte treatment with gemfibrozil, 36.2% and 27.9% repaglinide uptake inhibition at 3 and 10 min, respectively, was observed in rat hepatocytes co-dosed with repaglinide and gemfibrozil compared to vehicle-pretreated repaglinide-dosed hepatocytes. Gemfibrozil glucuronide pretreatment and co-dosing resulted in 54.4% repaglinide uptake inhibition at 3 min and 37.8% inhibition at 10 min. (Figure 6).

Repaglinide uptake in rat hepatocytes treated once with repaglinide in the presence and absence of 100 \( µM \) gemfibrozil or 100 \( µM \) gemfibrozil glucuronide.

In control rats, the vast majority of the repaglinide-related material was excreted in bile (Figure 3a). The excretion profile for repaglinide in these rats is consistent with the results observed in repaglinide-dosed rats (Figure 3b). Following gemfibrozil treatment, more of both repaglinide and metabolite M1 was excreted in urine than in bile. Consistent with the control rat data, both repaglinide and MET were almost completely eliminated via bile excretion. In contrast, approximately 20-50% of metabolites M0, M2, and M4 were excreted in urine by the gemfibrozil-treated rats. Neither M0-M4 nor M4 was detected in the control rats (Kalhspe et al., 2008). These factors have been implicated in the clinical interaction.

Recently, interest in animal models for DDI potential assessments has increased as utility has been demonstrated (Marathe and Rodrigues, 2010). A gemfibrozil and repaglinide DDI resulting in increased repaglinide and metabolite plasma exposure (>3-fold) and a vectoral shift in elimination pathways from biliary to urinary excretion following administration of gemfibrozil was previously established in male Sprague-Dawley rats (Barbara et al., 2014). Rats were dosed according to Scheme 1. In that study, negligible effects on drug metabolizing liver enzyme activity were observed following gemfibrozil treatment, suggesting that the DDI in rats was unlikely to be caused by gemfibrozil altering drug metabolizing enzyme activity (e.g., by P450 or UGT inhibition). Repaglinide uptake in hepatocytes was recently characterized, with a derived active uptake clearance value of 299 \( µM/\text{min/mL} \) at baseline, and passive permeability clearance of 58.2 \( µM/\text{min/mL} \) at baseline (Table 1, 2011). In the present study, the potential role of hepatic uptake transporter proteins in the rat repaglinide / gemfibrozil interaction was explored in vitro.

### Materials & Methods

Experiments were performed to evaluate the effects of gemfibrozil and repaglinide on glucose transport in cultured cryopreserved rat primary hepatocytes.

#### Single dose experiment:
Hepatocytes were seeded on 24-well biocoat plates at approximately 1.2 million cells/well. The hepatocytes were allowed to adapt for 48 h in culture with a Matrigel overlay, after which they were treated once daily for two consecutive days with vehicle control (0.1% DMSO) or 100 \( µM \) repaglinide or gemfibrozil glucuronide. On day 3, the hepatocytes were treated with repaglinide (0.5, 5.0 \( µM \)) or gemfibrozil (50 \( µM \)), and the hepatocytes were treated with repaglinide coadministered with 100 \( µM \) gemfibrozil. Repaglinide uptake was evaluated at 1, 3, 5, 10, 15 and 30 min.

#### Pre-treatment experiment:
Hepatocytes were seeded on 24-well collagen-coated plates at approximately 1.2 million cells/well. The hepatocytes were allowed to adapt for 48 h in culture with a Matrigel overlay, after which they were treated once daily for two consecutive days with vehicle control or 100 \( µM \) gemfibrozil. The hepatocytes were treated with repaglinide coadministered with 100 \( µM \) gemfibrozil. Repaglinide uptake was evaluated at 1, 10, 30, 60 and 120 min.

At each time point, incubation media was removed, cells were washed once with 0.2% BSA in PBS, twice with PBS, lysed with organic solvent containing repaglinide ethyl-d, internal standard, and analyzed for repaglinide uptake by LC/MS/MS against a calibration curve (1 to 5000 nM).

### Results

In the previous study, marked changes in repaglinide AUC were observed in rats treated with gemfibrozil (Figure 2). Gemfibrozil-treated rats exhibited a 4-fold higher repaglinide C_{max} and a 3.5-fold greater AUC_{0-24h}, than control rats, but the LTL (1.2 and 1.7 h) values were similar between treatment groups (Table 1). The observed plasma clearance and volume of distribution were both approximately 60% lower in repaglinide-treated rats than control rats (Table 1). Plasma levels of the selected metabolites M0-M4 did not change significantly between vehicle and gemfibrozil-treated rats than control rats. Previously, inhibition of CYP or UGT enzymes was ruled out as a possible cause for the observed DDI using multiple experimental approaches (Barbara et al., 2014).

### Conclusions

- Co-administration of gemfibrozil and its glucuronide metabolite with repaglinide slightly reduced repaglinide uptake in rat hepatocytes.
- The effects observed with the single-dose experiment were minimal and disproportionate to the observed 4-fold exposure increase and change in vectoral elimination pathway observed in vivo.
- Pretreatment of rat hepatocytes with gemfibrozil glucuronide for 48 h, along with co-administration, resulted in a marked time-dependent, inhibition of repaglinide glucuronidation.
- Gemfibrozil glucuronide pretreatment and co-administration caused more extensive repaglinide uptake inhibition than gemfibrozil pretreatment and co-administration. The authors would like to acknowledge Dr. Brian Ogilvie for critical scientific review and discussion.