Pumping new life into preclinical pharmacokinetics: exploring the pharmacokinetic application of ex vivo organ perfusion

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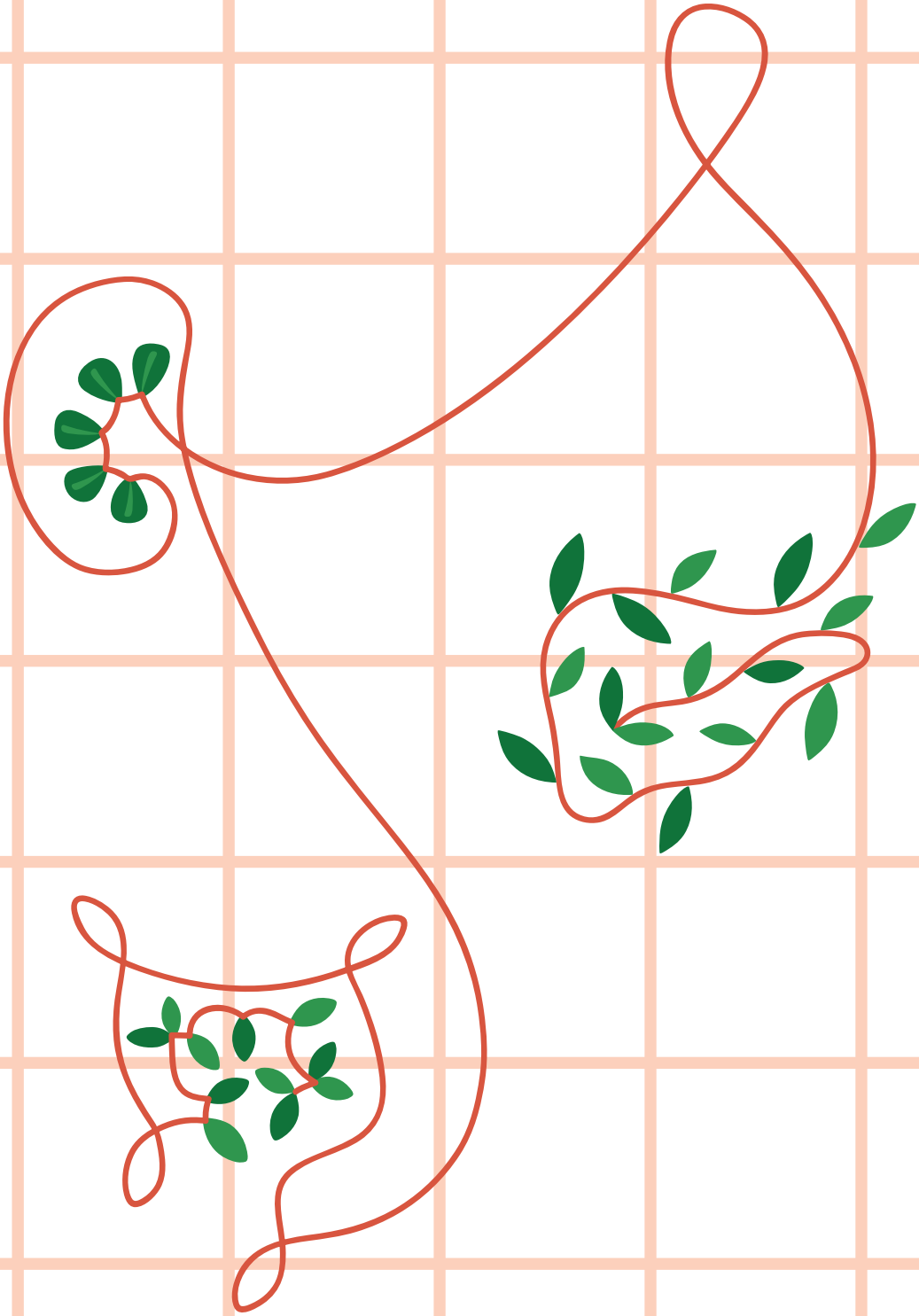
PART IV

Summary, future perspectives and conclusions



CHAPTER 07

Summary, future perspectives and conclusions

Summary

The ability to predict the PK profile of drugs in development remains a challenging process with a very poor success rate[1]. Preclinical studies are an important part of the drug discovery process, which aim to provide information regarding the efficacy and safety of the drug[2]. To this end, a comprehensive understanding of the ADME and PK profile is essential. To study ADME processes, *ex vivo* preclinical models can be regarded as the bridge between *in vitro* and *in vivo* models which is discussed in Part I of this thesis. In chapter 2 we provided an overview of the experimental predictive *ex vivo* models available to study drug ADME processes as well as DDI, in health and disease. The use of normothermic machine perfusion facilitates to study organ function under dynamic and as close as possible to the *in vivo* setting. The intact morphological structure, application of physiological blood flow rates and presence of intact elimination pathways are important characteristics in the field of pharmacology. These aspects, which cannot be adequately simulated in simplified *in vitro* models, provide valuable insights into substrate affinity for transporters, DDI and elimination routes. The objective of this thesis was to explore the applicability of pressure driven normothermic organ perfusion to study pharmacological processes in liver, intestine and kidney. In the different chapters we have shown the use of liver perfusion and the multi-organ model to characterize pharmacokinetic processes like DDI, endogenous substrate handling, pre-systemic intestinal and hepatic metabolism and excretion profiles.

In part II, we studied the applicability of normothermic machine perfusion of the liver to study drug pharmacokinetics and endogenous substrate handling. As a first step, in chapter 3, we used the porcine NMP model to investigate whether the perfusion model is a suitable platform to mimic clinical observed OATP mediated DDI[3,4]. We have demonstrated that NMP of porcine livers is a potential novel and reliable model to study OATP-mediated DDI and we showed its effect on hepatic clearance, biliary excretion and perfusate (metabolite) profile of statins. Overall, the rank order of DDI magnitude indicated in our experiments was in good agreement with clinical data. The lowest DDI for pitavastatin (AUC ratio 2.6) and the highest for atorvastatin (AUC ratio 7.2), indicating the potential importance of this new *ex vivo* model in early drug discovery.

Translation of preclinical findings using animal derived tissue like the porcine liver model to clinical practice remains challenging due to, among others, species differences in transporter expression[5,6]. On top of this, liver disease in humans leading to cirrhosis can affect liver morphology and transporter expression thereby affecting drug PK profiles. However, with the currently available preclinical and clinical models, it continues to be difficult to study the effect of these pathological changes on drug PK. In chapter 4 we showed for the first time the use of explanted human diseased livers as a model to assess the effect of liver cirrhosis on drug PK by measuring hepatic extraction, biliary clearance, DDI transporter function using 4 model drugs. We successfully perfused 7 cirrhotic livers and 4 non-cirrhotic livers for a period of 360 min, maintaining liver viability and functionality. Hepatic clearance of rosuvastatin and digoxin showed to be the most affected by cirrhosis with an increase in C_{max} of 11.5 and 2.9 times, respectively, compared to non-cirrhotic livers. No major differences were observed for metformin and furosemide. Interaction of rosuvastatin or digoxin with perpetrator drugs were more pronounced in non-cirrhotic livers (AUC ratio of 5.6 and 8.1 respectively) compared to cirrhotic livers (AUC ratio of 1.4 and 2.2 respectively). Studying drug pharmacokinetics using explanted human livers can serve as a basis to explore the differences in hepatic handling of drugs for patients with different types of hepatic impairment.

An advantage of the perfusion model is to determine specific functions of the whole organ such as the hepatic first pass effect and biliary excretion in an isolated environment in the absence of other systemic effects. However, the liver is a central organ in the human body and is in close connection to the intestines linked by the portal blood flow receiving nutrients, bile acids and hormones which activate or inhibit certain pathways[7-9]. Bile acids regulate their own homeostasis by providing negative feedback on bile acid biosynthesis. Bile acids inhibit CYP27A1, CYP7A1 and CYP8B1 by activating FXR, which upon activation also prevents toxic intracellular accumulation of bile acids by inhibiting bile acid uptake and stimulating bile acid export[10,11]. The currently used NMP protocols, which are widely applied in clinical as well as research settings, fall short of mimicking the natural functioning of the liver. This limitation arises from the absence of a recirculating bile acid pool as they rely solely on the infusion of taurocholic acid. This places a substantial burden on the liver during NMP as it is forced to engage in the *de novo* synthesis without the support of endogenous bile acids. In chapter 5, we addressed this gap and

aimed to characterize the *de novo* bile acid synthesis by profiling the biliary bile acid excretion, cholesterol homeostasis and transporter expression during *ex vivo* liver NMP. We showed that in porcine and human perfused livers, bile acid synthesis rates were above average reported values *in vivo* and decreased cholesterol perfusate levels were observed. Additionally, a decreased expression of bile acid synthesis related genes, increased gene expression of cholesterol metabolism related genes and a decreased expression in bile acid-dependent uptake and efflux transporters was observed after 360 min of human and porcine liver perfusion. Replacing taurocholate infusion with a more representative bile acid pool for the enterohepatic circulation has yielded promising results. The infusion of a bile acid mixture containing (un)conjugated bile acids showed a decreased release of hepatic injury markers and the maintenance of stable cholesterol levels in the perfusate. This approach has also shown that the infusion of (un)conjugated bile acids enhanced liver function pointing towards potential advancements in liver preservation and transplantation techniques.

In Part III, we studied PK processes through the perfusion of en-bloc porcine *ex vivo* abdominal organs. Real time characterization of the first-pass effect of orally administered drugs consisting of local intestinal absorption and metabolism, portal vein transport and hepatobiliary processes remains challenging[12]. In chapter 6, we showed the development of a porcine *ex vivo* perfusion model consisting of multiple abdominal organs and demonstrated its capabilities and potential use in studying ADME processes. Using this model, we were able to characterize pre-systemic extraction of midazolam by measuring the intestinal (E_G of 0.22) as well as hepatic extraction (E_H 0.65). As a result, oral bioavailability showed to be 0.27±0.05 which is in line with pig *in vivo* data. By employing this approach, valuable insights can be generated into the absorption and metabolism of new drugs, thereby facilitating the development and optimization of drug candidates for human use.

Future perspectives

NMP holds major potential for the field of pharmacology and drug development. Besides offering the opportunity to enhance the mechanistic understanding of ADME and PK processes of known and marketed drugs, it may also serve a platform to study the PK and efficacy of novel types of drugs.

The close to physiology representation and ability to control experimental settings is a hugh asset over conventional preclinical models. *However, can ex vivo models, particularly normothermic machine perfusion, provide a better understanding of DDI?* In this thesis, we showed the development and application of novel perfusion models like the human explanted liver model and the multi-organ perfusion model. *How can multi-organ perfusion models enhance our understanding of drug pharmacokinetics?* And *the future potential of explanted human diseased organs for ex vivo perfusion researc*h will be discussed. Finally, the key question remains; *How do ex vivo models translate to in vivo PK profiles?*

Better understanding of DDI through *ex vivo* perfusion models?

In chapter 3 and 4 of this thesis, the applicability to study DDI in perfused porcine and human livers was studied. The FDA guidance for industry, for *in vitro* and *in vivo* drug interaction studies, states that it is important to determine if a new drug is a substrate for Pgp, BCRP, OATP1B1/1B3, MATE and/or OCT2 since these transporters interact with drugs in clinical use[13,14]. In chapter 3, we showed that it was possible to mimic DDI at the transporter level and showed that the rank-order of DDI between statins was in good agreement with clinical data[4,15]. The porcine liver model showed to be a suitable platform to study transporter mediated hepatic uptake and/or transporter mediated biliary excretion. This is particularly valuable for drugs in development that are suspected to have the potential to induce or inhibit transporters or face other potential transporter mediated challenges. To illustrate, compound X, a drug in development, showed non-linear kinetics upon increasing dose levels in a phase I study. The underlying mechanism was suspected to be Pgp mediated saturation of biliary excretion. This was evaluated in our normothermic perfusion model using pig livers (Figure 7.1). Upon a step wise 3-fold increasing dose levels (0.56 mg, 1.67 mg, 5.0 mg, and 15.0 mg), we demonstrated that the AUC increased 3.3, 3.9, and 7.1 times, respectively (Figure 7.1A). This non-linearity effect was observed at dose levels >1.67 mg of compound X. Additionally, an increase in T_{max} from 6 to 15 min was observed in this study, also pointing towards decreased excretion rate at higher dose levels. The excretion of compound X into the bile decreased upon increasing the dose level from 0.56 mg (26% of dose excreted into bile) to 15.0 mg (15% of dose excreted into bile) (Figure 7.1B). Additionally, compound X showed to accumulate in the liver upon higher dose levels (Figure 7.1C). These results

indicated saturation of biliary excretion of compound X at dose levels >1.67 mg, which might be caused by transporter mediated saturation of biliary excretion.

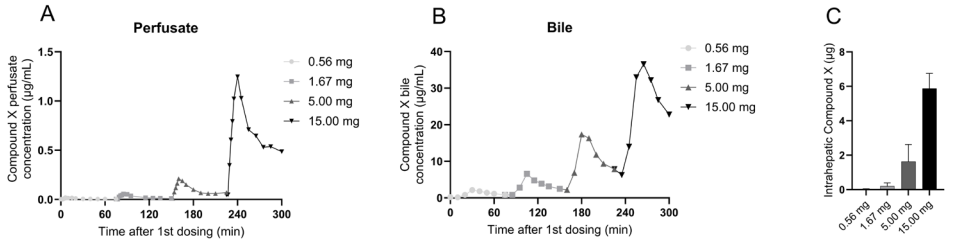


Figure 7.1 - Studying increasing dose levels to determine non-linear kinetics in the ex vivo porcine liver perfusion model. Increasing dose levels of 0.56 mg, 1.67 mg, 5.00 mg and 15.00 mg in (A) perfusate, (B) Biliary excretion of compound X upon increasing dose levels and (C) intrahepatic accumulation of compound X in biopsies taken at the end of each dosing.

To confirm Pgp mediated biliary efflux of compound X, a DDI experiment was designed like in chapter 3 was performed of this thesis. The Pgp inhibitor quinidine was applied as perpetrator drug and upon co-infusion with compound X a potential DDI was simulated (Figure 7.2A-C). Upon co-administration of Pgp inhibitor quinidine (22.4 mg), the plasma AUC increased 1.37-fold (Figure 7.2A) compared to the PK profile of compound X alone. The increased plasma AUC can be explained by diminished biliary excretion of compound X in the presence of Pgp inhibitor quinidine, resulting in 44% decrease in biliary excretion (AUC ratio 0.56) (Figure 7.2B). These results suggest that Pgp is actively involved as a biliary efflux transporter for clearance of compound X upon hepatic uptake. The results were also in line with digoxin, which was used as a positive control and known Pgp substrate (data not shown). After assessing Pgp involvement, in a follow up study also the OATP1B1/1B3 involvement was studied as OATP1B1/1B3 was suspected to be the main hepatic uptake transporter. In a separate study, this was studied by applying cyclosporin as inhibitor for OATP1B/1B3 (Figure 7.2D). Upon co-administration of cyclosporin A, the plasma AUC increased 1.27 times, demonstrating a slight inhibition of OATP-mediated hepatic uptake of compound X. To give more insight into OATP1B/1B3 involvement, bilirubin, the endogenous biomarker for OATP1B1/1B3 function was measured in perfusate (Figure 7.2G-H). The results illustrate that following each administration of compound X, there was a noticeable increase in bilirubin levels, indicating competition for hepatic uptake of bilirubin through the OATP transporter.

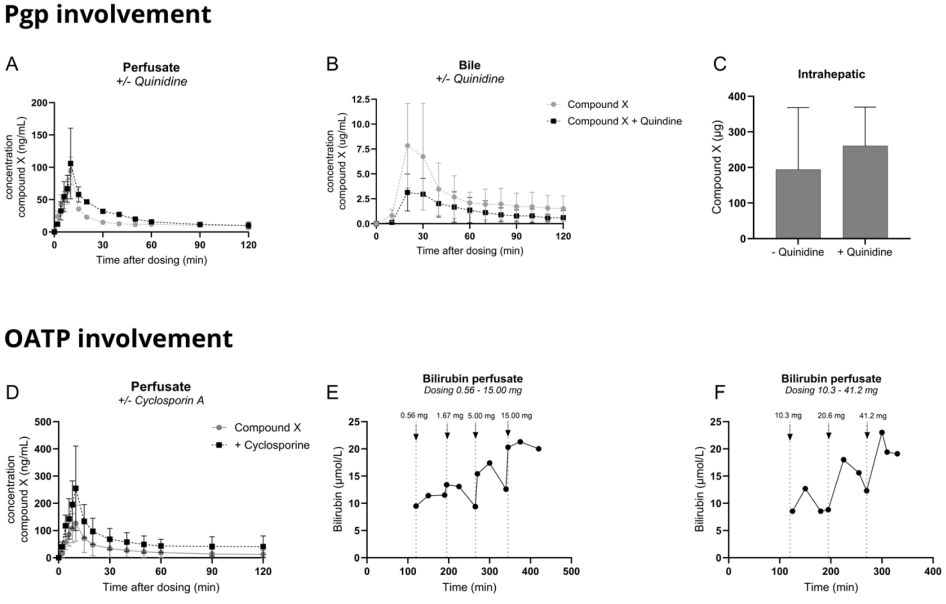


Figure 7.2 - Determination of Pgp and OATP involvement with the use of inhibitor dosing using the ex vivo porcine liver perfusion model. (A) perfusate (B) biliary excretion and (C) intrahepatic accumulation of compound X (dosed 1.67 mg) in the absence and presence of P-gp inhibitor quinidine dosed at 22.4 mg. Studying OATP involvement in (D) perfusate of compound X (dosed 1.67 mg) in the absence and presence of OATP inhibitor Cyclosporin A (22.4 mg). Bilirubin in perfusate was assessed as endogenous biomarker for OATP in (E) studies with infusion of 0.56 – 15.00 mg and (F) 10.3-41.2 mg.

Together these data clearly show that the porcine liver perfusion model holds great potential to study transporter involvement in a DDI design, which helps towards a better understanding of the uptake and excretion of drugs. Moreover, the ability to study DDI is crucial because it helps to ensure safe and effective medication use, minimizing potential risk and optimizing patient outcomes.

During the liver perfusion studies we infused the perpetrator drug, 5 minutes prior to the victim drug thereby simulating DDI. This setup has been employed in various other isolated liver perfusion studies as well[16]. However, *in vivo*, following oral administration of a drug, the rate and extent of intestinal absorption determines the portal vein concentration which differs between drugs. Bioavailability of a drug is therefore also affected by factors as dissolution, intestinal transit time and permeability, biotransformation by the

intestinal flora and gut wall metabolism[17]. Consequently, measuring DDI in a multi-organ perfusion model approach by dosing both drugs via the duodenum would result in an even more physiological representation of the biology. As demonstrated in chapter 3, varying perfusate rifampicin levels were observed in the condition with atorvastatin, leading to variation in the degree of DDI. The intestine thus plays a crucial role in the regulating the portal vein concentration and thus regulating the magnitude of hepatic DDI[18].

Besides DDI at the transporter level, the interaction can also occur at the (metabolizing) enzyme level as drugs can be an inhibitor or an inducer of these enzymes[13]. Rifampicin, for instance, showed to interact with the PK of several statins via interaction with drug transporters, while long term rifampicin administration (>7 days) results in the induction of the metabolizing CYP3A4 enzyme, thereby also showing that studying long-term effects can be crucial[19]. Interestingly, the first few reports in literature demonstrated the ability to extent normothermic perfusion times which offer a promising avenue to investigate drug induced CYP450 modulation over time[20-22]. Bridging the field of transplantation and pharmacology highlights the importance of long-term liver perfusion as a valuable approach to study CYP450 modulation. To take it one step further, several publications now report the possibility to perfuse split livers[21,23-25] which is an interesting and safe approach for studying drug induced CYP450 enzyme expression. Splitting the liver into two parts enables exposure of one half to the drug while the other half serves as a control. The use of for instance the 'Basel cocktail' containing specific substrates for: CYP1A2 (caffeine), CYP2B6 (Efavirenz), CYP2C9 (Flurbiprofen), CYP2C19 (omeprazole), CYP2D6 (metoprolol) and CYP3A4 (midazolam) can be applied to determine effects on the PK of these certain compounds[26].

Next to the potential that drugs can modulate drug metabolizing enzymes, some drugs have the potential to modulate cytokine release and induce an inflammatory environment which subsequently can suppress of elevate CYP450 enzymes which is known as a drug-biologic interaction. These type of drugs are commonly used for the treatment of cancer as well as inflammatory and immunologic diseases indicating a broad therapeutic range and use[27]. Drug-biological interactions are currently being studied in primary hepatocyte cultures, which are treated with different concentrations of cytokines to study the effect on CYP450 activity and mRNA/protein expression[28-30]. To illustrate, multiple *in vitro* studies show effects of IL-6, IL-1β, TNF-α and IFN-γ on the

expression of different CYP450 enzymes in primary hepatocytes[28,31-34]. Klein et al.[35] showed a significant reduction in the formation of the CYP3A4-derived atorvastatin metabolite after 48h and 72h incubation upon dosing 10 ng/mL of IL-6 in HepaRG cells. Additionally, a significant reduction was shown in the CYP3A4 gene expression. The liver perfusion model described in this thesis would be an interesting platform to study whether certain biologics modulate cytokine levels and subsequently alter CYP450 status and function. However, crucial in these studies is the understanding that the cytokine releasing effect of the biologic drug comes specifically from the drug itself and is not elicited by the perfusion process. Conducting a literature search on cytokine release during *ex vivo* organ perfusion highlighted the complexities inherent in this process, revealing that this subject is more challenging than initially anticipated. In a study by Gravante et al.[36], the researchers studied the potential cytokine response to ischemia reperfusion injury in an *ex vivo* porcine liver perfusion model. Significant elevation of IL-6 and IL-8 was observed after 6 hours of perfusion. Additionally, Chung et al.[37], Weissenbacher et al.[38] and Hosgood et al.[39] also showed release of a subset of cytokines (e.g. IL-6, IL-8, IFN-y) during liver and/or kidney perfusion[40]. In healthy individuals, baseline IL-6, IL-8 and IFN-y concentrations are around 5, 12 and 50 pg/mL respectively[41]. IL-6 levels reported in perfusion studies are in the range of ng/mL showing a thousand-fold difference between *in vivo* conditions and *ex vivo* perfusion studies[36,38,39]. To put this in perspective, IL-6 levels in the ng/mL range have been reported for critically ill patients with severe infections like sepsis[42]. This indicates ongoing cytokine release and inflammatory environment which could also influence the CYP450 expression. The use of a hemoadsorption filter has been recommended for eliminating cytokines in the treatment of severe inflammatory driven medical conditions. Hosgood et al.[43] showed that the use of a hemoadsorption filter during kidney perfusion resulted in lower and stable cytokine levels during 6 hours of perfusion. The addition of the cytosorb filter reduced the IL-6 and IL-8 concentration by 87% and 59% respectively[43]. From a pharmacological perspective and for future PK perfusion studies, it would be recommended to include an adsorbent membrane to diminish the inflammatory environment and thereby not affecting the CYP450 enzyme abundance and activity.

How can multi-organ perfusion models enhance our understanding of drug pharmacokinetics?

In chapter 6, we showed the possibility to perfuse multiple organs and subsequently study the ADME profile of midazolam. The ability to study the gut-liver axis offers a unique opportunity to unravel the dynamic interplay between gut-wall metabolism and hepatic uptake and metabolism. We showed the ability to measure pre-systemic intestinal and hepatic metabolism for midazolam, a widely applied CYP3A4 substrate model compound. Up to now, no preclinical models have been developed which directly give insight into the gut wall absorption and metabolism. This is mainly because the intestine is a heterogenous organ and therefore difficult to capture all its function into one *in vitro* model[44,45]. Therefore, PBPK modeling is often needed to generate insight into the extent of the fraction escaping first pass gut wall metabolism, the F_G. Gertz et al.[46] did build a PBPK model to predict the F_G, using microsomal fractions as input data. The authors showed that drugs with a low intestinal extraction could in general be well predicted, however the prediction of high intestinal extraction drugs was less accurate[46]. Current assessment of the F_G is based on plasma concentration time profiles of IV versus oral dosing or concentration time profiles after dosing an inhibitor[18,46,47]. Although the abundance of CYP3A4 in the intestine is around 1% of the abundance in liver, CYP3A4 substrate drugs as midazolam show extensive intestinal wall metabolism[48]. The lower blood flow in the intestinal mucosa compared to the liver blood flow, results in an extended duration of a compounds presence in the intestinal tissue and thereby increasing the likelihood of CYP450 mediated metabolism in the intestine compared to the liver which underscores the difficulty to predict CYP450 mediated metabolism in *in vitro* models[18]. The utilization of the multi-organ perfusion model can provide helpful insights into determination of the F_G since it allows the opportunity to collect samples from, among others, the portal vein. The ability to take portal vein samples has only been described by Paine et al.,[47] who studied the intestinal midazolam metabolism in patients undergoing liver transplant surgery in the anhepatic phase. Interestingly, the researchers demonstrated that after IV dosing, there is a higher concentration of the midazolam metabolite 1-OH midazolam in the portal vein compared to the systemic circulation. This indicates that there is basolateral uptake of midazolam with subsequent midazolam oxidation to 1-OH midazolam which is transported back to the portal vein. Studies comparing IV versus oral dosing, like those exemplified here, represent a future application

of the multi-organ model. Such investigations offer valuable insights into the precise metabolism of (new) drugs and serve as input for PBPK modeling.

Besides phase I metabolism, the liver and intestine are also involved in phase II metabolism e.g. glucuronidation and sulfation[49]. After CYP450 mediated metabolism, compounds can undergo further biotransformation, for instance by glucuronidation, whereafter the glucuronidated product can be excreted via the biliary system. Interestingly, it is observed in *ex vivo* fermentation platforms that the gut microbiota also can also contribute to metabolism[50]. An example is the metabolism of irinotecan. Irinotecan is a pro-drug and is metabolized by the liver to SN-38 and subsequently glucuronidated to SN38-glucuronide and eliminated via biliary excretion[51]. After biliary excretion, the intestinal microbiota can deconjugate the SN38-G to SN-38. This is followed by intestinal absorption of SN38 to the portal venous blood, whereafter again glucuronidation can occur, thereby resulting in a prominent secondary plasma peak. This process involving phase I, phase II metabolism, biliary excretion and intestinal absorption is extremely difficult to capture in *in vitro* models as well as via PBPK modelling. Nevertheless, its significance is exemplified by Gupta et al.,[51] who showed that patients with lower rates of hepatic glucuronidation would have higher concentrations of biliary SN38, leading to gastrointestinal toxicity. The multi-organ perfusion model presented in this thesis holds potential to study these dynamic processes to understand the specific role of each organ contributing to the metabolism of the drug. To gain even a better understanding of organ specific drug metabolism during multi-organ perfusion, microdialysis emerges as a powerful tool as it allows for real-time monitoring and in-depth insights into the metabolic pathways. Microdialysis sampling is a technique often used in the field of neurosciences to study biochemical conversions in the extracellular fluid[52]. The techniques consists of a probe with a hollow fiber dialysis membrane which can easily be implanted in a (perfused) organ. This allows for real time monitoring of the extracellular fluid and thus real-time monitoring of PK processes like phase I and II metabolism[53], study drug unbound concentrations[54] or (blood flow dependent) tissue penetration. Until now, the use of microdialysis in organ perfusion has only be described in the field of *ex vivo* lung perfusion. Mazzeo et al.[55] described the use of microdialysis during *ex vivo* lung perfusion and reported that microdialysis was more effective and specific in studying lung metabolism compared to perfusate levels. Continuous sampling from the microdialysis flow in the intestine and liver would be beneficial and informative to study the distribution and

metabolism profile of drugs with complex ADME processes (e.g. phase I, phase II, EHC). Thereby in depth characterization of the metabolic pathway will enable better PBPK predictions.

Use of explanted human diseased organs for *ex vivo* perfusion research

The use of human tissues for pharmacological studies is superior over other species. In chapter 4, we showed the use of explanted human diseased livers for PK research. So far, the utilization of human diseased explanted livers is mentioned in a limited number of publications[56-58]. However, the applicability of human diseased livers for *ex vivo* perfusion research has major potential for instance to gain in depth information on disease-specific processes and the role in PK and even pharmacodynamic processes. For example, MAFLD is one of the most important causes of liver disease worldwide[59]. Non-alcoholic liver disease (NASH) is an advanced form of MAFLD and can potentially progress to cirrhosis and hepatocellular carcinoma. NASH is one of the most common indications for liver transplant, alongside alcoholic cirrhosis, hepatocellular carcinoma, hepatitis C related cirrhosis and cholestatic disease[59,60]. There are currently no therapies available for the treatment of MAFLD, NASH or ALD. Nevertheless, notable progress is being made in drug development regarding oligonucleotide-based treatments[61]. Oligonucleotide- based therapeutics are currently an emerging class of drugs which include short interfering RNA (siRNA) that degrade target mRNA[61]. So far, only a limited number of oligonucleotides have progressed to clinical stages[62]. The predominant challenge thus far has been securing the safe and effective intracellular delivery of these compounds in human tissues. A disadvantage of lipid nanoparticle delivery is for instance the high concentration needed and inducing a pro-inflammatory effect[63,64]. Given the abundance of disease targets in the liver which are susceptible to modulation, the liver is an interesting target for oligonucleotides therapies[61,65]. Therefore, *ex vivo* organ perfusion and especially *ex vivo* perfusion using diseased human livers would be a first step bridging the gap between preclinical *in vitro* and clinical *in vivo* studies. Utilizing explanted diseased human livers with NASH or ALD, uptake and gene modification can be assessed by leveraging the disease characteristics. Several oligonucleotides have been described which target NASH[63,66,67]. Linden et al.[67] for instance, demonstrated in a mice model the use of a conjugated antisense oligonucleotide which mediated silencing of the gene Pnpla3 and subsequently reduced liver steatosis score and fibrosis[67]. Exploring the application of these type of therapeutics in a liver perfusion model with explanted NASH or MAFLD

livers would provide a valuable opportunity to study tissue uptake, potential local toxicity effects or immune effects. First reports already describe the use of siRNA during *ex vivo* liver perfusion[68-70]. Bonaccorsi et al.[69] aimed to inhibit an apoptosis-associated gene using an siRNA approach in a rat transplant model to reduce ischemia reperfusion injury. The siRNA was administered during hypothermic machine perfusion (HMP) followed by liver transplantation. While the results on apoptosis inhibition by the siRNA remained inconclusive, the researchers were able to show hepatic uptake of the siRNA[71]. Recent studies have adapted machine perfusion to demonstrate the possibility to prolong organ perfusion duration[72-74], with perfusion of human and porcine livers for up to 7 days[75]. Prolonged perfusion would allow to study hepatic uptake of an oligonucleotide-based therapeutic and at the same time study changes in RNA and protein levels in time. Besides liver perfusion, first studies have also been reported with kidney perfusion. Thompson et al.[76] demonstrated the delivery of antisense oligonucleotide in a human kidney during perfusion and showed to block microRNAs function implicated in ischemia reperfusion injury.

Explantation of diseased organs followed by *ex vivo* organ perfusion for PK research is a concept which can be extrapolated to other research fields. The application of *ex vivo* organ perfusion may also find relevance in pediatrics as livers and kidneys are explanted due to conditions such as cancer[77-79]. In pediatric research, key research questions involve understanding the ontogeny of drugs transporters and drug metabolizing enzymes as well as studying age-related variations in the pharmacokinetics of specific drug classes in children[80,81]. *Ex vivo* organ perfusion complemented with PBPK modelling can subsequently contribute to the development of age-appropriate dosing guidelines. This concept can also be applied for other special population groups such as morbidly obese individuals for investigating specific pathophysiological changes related to obesity that impact drug metabolism[82,83]. An illustrative example is the study conducted by de Hoogd et al.[84] demonstrating reduced elimination of morphine glucuronide metabolites (morphine-3-glucuronide and morphine-6-glucuornide) in morbidly obese patients in comparison to healthy volunteers. Although the primary route of the glucuronide metabolites elimination is via renal excretion (80%), there was no difference in kidney function between the morbidly obese and healthy subject group. Therefore the researchers hypothesized, based on reports on Dubin-Johnson syndrome where dysfunctional mutations in the MRP2 gene caused impairment in biliary excretion of bilirubin glucuronides, that hepatic transporters in the biliary

elimination of morphine glucuronide metabolites plays a significant role in the morbidly obese subject group[85]. To test this hypothesis, *ex vivo* liver perfusion could be conducted if there are morbidly obese patients with an underlying liver disease awaiting transplantation[86]. With this approach, the metabolism and excretion as well as transporter abundance can be studied to investigate and characterize the underlying mechanism. Besides changes in CYP450 enzymes and uridine diphosphate glucuronosyltransferase (UGT) enzyme activity in morbidly obese patients affecting drug metabolism[82,87], hepatic blood flow can also be altered[87,88]. Hepatic clearance is a result of an interplay between CYP450 abundance and activity and hepatic flow. To understand the observed differences in midazolam clearance in morbidly obese adults or obese adolescents compared to healthy subjects[89,90], it would be of great value to study the impact of obesity on hepatic flow. *Ex vivo* perfusion using pressure driven perfusion machines is a solution to study the effect of obesity on hepatic flow as well as CYP3A4 activity.

How do *ex vivo* models translate to *in vivo* PK profiles?

Compared to traditional *in vitro* models, *ex vivo* (whole organ) models are a promising platform and thereby paving the way to apply PBPK modeling in a more reliable and accurate way. It is hypothesized that accurate predictions of PK profiles would result in better translation of preclinical data to *in vivo*, which is accompanied by a lower attrition rate[91-93]. In this thesis, multiple *ex vivo* liver perfusions were performed, generating concentration-time profiles of the disappearance of the drug from the perfusate and appearance of the drug into the bile. The concentration-time profiles give an estimate regarding the hepatic elimination rate and percentage biliary clearance. However, interpretation to clinical *in vivo* profiles lack as 'only' the hepatic extraction of a drug and the biliary clearance can be determined. This is also true for the previously published InTESTine system; a platform with *ex vivo* tissue explants to study (regional) intestinal absorption and permeability[94,95]. As mentioned in the introduction of this thesis, the intestines, liver and kidneys are key organs involved in ADME processes and together define the PK profile of a drug.

To study the potential of *ex vivo* platforms to predict the *in vivo* PK profile of a drug, we here combined preclinical *ex vivo* data from the InTESTine system, combined liver-kidney perfusion all integrated by PBPK modeling to predict *in vivo* PK profiles using the drug cocktail rosuvastatin, digoxin, metformin and furosemide. First, regional intestinal transport was assessed using the

InTESTine system. Figure 7.3 shows the regional transport P_{app} values derived from the InTESTine system with porcine intestinal tissue.

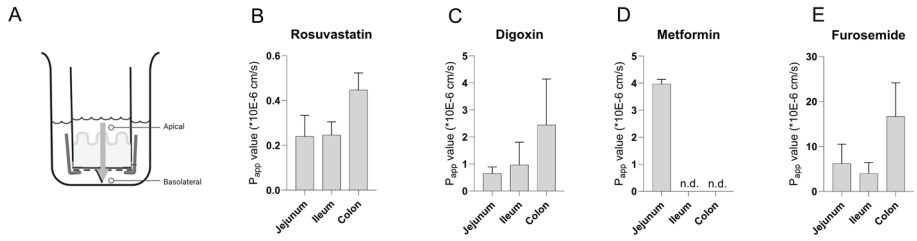


Figure 7.3 - Assessment of the regional intestinal transport of rosuvastatin, digoxin, metformin and furosemide using the InTESTine system with porcine intestinal tissue. (A) Schematic representation of the InTESTine system where porcine intestinal tissue is mounted in the system creating an apical (lumen) and basolateral (portal blood side) compartment. Determination of the regional permeability of (B) Rosuvastatin in jejunum (n=1), ileum (n=3), colon (n=2) tissue (C) Digoxin jejunum (n=6), ileum (n=3) and colon (n=4) tissue, (E) Metformin jejunum tissue (n=2) and (E) Furosemide jejunum (n=1), ileum (n=1) and colon (n=1) tissue.

The reported observed fraction absorbed of rosuvastatin, digoxin, metformin and furosemide *in vivo* is 0.50, 0.81, 0.55 and 0.53 respectively[95-98]. The reported range of the InTESTine system showed P_{app} values of 0 to ~18 (x10 ^{6}cm/s) translating to a F_a of 0 – 1[94]. Intestinal permeability of rosuvastatin was limited in the InTESTine system (mainly due to the fact that it is a strong BCRP substrate), with average P_{app} value of 0.21±0.09 for more proximal GI tract and 0.44±0.07 for distal GI tract, which does not correspond to the reported fraction absorbed of 0.50[98]. Comparable results were found for digoxin (average P_{app} 0.65±0.23, due to high affinity of digoxin for Pgp), and also showing higher P_{app} values in the distal parts of GI tract (P_{app}: 2.44±1.69) (Figure 7.3C). The absorption of metformin and furosemide across the intestinal wall showed to be faster with 3.98±0.16 for metformin jejunum and 8.74±6.01, 4.06±2.41 and 16.67±7.49 in jejunum, ileum and colon respectively for furosemide (Figure 7.3D-E).

To assess hepatic and renal clearance and subsequent biliary and renal excretion, the dual perfusion of liver+kidney was explored using the LiverAssist perfusion device (XVIVO, the Netherlands) (Figure 7.4). In this perfusion model the arterial blood supply is splitted to the 1) hepatic artery and 2) the renal artery. The liver receives also blood via the portal vein. The simultaneous perfusion of *ex vivo* kidney and liver is a novel and unique approach to determine the hepatic and renal clearance and excretion of drugs within one experiment[99]. The drug cocktail was dosed via the portal vein (1.4 mg

rosuvastatin, 0.056 mg digoxin, 74.2 mg metformin and 0.77 mg furosemide) and samples were taken from the perfusate, bile and urine.

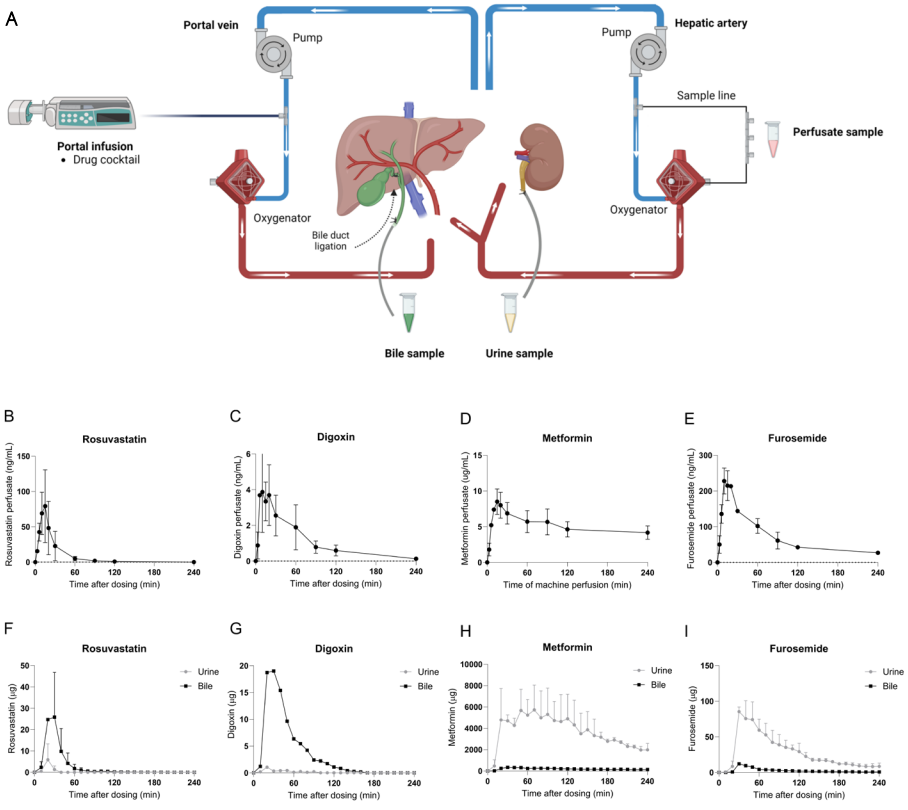


Figure 7.4 - Schematic representation of the perfused liver+kidney model using porcine organs and the perfusate and excretion profiles of rosuvastatin, digoxin, metformin and furosemide using the combined liver+kidney perfusion model. (A) The liver+kidney perfusion model: applied portal pressure of 8-11 mmHg and arterial pressure of 75-85 mmHg, with a total circulating volume of 2.5 L of perfusate. Drug cocktail was dosed to the portal vein of the liver mimicking oral dosing. Systemic perfusate profiles of (B) rosuvastatin dosed 1.4 mg, (C) Digoxin dosed 0.056 mg, (D) Metformin dosed 74.2 mg and (E) Furosemide dosed 0.77 mg. Urine and biliary elimination of (F) Rosuvastatin, (G) Digoxin, (H) Metformin (I) Furosemide. Data represents n=2 mean ± SD.

The combined liver+kidney perfusion model showed stable arterial and portal flow during 360 min of perfusion with constant bile and urine production (data not shown). Figure 7.4 shows the systemic profiles and excretion patterns after a single administration of the drug cocktail. Rosuvastatin was rapidly cleared from the circulation (Figure 7.4B) and was mainly eliminated via bile (20.2±5.8 %) and only a minor part was excreted into urine (2.1±1.5%). Digoxin was highly

biliary excreted (100%) and only a minor part was eliminated via urine (5.6%). Metformin clearance demonstrated slow uptake from the perfusate suggesting saturation of the OCT2 and MATE1/2 transporters, however was rapidly excreted into urine (120±29%) and to a minor extent via bile (6.5±0.3%). Furosemide was mainly excreted into urine (80.3±6.4%) and only a minor part in bile (7.7±1.8%). Data is in line with literature showing rosuvastatin and digoxin being mainly eliminated via bile and minorly into urine while metformin and furosemide are known to be mainly renally excreted[100-103]. To integrate *ex vivo* data into PBPK modeling, as a first step the concentration-time profiles of perfusate and cumulative amounts of bile and urine were fitted (Figure 7.5) to the developed liver+kidney PBPK model using R programming (R Studio, version 4.3.2). This liver+kidney PBPK model presented in Figure 7.6, generating the model-specific PK parameters CL_{bile}, CL_{urine}, K_{urine} and K_{bile} (Figure 7.6B).

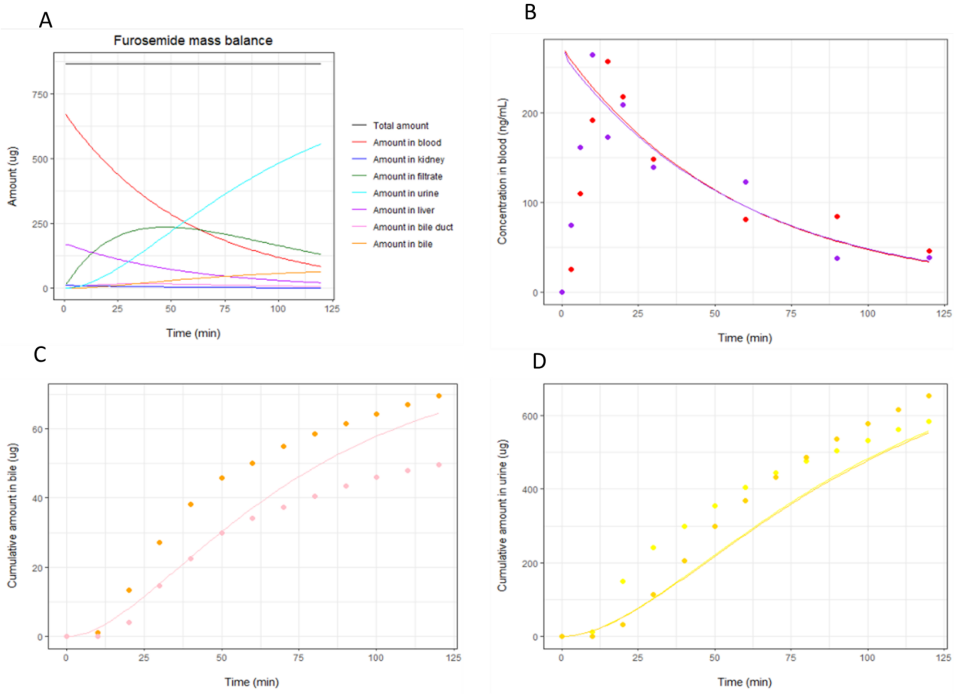
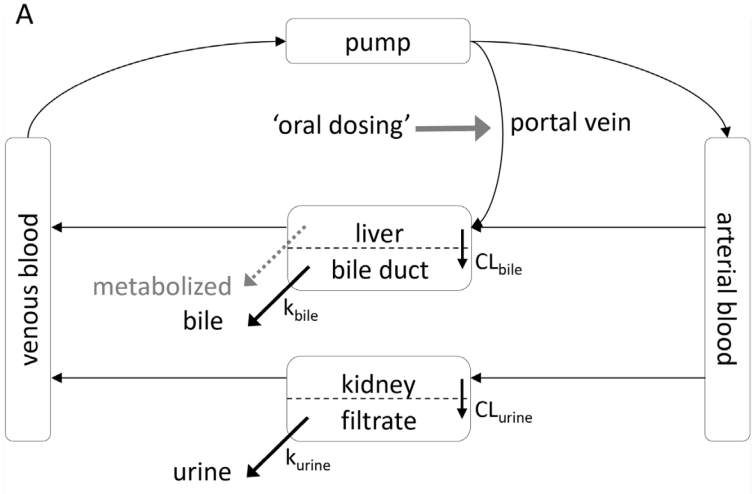


Figure 7.5 - Fitted concentration-time profiles of furosemide. (A) Furosemide mass balance per compartment (B) Concentration-time profile of furosemide perfusate perfusion experiment (C) cumulative amount of furosemide in bile and (D) Cumulative amount of furosemide in urine.

A



B

	Rosuvastatin	Digoxin	Metformin	Furosemide
CL_{bile} (mL/min)	654.7	98.5	2.6	24.4
K_{bile} (min^{-1})	0.027	0.052	0.023	0.043
CL_{urine} (mL/min)	48.8	59.1	61.9	456.3
K_{urine} (min^{-1})	0.13	0.0047	0.017	0.025

Figure 7.6 - Representation of the ex vivo liver+kidney PBPK model and simulated values derived from the sub PBPK model. (A) Schematic representation of the ex-vivo liver+kidney PBPK model and (B) Simulated pharmacokinetic parameters derived from the fitted ex vivo perfusion data of perfusate bile and urine determined in the generated ex vivo liver+kidney PBPK model.

Secondly, the PK parameters from the liver+kidney PBPK model (Figure 6A-B) together with the P_{app} data from the InTESTine system were integrated into a generic PBPK model using R programming. Figure 7.7 demonstrates the predicted concentration time profiles of the arterial blood following oral intake of the rosuvastatin, digoxin, metformin and furosemide. The predicted C_{max} and T_{max} of rosuvastatin showed to be within the range of the (lower) observed clinical values. Digoxin, displayed a systemic profile with a 7-fold lower C_{max} and a delay in T_{max} (T_{max} of 5 hours vs. 1.2 hours in vivo). Predictions for furosemide reached a maximum concentration at 0.105 mg/L which is nicely within the range of the clinically observed profiles. The T_{max} was predicted after 5 hours which was compared to clinical *in vivo* data showing a T_{max} of 1.2 hours, slightly delayed. In the case of metformin, our predictions showed a C_{max} level of 1.5 mg/L which is a 2-fold overestimation of the average C_{max} observed in clinical

profiles. Tmax was close to reported in vivo data (T_{max} of 4 hours vs 3.35 hours in vivo). Both furosemide and metformin showed an underestimation of the elimination of the drugs from the circulation.

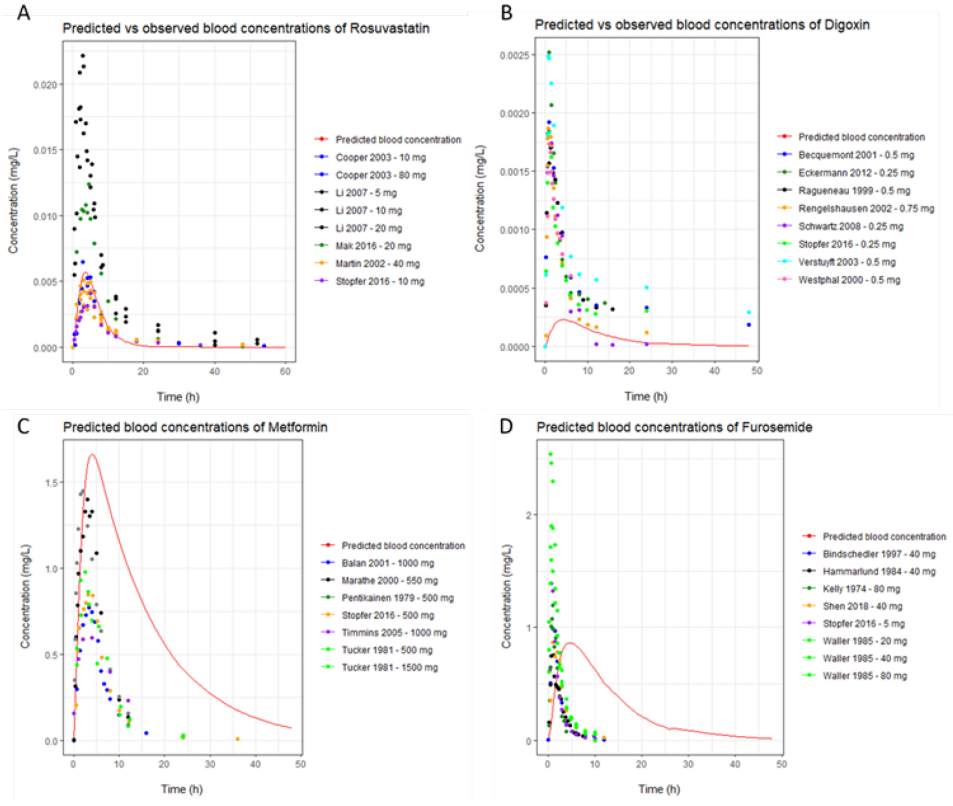


Figure 7.7 - Simulated blood concentration profiles after single oral dose of the drug cocktail. Simulation profiles of oral dosing of (A) 10 mg rosuvastatin, (B) 0.5 mg digoxin (C) 500 mg Metformin and (D) 40 mg furosemide. The simulation duration was 48 hours.

The prediction for C_{max} of rosuvastatin showed to be within the range of clinically observed data, but on the lower side. The intestinal transport of rosuvastatin showed to be relatively low when using porcine intestinal tissue. Rosuvastatin is a substrate for the BCRP efflux transporter, limiting the influx into while facilitating the efflux out of the cells[104]. Sjoberg et al.[105] reported a P_{app} value of 6.95±1.05 in human jejunum tissue while in porcine jejunum tissue a P_{app} value of 0.24 was measured. Vaessen et al.[44] demonstrated differences in abundance of BCRP expression between pig and human. A significant higher

expression of the protein was measured in jejunum tissue of pigs (0.75 pmol protein/g tissue) compared to human (0.45 pmol protein/g tissue), hypothesizing more efflux of rosuvastatin by BCRP in porcine intestinal tissue resulting in a lower permeability. Underestimation of the intestinal absorption resulted in a slightly lower C_{max} compared to clinical *in vivo* data[106-109]. However, important to note is the variation observed in clinical studies. Cooper et al.[106] demonstrated a C_{max} of 53.5 ng/mL after an oral dose of 80 mg rosuvastatin, while Li et al.[107] showed a C_{max} of 45 ng/mL after an oral dose of 40 mg rosuvastatin. These differences highlight the importance of considering and understanding the variability in drugs response among individuals. Similar as rosuvastatin, the same effect was observed for digoxin regarding intestinal transport and regional variability[110]. Although a fraction absorbed of 80% is observed in human, *ex vivo* tissue models and the Caco-2 model report rather low values of intestinal permeability which are not in line with a fraction absorbed of 80%[94,95,105]. Using the *ex vivo* data, the prediction of C_{max} showed to be 7 fold lower[111-116]. The protein expression of OATP2B1 (furosemide) and OCT1 (metformin) showed no major difference between intestinal regions[44,110] and the P_{app} values were in line with literature[95,105,117]. The predicted C_{max} levels showed to be close to *in vivo* observed data for metformin[118-122] as well as furosemide[103,120,123-126]. However, the predicted systemic profiles for metformin and furosemide showed delayed elimination compared to *in vivo* profiles with CL_{urine} as a factor contributing to the elimination. In a clinical study by Stopfer et al.[120] human subjects receiving the drug cocktail showed complete elimination of furosemide after 8 hours and metformin after 24 hours while in our model this process takes approximately 48 hours. This indicates an underestimated rate of renal elimination during *ex vivo* experiments compared to *in vivo* which could be the result of diminished *ex vivo* kidney function during normothermic perfusion. However, there are currently no established parameters for *ex vivo* kidney function. Parameters such as flow, urine output, creatine clearance and fractional sodium excretion are commonly investigated[127-130]. Multiple studies demonstrate the creatine clearance (GFR) during *ex vivo* kidney perfusion. In these studies, using slaughterhouse kidneys, GFR values around the 1.0 – 5.0 mL/min are measured[130-132]. In contrast, Lødrup et al.[133] measured the GFR from a single kidney *in vivo* and showed an average GFR of 33.9±8.9 mL/min thus indicating that *ex vivo*, kidney GFR is diminished compared to *in vivo*. Inulin is often used as a model compound to measure the GFR and Markgraf et al.[130] studied inulin clearance as function assessment test in perfused kidneys derived from laboratory pigs and slaughterhouse pigs with different time of

warm ischemia (WIT). The researchers demonstrated that none of the slaughterhouse derived kidneys were within the limits for consideration of 'functional kidneys' which was determined by the inulin uptake and excretion behavior independent of the WIT[130]. Together, the combination of *ex vivo* data with PBPK modeling provides a first 'real' insight into *ex vivo* kidney function by studying the clearance of transporter mediated drugs and subsequent translation to in vivo situation. This could aid in the generation of function assessment tests or parameters to study kidney function in an *ex vivo* environment which is needed in the field of transplantation.

The *ex vivo* models are complex models and may better represent physiological conditions than purely *in vitro* data and thereby enhancing the models' predictive capabilities. This is particularly relevant when studying transporter mediated processes as transporter mediated processes are typically more complicated than that of drug metabolizing enzymes. However, translation of the *ex vivo* data towards *in vivo* profiles with PBPK modeling has some challenges. First, we used a perfusate with at total concentration of 1% albumin which is lower compared to the physiological concentrations ranging between 3.5 - 5.0% albumin[134]. Many of our kidney perfusion experiments showed the inability to produce urine when using a perfusate consisting of red blood cells with plasma. Lowering the albumin concentration in the perfusate showed urine production by the *ex vivo* kidneys. Since urine production is essential to measure the renal elimination of a compound, we chose to use these sub-physiological concentrations. Many drugs are however highly bound to plasma proteins and changes into plasma protein concentration will therefore affect the elimination rate from the perfusate into the organ[135]. Although e*x vivo* to *in vivo* extrapolation was performed to adjust for the percentage of albumin in the system, it introduces additional uncertainties to the data. Moreover, to better fit the experimental data, tissue concentrations are needed which can easily be obtained from the *ex vivo* perfused organ.

Here we present for the first time the use of porcine *ex vivo* tissue models in combination with PBPK modeling, predicting PK profiles which are close to clinical observed human profiles. Currently, more abundant data is available regarding transporter abundance in intestine, liver and kidney between pig and humans[44,136,137] which can be used to refine profiles with greater accuracy. The use of human tissues in *ex vivo* models have been described before by our

group for intestine[94] and liver[138], showing the potential for even further enhancement of PK prediction in human.

Conclusion

In conclusion, in this thesis we aimed to study to pharmacokinetic application of pressure driven normothermic organ perfusion. Using a novel pressure driven perfusion machine, we endeavored to bridge the fields of transplantation and pharmacology. Our studies demonstrated the utilization of NMP to examine various drug PK processes such as the hepatic first pass effect, hepatic clearance, biliary excretion, transporter function and DDI using porcine and human explanted diseased livers. The use of porcine livers was an appropriate substitute for human livers to mechanistically study transporter contribution in drug uptake, drug excretion and to study DDI. These studies enabled the investigation of OATP1B1/1B3 mediated DDI, with results aligning closely with clinical data. Moreover, explanted diseased human livers showed to be suitable for perfusion research and can serve as a basis to explore the differences in hepatic handling of drugs for patients with different types of hepatic impairment. Hepatic clearance of rosuvastatin and digoxin showed to be the most affected by cirrhosis while no major differences were observed for the renally cleared drugs metformin and furosemide. The 3-fold lower portal flow in cirrhotic livers showed to diminish the hepatic extraction of rosuvastatin showing the importance of portal flow in a preclinical model to determine hepatic clearance. Furthermore, optimalisation of the liver perfusion model was studied by infusion of a (un)conjugated bile acid pool to replicate physiological conditions for a more accurate assessment of hepatic PK processes. This approach demonstrated that the infusion of (un)conjugated bile acids alleviated the burden of the *de novo* bile acid synthesis and enhanced liver function pointing towards potential advancements in liver preservation and transplantation techniques. The possibilities of the pressure driven perfusion system are numerous, as we demonstrated the development and application of multi-organ perfusion to understand the interplay between the intestine and liver by characterization of the first-pass effect and pre-systemic CYP3A4 metabolism. Use of perfusion showed to be an excellent tool to study drug concentrations in blood flows and tissues which are otherwise impossible to reach, thereby generating a novel and in depth insights into the ADME profile of drugs.

References

1. Benet LZ, Sodhi JK. Investigating the theoretical basis for in vitro–in vivo extrapolation (ivive) in predicting drug metabolic clearance and proposing future experimental pathways. The AAPS journal 2020;22:120.
2. Honek J. Preclinical research in drug development. Medical Writing 2017;26:5-8.
3. Mori D, Kashihara Y, Yoshikado T, Kimura M, Hirota T, Matsuki S, Maeda K, et al. Effect of OATP1B1 genotypes on plasma concentrations of endogenous OATP1B1 substrates and drugs, and their association in healthy volunteers. Drug metabolism and pharmacokinetics 2019;34:78-86.
4. Takehara I, Yoshikado T, Ishigame K, Mori D, Furihata K-i, Watanabe N, Ando O, et al. Comparative study of the dose-dependence of OATP1B inhibition by rifampicin using probe drugs and endogenous substrates in healthy volunteers. Pharmaceutical research 2018;35:1-13.
5. Nevzorova YA, Boyer-Diaz Z, Cubero FJ, Gracia-Sancho J. Animal models for liver disease–A practical approach for translational research. Journal of hepatology 2020;73:423-440.
6. Guillouzo A. Liver cell models in in vitro toxicology. Environmental health perspectives 1998;106:511-532.
7. Chiang JY. Bile acid regulation of gene expression: roles of nuclear hormone receptors. Endocrine reviews 2002;23:443-463.
8. Chiang JY. Bile acids: regulation of synthesis: thematic review series: bile acids. Journal of lipid research 2009;50:1955-1966.
9. Chiang JY. Bile acid metabolism and signaling. Comprehensive physiology 2013;3:1191.
10. De Fabiani E, Mitro N, Gilardi F, Caruso D, Galli G, Crestani M. Coordinated control of cholesterol catabolism to bile acids and of gluconeogenesis via a novel mechanism of transcription regulation linked to the fasted-to-fed cycle. Journal of Biological Chemistry 2003;278:39124-39132.
11. Halilbasic E, Claudel T, Trauner M. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. Journal of hepatology 2013;58:155-168.
12. Alqahtani S, Mohamed LA, Kaddoumi A. Experimental models for predicting drug absorption and metabolism. Expert Opinion on Drug Metabolism & Toxicology 2013;9:1241-1254.
13. FDA U. In vitro drug interaction studies—cytochrome P450 enzyme-and transporter-mediated drug interactions guidance for industry. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Silver Spring, MD 2020.
14. Giacomini K, Huang SM. Transporters in drug development and clinical pharmacology. In: Wiley Online Library; 2013. p. 3-9.
15. Stevens LJ, Zhu AZ, Chothe PP, Chowdhury SK, Donkers JM, Vaes WH, Knibbe CA, et al. Evaluation of normothermic machine perfusion of porcine livers as a novel preclinical model to predict biliary clearance and transporter-mediated drug-drug interactions using statins. Drug Metabolism and Disposition 2021;49:780-789.
16. Lau YY, Okochi H, Huang Y, Benet LZ. Pharmacokinetics of atorvastatin and its hydroxy metabolites in rats and the effects of concomitant rifampicin single doses: relevance of first-pass effect from hepatic uptake transporters, and intestinal and hepatic metabolism. Drug metabolism and disposition 2006;34:1175-1181.
17. Luo Z, Liu Y, Zhao B, Tang M, Dong H, Zhang L, Lv B, et al. Ex vivo and in situ approaches used to study intestinal absorption. Journal of pharmacological and toxicological methods 2013;68:208-216.
18. Galetin A, Gertz M, Houston JB. Potential role of intestinal first-pass metabolism in the prediction of drug–drug interactions. Expert opinion on drug metabolism & toxicology 2008;4:909-922.

19. Kanebratt K, Diczfalusy U, Bäckström T, Sparve E, Bredberg E, Böttiger Y, Andersson T, et al. Cytochrome P450 induction by rifampicin in healthy subjects: determination using the Karolinska cocktail and the endogenous CYP3A4 marker 4β-hydroxycholesterol. Clinical Pharmacology & Therapeutics 2008;84:589-594.
20. Eshmuminov D, Becker D, Borrego LB, Hefti M, Schuler MJ, Hagedorn C, Muller X, et al. An integrated perfusion machine preserves injured human livers for 1 week. Nature biotechnology 2020;38:189-198.
21. Lau N-S, Ly M, Dennis C, Jacques A, Cabanes-Creus M, Toomath S, Huang J, et al. Long-term ex-vivo normothermic perfusion of human split livers: a unique model to study new therapeutics and increase the number of available organs. 2023.
22. Schuler MJ, Becker D, Mueller M, Bautista Borrego L, Mancina L, Huwyler F, Binz J, et al. Observations and findings during the development of a subnormothermic/normothermic long-term ex vivo liver perfusion machine. Artificial Organs 2023;47:317-329.
23. Attard JA, Osei-Bordom D-C, Boteon Y, Wallace L, Ronca V, Reynolds G, Perera M, et al. Ex situ normothermic split liver machine perfusion: protocol for robust comparative controls in liver function assessment suitable for evaluation of novel therapeutic interventions in the pre-clinical setting. Frontiers in surgery 2021;8:627332.
24. Huang V, Karimian N, Detelich D, Raigani S, Geerts S, Beijert I, Fontan FM, et al. Split-liver ex situ machine perfusion: a novel technique for studying organ preservation and therapeutic interventions. Journal of Clinical Medicine 2020;9:269.
25. Thorne AM, Lantinga V, Bodewes S, de Kleine RH, Nijkamp MW, Sprakel J, Hartog H, et al. Ex situ dual hypothermic oxygenated machine perfusion for human split liver transplantation. Transplantation direct 2021;7.
26. Derungs A, Donzelli M, Berger B, Noppen C, Krähenbühl S, Haschke M. Effects of cytochrome P450 inhibition and induction on the phenotyping metrics of the Basel cocktail: a randomized crossover study. Clinical Pharmacokinetics 2016;55:79-91.
27. Chen K-F, Jones HM, Gill KL. Physiologically based pharmacokinetic modeling to predict drug-biologic interactions with cytokine modulators: are these relevant and is interleukin-6 enough? Drug Metabolism and Disposition 2022;50:1322-1331.
28. Abdel-Razzak Z, Loyer P, Fautrel A, Gautier J-C, Corcos L, Turlin B, Beaune P, et al. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. Molecular pharmacology 1993;44:707-715.
29. Dallas S, Sensenhauser C, Batheja A, Singer M, Markowska M, Zakszewski C, NVS Mamidi R, et al. De-risking bio-therapeutics for possible drug interactions using cryopreserved human hepatocytes. Current drug metabolism 2012;13:923-929.
30. Mimura H, Kobayashi K, Xu L, Hashimoto M, Ejiri Y, Hosoda M, Chiba K. Effects of cytokines on CYP3A4 expression and reversal of the effects by anti-cytokine agents in the three-dimensionally cultured human hepatoma cell line FLC-4. Drug metabolism and pharmacokinetics 2015;30:105-110.
31. Abdel-Razzak Z, Corcos L, Fautrel A, Campion J-P, Guillouzo A. Transforming growth factor-beta 1 down-regulates basal and polycyclic aromatic hydrocarbon-induced cytochromes P-450 1A1 and 1A2 in adult human hepatocytes in primary culture. Molecular Pharmacology 1994;46:1100-1110.
32. Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metabolism and Disposition 2007;35:1687-1693.
33. Guillén MI, Donato MT, Jover R, Castell J, Fabra R, Trullenque R, Gómez-Lechón MJ. Oncostatin M down-regulates basal and induced cytochromes P450 in human hepatocytes. Journal of Pharmacology and Experimental Therapeutics 1998;285:127-134.
34. de Jong LM, Jiskoot W, Swen JJ, Manson ML. Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: lessons from experimental models and a potential role for pharmacogenetics. Genes 2020;11:1509.

35. Klein M, Thomas M, Hofmann U, Seehofer D, Damm G, Zanger UM. A systematic comparison of the impact of inflammatory signaling on absorption, distribution, metabolism, and excretion gene expression and activity in primary human hepatocytes and HepaRG cells. Drug Metabolism and Disposition 2015;43:273-283.
36. Gravante G, Ong S, Metcalfe M, Sorge R, Sconocchia G, Orlando G, Lloyd D, et al. Cytokine response to ischemia/reperfusion injury in an ex vivo perfused porcine liver model. In: Transplantation proceedings; 2009: Elsevier; 2009. p. 1107-1112.
37. Chung WY, Gravante G, Al-Leswas D, Alzaraa A, Sorge R, Ong SL, Pollard C, et al. Addition of a kidney to the normothermic ex vivo perfused porcine liver model does not increase cytokine response. Journal of Artificial Organs 2012;15:290-294.
38. Weissenbacher A, Stone JP, Lo Faro ML, Hunter JP, Ploeg RJ, Coussios CC, Fildes JE, et al. Hemodynamics and metabolic parameters in normothermic kidney preservation are linked with donor factors, perfusate cells, and cytokines. Frontiers in medicine 2022;8:801098.
39. Hosgood SA, Moore T, Qurashi M, Adams T, Nicholson ML. Hydrogen gas does not ameliorate renal ischemia reperfusion injury in a preclinical model. Artificial Organs 2018;42:723-727.
40. De Beule J, Keppens D, Korf H, Jochmans I. Differential Cytokine Levels during Normothermic Kidney Perfusion with Whole Blood-or Red Blood Cell-Based Perfusates—Results of a Scoping Review and Experimental Study. Journal of Clinical Medicine 2022;11:6618.
41. Volpin G, Cohen M, Assaf M, Meir T, Katz R, Pollack S. Cytokine levels (IL-4, IL-6, IL-8 and TGFβ) as potential biomarkers of systemic inflammatory response in trauma patients. International orthopaedics 2014;38:1303-1309.
42. Damas P, Ledoux D, Nys M, Vrindts Y, De Groote D, Franchimont P, Lamy M. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. Annals of surgery 1992;215:356.
43. Hosgood SA, Moore T, Kleverlaan T, Adams T, Nicholson ML. Haemoadsorption reduces the inflammatory response and improves blood flow during ex vivo renal perfusion in an experimental model. Journal of Translational Medicine 2017;15:1-10.
44. Vaessen SF, van Lipzig MM, Pieters RH, Krul CA, Wortelboer HM, van de Steeg E. Regional expression levels of drug transporters and metabolizing enzymes along the pig and human intestinal tract and comparison with Caco-2 cells. Drug metabolism and disposition 2017;45:353-360.
45. Englund G, Rorsman F, Rönnblom A, Karlbom U, Lazorova L, Gråsjö J, Kindmark A, et al. Regional levels of drug transporters along the human intestinal tract: co-expression of ABC and SLC transporters and comparison with Caco-2 cells. European Journal of Pharmaceutical Sciences 2006;29:269-277.
46. Gertz M, Harrison A, Houston JB, Galetin A. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. Drug Metabolism and Disposition 2010;38:1147-1158.
47. Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, Barr DM, et al. First-pass metabolism of midazolam by the human intestine. Clinical Pharmacology & Therapeutics 1996;60:14-24.
48. Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD, et al. Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. Journal of Pharmacology and Experimental Therapeutics 1997;283:1552-1562.
49. Bachmann F, Duthaler U, Krähenbühl S. Effect of deglucuronidation on the results of the Basel phenotyping cocktail. British Journal of Clinical Pharmacology 2021;87:4608-4618.
50. van de Steeg E, Schuren FH, Obach RS, van Woudenbergh C, Walker GS, Heerikhuisen M, Nooijen IH, et al. An ex vivo fermentation screening platform to study drug metabolism by human gut microbiota. Drug Metabolism and Disposition 2018;46:1596-1607.
51. Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. Cancer research 1994;54:3723-3725.
52. Davies MI. A review of microdialysis sampling for pharmacokinetic applications. Analytica Chimica Acta 1999;379:227-249.

53. Gunaratna C, Kissinger PT. Application of microdialysis to study the in vitro metabolism of drugs in liver microsomes. Journal of pharmaceutical and biomedical analysis 1997;16:239-248.
54. Brill MJ, Houwink AP, Schmidt S, Van Dongen EP, Hazebroek EJ, van Ramshorst B, Deneer VH, et al. Reduced subcutaneous tissue distribution of cefazolin in morbidly obese versus non-obese patients determined using clinical microdialysis. Journal of Antimicrobial Chemotherapy 2014;69:715-723.
55. Mazzeo AT, Fanelli V, Boffini M, Medugno M, Filippini C, Simonato E, Costamagna A, et al. Feasibility of lung microdialysis to assess metabolism during clinical ex vivo lung perfusion. The Journal of Heart and Lung Transplantation 2019;38:267-276.
56. Schreiter T, Sowa J, Mathé Z, Treckmann J, Bröcker-Preuß M, Baba H, Gieseler R, et al. Long-term maintenance of human liver tissue by ex-vivo perfusion of liver sections. Zeitschrift für Gastroenterologie 2014;52:P_3_19.
57. Melgert BN, Olinga P, Weert B, Slooff MJ, Meijer DK, Poelstra K, Groothuis GM. Cellular distribution and handling of liver-targeting preparations in human livers studied by a liver lobe perfusion. Drug metabolism and disposition 2001;29:361-367.
58. Villeneuve J-P, Dagenais M, Huet P-M, Lapointe R, Roy A, Marleau D. Clearance by the liver in cirrhosis. III. Propranolol uptake by the isolated perfused human liver. Canadian journal of physiology and pharmacology 1996;74:1327-1332.
59. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nature reviews Gastroenterology & hepatology 2018;15:11-20.
60. Müller PC, Kabacam G, Vibert E, Germani G, Petrowsky H. Current status of liver transplantation in Europe. International Journal of Surgery 2020;82:22-29.
61. Sehgal A, Vaishnaw A, Fitzgerald K. Liver as a target for oligonucleotide therapeutics. Journal of hepatology 2013;59:1354-1359.
62. Roberts TC, Langer R, Wood MJ. Advances in oligonucleotide drug delivery. Nature Reviews Drug Discovery 2020;19:673-694.
63. An G. Pharmacokinetics and Pharmacodynamics of GalNAc-Conjugated siRNAs. The Journal of Clinical Pharmacology 2023.
64. Andersson P, den Besten C: Preclinical and clinical drug-metabolism, pharmacokinetics and safety of therapeutic oligonucleotides. In: Advances in nucleic acid therapeutics, 2019; 474-531.
65. Griffett K, Burris TP. Development of LXR inverse agonists to treat MAFLD, NASH, and other metabolic diseases. Frontiers in medicine 2023;10:1102469.
66. Alkhouri N, Reddy GK, Lawitz E. Oligonucleotide-Based Therapeutics: An Emerging Strategy for the Treatment of Chronic Liver Diseases. Hepatology 2021;73:1581-1593.
67. Lindén D, Ahnmark A, Pingitore P, Ciociola E, Ahlstedt I, Andréasson A-C, Sasidharan K, et al. Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148M knock-in mice. Molecular metabolism 2019;22:49-61.
68. Bonaccorsi-Riani E, Gillooly A, Brüggenwirth IM, Martins PN. Delivery of genetic load during ex situ liver machine perfusion with potential for CRISPR-Cas9 gene editing: an innovative strategy for graft treatment. Hepatobiliary Pancreat Dis Int 2021;20:503-505.
69. Bonaccorsi-Riani E, Gillooly AR, Iesari S, Brüggenwirth IM, Ferguson CM, Komuta M, Xhema D, et al. Delivering siRNA compounds during HOPE to modulate organ function: a proof-of-concept study in a rat liver transplant model. Transplantation 2022;106:1565-1576.
70. Gillooly AR, Perry J, Martins PN. First report of siRNA uptake (for RNA interference) during ex vivo hypothermic and normothermic liver machine perfusion. In: LWW; 2019.
71. Thijssen MF, Brüggenwirth IM, Gillooly A, Khvorova A, Kowalik TF, Martins PN. Gene silencing with siRNA (RNA interference): a new therapeutic option during ex vivo machine liver perfusion preservation. Liver Transplantation 2019;25:140-151.
72. Butler AJ, Rees MA, Wight DG, Casey ND, Alexander G, White DJ, Friend PJ. Successful extracorporeal porcine liver perfusion for 72 hr1. Transplantation 2002;73:1212-1218.

73. Liu Q, Nassar A, Buccini L, Iuppa G, Soliman B, Pezzati D, Hassan A, et al. Lipid metabolism and functional assessment of discarded human livers with steatosis undergoing 24 hours of normothermic machine perfusion. Liver Transplantation 2018;24:233-245.
74. Vogel T, Brockmann JG, Pigott D, Neil DA, Muthusamy ASR, Coussios CC, Friend PJ. Successful transplantation of porcine liver grafts following 48-hour normothermic preservation. PLoS One 2017;12:e0188494.
75. Eshmuminov D, Becker D, Bautista Borrego L, Hefti M, Schuler MJ, Hagedorn C, Muller X, et al. An integrated perfusion machine preserves injured human livers for 1 week. Nature biotechnology 2020;38:189-198.
76. Thompson ER, Sewpaul A, Figuereido R, Bates L, Tingle SJ, Ferdinand JR, Situmorang GR, et al. MicroRNA antagonist therapy during normothermic machine perfusion of donor kidneys. American Journal of Transplantation 2022;22:1088-1100.
77. Finegold MJ, Egler RA, Goss JA, Guillerman PR, Karpen SJ, Krishnamurthy R, O'Mahony CA. Liver tumors: pediatric population. Liver Transplantation 2008;14:1545-1556.
78. Mangus RS, Tector AJ, Kubal CA, Fridell JA, Vianna RM. Multivisceral transplantation: expanding indications and improving outcomes. Journal of gastrointestinal surgery 2013;17:179-187.
79. Dhakal P, Giri S, Siwakoti K, Rayamajhi S, Bhatt VR. Renal cancer in recipients of kidney transplant. Rare tumors 2017;9:9-13.
80. HJ Krekels E, Danhof M, Tibboel D, AJ Knibbe C. Ontogeny of hepatic glucuronidation; methods and results. Current drug metabolism 2012;13:728-743.
81. Thakur A, Parvez MM, Leeder JS, Prasad B. Ontogeny of drug-metabolizing enzymes. Enzyme Kinetics in Drug Metabolism: Fundamentals and Applications 2021:551-593.
82. van Rongen A, Välitalo PA, Peeters MY, Boerma D, Huisman FW, van Ramshorst B, van Dongen EP, et al. Morbidly obese patients exhibit increased CYP2E1-mediated oxidation of acetaminophen. Clinical pharmacokinetics 2016;55:833-847.
83. Smit C, De Hoogd S, Brüggemann RJ, Knibbe CA. Obesity and drug pharmacology: a review of the influence of obesity on pharmacokinetic and pharmacodynamic parameters. Expert opinion on drug metabolism & toxicology 2018;14:275-285.
84. de Hoogd S, Välitalo PA, Dahan A, van Kralingen S, Coughtrie MM, van Dongen EP, van Ramshorst B, et al. Influence of morbid obesity on the pharmacokinetics of morphine, morphine-3-glucuronide, and morphine-6-glucuronide. Clinical pharmacokinetics 2017;56:1577-1587.
85. König J, Rost D, Cui Y, Keppler D. Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. Hepatology 1999;29:1156-1163.
86. Delacôte C, Favre M, El Amrani M, Ningarhari M, Lemaitre E, Ntandja-Wandji LC, Bauvin P, et al. Morbid obesity increases death and dropout from the liver transplantation waiting list: A prospective cohort study. United European Gastroenterology Journal 2022;10:396-408.
87. Zhang T, Krekels EH, Smit C, Knibbe CA. Drug pharmacokinetics in the obese population: challenging common assumptions on predictors of obesity-related parameter changes. Expert Opinion on Drug Metabolism & Toxicology 2022;18:657-674.
88. Rolle A, Paredes S, Cortinez L, Anderson B, Quezada N, Solari S, Allende F, et al. Dexmedetomidine metabolic clearance is not affected by fat mass in obese patients. British Journal of Anaesthesia 2018;120:969-977.
89. Brill MJ, Välitalo PA, Darwich AS, van Ramshorst B, Van Dongen H, Rostami–Hodjegan A, Danhof M, et al. Semiphysiologically based pharmacokinetic model for midazolam and CYP3A mediated metabolite 1-OH-midazolam in morbidly obese and weight loss surgery patients. CPT: pharmacometrics & systems pharmacology 2016;5:20-30.
90. van Rongen A, Brill MJ, Vaughns JD, Välitalo PA, van Dongen EP, van Ramshorst B, Barrett JS, et al. Higher midazolam clearance in obese adolescents compared with morbidly obese adults. Clinical pharmacokinetics 2018;57:601-611.
91. DiMasi JA, Hansen RW, Grabowski HG. The price of innovation: new estimates of drug development costs. Journal of health economics 2003;22:151-185.

92. Zhang Y, Chan HF, Leong KW. Advanced materials and processing for drug delivery: the past and the future. Advanced drug delivery reviews 2013;65:104-120.
93. Waring MJ, Arrowsmith J, Leach AR, Leeson PD, Mandrell S, Owen RM, Pairaudeau G, et al. An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nature reviews Drug discovery 2015;14:475-486.
94. Stevens LJ, van Lipzig MM, Erpelinck SL, Pronk A, van Gorp J, Wortelboer HM, van de Steeg E. A higher throughput and physiologically relevant two-compartmental human ex vivo intestinal tissue system for studying gastrointestinal processes. European Journal of Pharmaceutical Sciences 2019;137:104989.
95. Westerhout J, van de Steeg E, Grossouw D, Zeijdner EE, Krul CA, Verwei M, Wortelboer HM. A new approach to predict human intestinal absorption using porcine intestinal tissue and biorelevant matrices. European Journal of Pharmaceutical Sciences 2014;63:167-177.
96. Harrison LI, Gibaldi M. Physiologically based pharmacokinetic model for digoxin disposition in dogs and its preliminary application to humans. Journal of pharmaceutical sciences 1977;66:1679-1683.
97. Hu L-D, Liu Y, Tang X, Zhang Q. Preparation and in vitro/in vivo evaluation of sustained-release metformin hydrochloride pellets. European journal of pharmaceutics and biopharmaceutics 2006;64:185-192.
98. Jamei M, Bajot F, Neuhoff S, Barter Z, Yang J, Rostami-Hodjegan A, Rowland-Yeo K. A mechanistic framework for in vitro–in vivo extrapolation of liver membrane transporters: prediction of drug–drug interaction between rosuvastatin and cyclosporine. Clinical pharmacokinetics 2014;53:73-87.
99. Stevens LJ, Donkers JM, Dubbeld J, Vaes WH, Knibbe CA, Alwayn IP, van de Steeg E. Towards human ex vivo organ perfusion models to elucidate drug pharmacokinetics in health and disease. Drug metabolism reviews 2020;52:438-454.
100. Bergman E, Lundahl A, Fridblom P, Hedeland M, Bondesson U, Knutson L, Lennernäs H. Enterohepatic disposition of rosuvastatin in pigs and the impact of concomitant dosing with cyclosporine and gemfibrozil. Drug metabolism and disposition 2009;37:2349-2358.
101. Lau YY, Wu C-Y, Okochi H, Benet LZ. Ex situ inhibition of hepatic uptake and efflux significantly changes metabolism: hepatic enzyme-transporter interplay. Journal of Pharmacology and Experimental Therapeutics 2004;308:1040-1045.
102. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong J, Furlong TJ, et al. Clinical pharmacokinetics of metformin. Clinical pharmacokinetics 2011;50:81-98.
103. Waller ES, Massarella JW, Tomkiw MS, Smith RV, Doluisio JT. Pharmacokinetics of furosemide after three different single oral doses. Biopharmaceutics & drug disposition 1985;6:109-117.
104. Takano M, Yumoto R, Murakami T. Expression and function of efflux drug transporters in the intestine. Pharmacology & therapeutics 2006;109:137-161.
105. Sjöberg Å, Lutz M, Tannergren C, Wingolf C, Borde A, Ungell A-L. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. European Journal of Pharmaceutical Sciences 2013;48:166-180.
106. Cooper KJ, Martin PD, Dane AL, Warwick MJ, Schneck DW, Cantarini MV. Effect of itraconazole on the pharmacokinetics of rosuvastatin. Clinical Pharmacology & Therapeutics 2003;73:322-329.
107. Li Y, Jiang X, Lan K, Zhang R, Li X, Jiang Q. Pharmacokinetic properties of rosuvastatin after single-dose, oral administration in Chinese volunteers: a randomized, open-label, three-way crossover study. Clinical therapeutics 2007;29:2194-2203.
108. Mak W, Tan S, Wong J, Chin S, Lim A. Pharmacokinetic Comparison and Bioequivalence Study of Two Rosuvastatin 20 mg Formulations in Healthy Volunteers. J Bioequiv Availab 2016;8:095-098.
109. Martin PD, Warwick MJ, Dane AL, Brindley C, Short T. Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers. Clinical therapeutics 2003;25:2553-2563.
110. Drozdzik M, Gröer C, Penski J, Lapczuk J, Ostrowski M, Lai Y, Prasad B, et al. Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine. Molecular pharmaceutics 2014;11:3547-3555.

111. Becquemont L, Verstuyft C, Kerb R, Brinkmann U, Lebot M, Jaillon P, Funck-Brentano C. Effect of grapefruit juice on digoxin pharmacokinetics in humans. Clinical Pharmacology & Therapeutics 2001;70:311-316.
112. Eckermann G, Lahu G, Nassr N, Bethke TD. Absence of pharmacokinetic interaction between roflumilast and digoxin in healthy adults. The Journal of Clinical Pharmacology 2012;52:251-257.
113. Ragueneau I, Poirier JM, Radembino N, Sao AB, Funck-Brentano C, Jaillon P. Pharmacokinetic and pharmacodynamic drug interactions between digoxin and macrogol 4000, a laxative polymer, in healthy volunteers. British journal of clinical pharmacology 1999;48:453-456.
114. Schwartz JI, Agrawal NG, Wehling M, Musser BJ, Gumbs CP, Michiels N, De Smet M, et al. Evaluation of the pharmacokinetics of digoxin in healthy subjects receiving etoricoxib. British journal of clinical pharmacology 2008;66:811-817.
115. Verstuyft C, Schwab M, Schaeffeler E, Kerb R, Brinkmann U, Jaillon P, Funck-Brentano C, et al. Digoxin pharmacokinetics and MDR1 genetic polymorphisms. European journal of clinical pharmacology 2003;58:809-812.
116. Westphal K, Weinbrenner A, Giessmann T, Stuhr M, Franke G, Zschiesche M, Oertel R, et al. Oral bioavailability of digoxin is enhanced by talinolol: evidence for involvement of intestinal P-glycoprotein. Clinical Pharmacology & Therapeutics 2000;68:6-12.
117. Rozehnal V, Nakai D, Hoepner U, Fischer T, Kamiyama E, Takahashi M, Yasuda S, et al. Human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for characterizing the intestinal absorption of drugs. European Journal of Pharmaceutical Sciences 2012;46:367-373.
118. Marathe PH, Wen Y, Norton J, Greene DS, Barbhaiya RH, Wilding IR. Effect of altered gastric emptying and gastrointestinal motility on metformin absorption. British journal of clinical pharmacology 2000;50:325-332.
119. Pentikäinen P, Neuvonen P, Penttilä A. Pharmacokinetics of metformin after intravenous and oral administration to man. European journal of clinical pharmacology 1979;16:195-202.
120. Stopfer P, Giessmann T, Hohl K, Sharma A, Ishiguro N, Taub M, Zimdahl-Gelling H, et al. Pharmacokinetic evaluation of a drug transporter cocktail consisting of digoxin, furosemide, metformin, and rosuvastatin. Clinical Pharmacology & Therapeutics 2016;100:259-267.
121. Timmins P, Donahue S, Meeker J, Marathe P. Steady-state pharmacokinetics of a novel extended-release metformin formulation. Clinical pharmacokinetics 2005;44:721-729.
122. Tucker G, Casey C, Phillips P, Connor H, Ward J, Woods H. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. British journal of clinical pharmacology 1981;12:235.
123. Hammarlund M, Paalzow L, Odlind B. Pharmacokinetics of furosemide in man after intravenous and oral administration. Application of moment analysis. European journal of clinical pharmacology 1984;26:197-207.
124. Shen H, Holenarsipur VK, Mariappan TT, Drexler DM, Cantone JL, Rajanna P, Gautam SS, et al. Evidence for the validity of pyridoxic acid (PDA) as a plasma-based endogenous probe for OAT1 and OAT3 function in healthy subjects. Journal of Pharmacology and Experimental Therapeutics 2019;368:136-145.
125. Bindschedler M, Degen P, Flesch G, De Gasparo M, Preiswerk G. Pharmacokinetic and pharmacodynamic interaction of single oral doses of valsartan and furosemide. European journal of clinical pharmacology 1997;52:371-378.
126. Michael RK, Ralph EC, Arden WF, Barbara MK. Pharmacokinetics of orally administered furosemide. Clinical Pharmacology & Therapeutics 1974;15:178-186.
127. Hosgood S, Barlow A, Hunter J, Nicholson M. Ex vivo normothermic perfusion for quality assessment of marginal donor kidney transplants. Journal of British Surgery 2015;102:1433-1440.
128. Pool MB, Hamelink TL, van Goor H, van den Heuvel MC, Leuvenink HG, Moers C. Prolonged ex-vivo normothermic kidney perfusion: The impact of perfusate composition. PLoS One 2021;16:e0251595.

129. Kaths JM, Hamar M, Echeverri J, Linares I, Urbanellis P, Cen JY, Ganesh S, et al. Normothermic ex vivo kidney perfusion for graft quality assessment prior to transplantation. American Journal of Transplantation 2018;18:580-589.
130. Markgraf W, Mühle R, Lilienthal J, Kromnik S, Thiele C, Malberg H, Janssen M, et al. Inulin clearance during ex vivo normothermic machine perfusion as a marker of renal function. ASAIO Journal 2022;68:1211-1218.
131. van Leeuwen LL, Ruigrok MJ, Kessler BM, Leuvenink HG, Olinga P. Targeted delivery of galunisertib using machine perfusion reduces fibrogenesis in an integrated ex vivo renal transplant and fibrogenesis model. British Journal of Pharmacology 2023.
132. Venema LH, Leuvenink HG. Development of a porcine slaughterhouse kidney perfusion model. Transplantology 2022;3:6-19.
133. Lødrup AB, Karstoft K, Dissing TH, Nyengaard JR, Pedersen M. The association between renal function and structural parameters: a pig study. BMC nephrology 2008;9:1-9.
134. Moman RN, Gupta N, Varacallo M. Physiology, albumin. 2017.
135. Gülden M, Seibert H. Influence of protein binding and lipophilicity on the distribution of chemical compounds in in vitro systems. Toxicology in vitro 1997;11:479-483.
136. Henze LJ, Koehl NJ, O'Shea JP, Kostewicz ES, Holm R, Griffin BT. The pig as a preclinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms: a PEARRL review. Journal of pharmacy and pharmacology 2019;71:581-602.
137. Elmorsi Y, Al Feteisi H, Al-Majdoub ZM, Barber J, Rostami-Hodjegan A, Achour B. Proteomic characterisation of drug metabolising enzymes and drug transporters in pig liver. Xenobiotica 2020;50:1208-1219.
138. Stevens LJ, Dubbeld J, Doppenberg JB, van Hoek B, Menke AL, Donkers JM, Alsharaa A, et al. Novel explanted human liver model to assess hepatic extraction, biliary excretion and transporter function. Clinical Pharmacology & Therapeutics 2023.