Regional Expression Levels of Drug Transporters and Metabolizing Enzymes along the Pig and Human Intestinal Tract and Comparison with Caco-2 Cells

Stefan F.C. Vaessen, Marola M.H. van Lipzig, Raymond H.H. Pieters, Cyrille A.M. Krul, Heleen M. Wortelboer, and Evita van de Steeg


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ABSTRACT

Intestinal transporter proteins and metabolizing enzymes play a crucial role in the oral absorption of a wide variety of drugs. The aim of the current study was to characterize better available intestinal in vitro models by comparing expression levels of these proteins and enzymes between porcine intestine, human intestine, and Caco-2 cells. We therefore determined the absolute protein expression of 19 drug transporters and the mRNA expression of 12 metabolic enzymes along the pig intestinal tract (duodenum, jejunum, ileum; \( N = 4 \)), in human intestine (jejunum; \( N = 9 \)), and Caco-2 cells. Expression of the included transporters and enzymes was in general well comparable between porcine and human intestinal tissue, although breast cancer resistance protein, monocarboxylate transporter, multidrug resistance gene, and organic anion-transporting polypeptide (OATP) 4A1 (\( \sim 6 \)-fold) was higher expressed in pig compared with human jejunum. Alternatively, expression level of relevant transporter proteins (glucose transporter 1, OATP4A1, MRP2, MRP1, and OATP2B1) was significantly higher (3- to 130-fold) in Caco-2 cells compared with human jejunum. Moreover, all examined CYPs showed at least a fivefold lower gene expression in Caco-2 cells compared with human jejunum, with the smallest differences for CYP1A1 and CYP3A4 and the largest difference for CYP3A4 (871-fold higher expression in human jejunum compared with Caco-2 cells). In conclusion, a comprehensive overview is provided of the expression levels of clinically relevant transporters and metabolic enzymes in porcine and human intestinal tissue and Caco-2 cells, which may assist in deciding upon the most suitable model to further improve our understanding of processes that determine intestinal absorption of compounds.

Introduction

An accurate prediction of the human intestinal absorption and oral bioavailability early in drug development is essential in the pharmaceutical and nutritional industry, as this codetermines the efficacy and/or toxicity of the active compound. Several in vitro (e.g., Caco-2 cells, HT-29 cells, Ussing chamber) and in silico methods (e.g., GastroPlus and SimCyp) are currently in use to predict human intestinal absorption and subsequently human oral bioavailability of compounds. Initial assays to study intestinal apparent permeability of the compound and the effect of efflux transporters [e.g., multidrug resistance protein 1 (MDR1), breast cancer resistance protein (BCRP)] on intestinal absorption are often performed with Caco-2 cells, originating from human epithelial colorectal adenocarcinoma cells (Yee, 1997; Yazdanian et al., 1998; Haslam et al., 2011). Whereas the use of Caco-2 cell monolayers as an intestinal barrier model is well established and provides a quick and inexpensive screening model, standard Caco-2 cells lack morphologic and physiologic features of complete intestinal tissue. For instance, standard Caco-2 cultures show differences with complete intestinal tissue with regard to mucus production, passive diffusion, carrier-mediated uptake and excretion, paracellular transport via tight junctions, and intestinal metabolism (Rozehnal et al., 2012). However, recent studies in which Caco-2 cells were cultured on porous membranes in a fluidic device with peristaltic movement also demonstrate the formation of villi-like structures and increased metabolizing activity (CYP3A mediated) compared with Caco-2 cells cultured on Transwell membranes under static conditions (Kim and Ingber, 2013) and thereby more closely mimicking the human physiology. We recently developed the InTESTine system (Westerhout et al., 2014) to enable study processes that determine (human) intestinal absorption in a physiologic relevant model. In this system, ex vivo intestinal tissue (human or porcine) is mounted into a two-compartment system, simulating luminal and blood compartments. The InTESTine system is currently optimized to keep the mounted tissue viable for a maximum of 4 days.

ABBREVIATIONS: BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CYP, cytochrome P450; GLUT1, glucose transporter 1; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IVIVE, in vitro-in vivo extrapolation; KRb, Krebs-Ringer buffer; LC-MS/MS, liquid chromatography tandem mass spectrometry; MCT, monocarboxylate transporter; MDR1, multidrug resistance gene; MRP, multidrug resistance-associated protein; NTCP, Na-taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; PEPT1, peptide transporter 1; UGT, uridine 5’-diphospho-glucuronyltransferase.

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4 hours. It provides some distinct advantages compared with both Caco-2 cell culture systems and the frequently used Ussing chamber model (Lennernas, 2007; Rozenhals et al., 2012). The main advantages compared with Ussing chamber is the higher throughput, using a disposable multi-well setting, and standardized culture conditions using a humidified high oxygen/CO2 incubator on a rocker platform. The key advantage compared with Caco-2 cells is the presence of complete mucosal tissue, including the presence of different intestinal epithelial cells (enterocytes, goblet cells, enteroendocrine cells, Paneth cells, and M-cells) and the lamina propria, including the intraepithelial lymphocytes and other immune cells (Mowat and Agace, 2014). This makes the InTESTine model suitable for studies relating to gut health and gut immune function. Additionally, the presence of a natural mucus barrier in the InTESTine system enables the direct combination of biorelevant luminal samples with an in vitro absorption model to better simulate the physiology of the human GI epithelial wall.

The intestinal absorption of compounds across the intestinal epithelium depends on their chemical characteristics, and compounds can be substrates for numerous transporter proteins and metabolizing enzymes. There is, however, limited information available on the absolute expression of transporter proteins and metabolizing enzymes in the currently used models including Caco-2 cells, human, and porcine intestinal tissue. Although there is some literature available on the gene expression of drug transporter genes in human intestine and Caco-2 cells (Taipalensuu et al., 2001; Englund et al., 2006; Hilgendorf et al., 2007), mRNA expression levels of transporter proteins are shown not to correlate well with protein abundance levels (Ohtsuki et al., 2012). A lack of absolute expression levels of active transporter proteins and metabolizing enzymes could potentially result in inaccurate classification of the permeability and intestinal absorption of compounds. As pointed out, the various in vitro intestinal models all have their applications and limitations, and it is important to emphasize that there is not one model available that can be used as the golden standard to predict human luminal processes and intestinal absorption. The aim of the current study was therefore to characterize further and compare these potential in vitro intestinal models to determine their feasibility for absorption of compounds. To that end, we quantified the absolute and regional protein expression of several uptake and efflux transporters [BCRP, bile salt export pump (BSEP), glucose transporter 1 (GLUT1), monocarboxylate transporter (MCT) 1, MCT5, MDR1, MRPI, MRP2, MRP3, Na+taurocholate cotransporting polypeptide (NTCP), organic anion-transporting polypeptide (OATP) 4A1, OATP1B1, OATP1B3, OATP2B1, OATP1C1, organic cation transporter (OCT) 1, OCT3, OCTN2, peptide transporter 1 (PEPT1)] at the plasma membrane along the pig intestinal tract and compared these data to expression levels in Caco-2 cells and ex vivo human intestinal tissue. Moreover, mRNA expression profiles of several metabolizing enzymes of important cytochrome P450 and uridine 5'-diphospho-glucuronosyltransferase (UGT) families were studied. In future studies, these abundance data will be integrated in in silico models to be able to better predict human oral bioavailability based on in vitro absorption studies.

### Materials and Methods

#### Chemicals and Reagents.

Kreb's-Ringer bicarbonate buffer and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, The Netherlands). Dulbecco's modified Eagle medium, minimum essential medium, L-glutamine, gentamycin, penicillin, streptomycin, and heat-inactivated fetal bovine serum were purchased from Gibco (Paisley, Scotland).

#### Culturing of Caco-2 Cells.

The human colon carcinoma cell line Caco-2 was obtained from the German Collection of Microorganisms and Cell cultures (DSMZ ACC 169, Braunschweig, Germany). Caco-2 cells were cultured in HEPES-buffered Dulbecco's modified Eagle medium containing 4.5 g/l glucose, supplemented with 1% (v/v) minimum essential medium nonessential amino acids, 6 mM l-glutamine, 50 mg/l gentamicin, and 10% (v/v) heat-inactivated fetal bovine serum. Cells were grown in 75 cm² flasks (Corning-Costar, Cambridge, MA) at approximately 37°C in a humidified incubator containing a 95% air:5% CO2 mixture. For protein expression analysis, Caco-2 cells were cultured for 14 days in 75 cm² flasks, which were fully confluent after 3–4 days, and when harvested (14 days after seeding) a full epithelial monolayer was formed. Approximately 70 × 10⁶ trypsinized and pelleted cells were used for single plasma membrane isolation (preparation in duplicate, n = 2). For gene expression analysis, Caco-2 cells were cultured for 21 days on permeable supports (polyester membrane with 0.4 μm pore size, Corning, NY) in a 12-well plate with medium replacement every 2–3 days.

#### Origin of Pig and Human Intestinal Tissue.

Five healthy domestic pigs (Sus scrofa domesticus, 2 male and 3 female, age 10–14 weeks and body weight between 15 and 25 kg) were used for the collection of intestinal tissue. These animals were additionally used for educational purposes at the Utrecht University (Utrecht, The Netherlands) with approval of the local animal welfare office, and in full compliance with the aim to contribute to the reduction, refinement, and replacement of animal experiments. Before euthanization, pigs had free access to food and water. Intestinal tissue of domestic pigs was collected only when defined healthy as judged by a veterinarian. Before the isolation of the intestine, 2000 ml Krebs-Ringer bicarbonate buffer (containing 10 mM glucose, 25 mM HEPES, 15 mM sodium bicarbonate, 2.5 mM calcium chloride, pH 7.4, and saturated with oxygen using a 95%/5% O2/CO2 mixture by gassing for 120 minutes, further indicated as KRB) was divided over different small volume flasks. After sedation, animals were euthanized and segments of duodenal tissue (the first 25 cm from the stomach), jejunal tissue (150 cm from the stomach), and ileal tissue (50 cm from the ileocecal valve) were excised, flushed with ice-cold KRB buffer, stored in ice-cold KRB, transported to the laboratory, and immediately used for ex vivo protein analysis. Once in the laboratory, the intestinal tissue segments were cut into pieces of 10 to 20 cm and cut open longitudinally continuously submerged under ice-cold KRB buffer during further preparation. Then the upper villus layer of the mucosa was removed with the edge of a glass slide and mucosal cells were collected and quickly stored lower than −70°C until further processing.

Human jejunum samples derived from 9 individuals (4 women, 5 men) were collected at the University Medical Center of Groningen (UMCG, Groningen, The Netherlands) and were kindly provided by Prof. Dr. G.M.M. Groothuis (University of Groningen, The Netherlands). Collection of redundant intestinal tissue from surgeries (collected as waste material) was approved by the Medical Ethical Committee of the UMCG. No clinically relevant or identifiable information from the patients was collected. Intestinal tissue samples were directly snap frozen and stored lower than −70°C until further processing. The weight of four of these tissue samples was sufficient for plasma membrane protein analysis and subsequent quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis; the remaining samples were only used for gene expression analysis.

#### Protein Isolation for Quantitative LC-MS/MS Analysis.

To determine absolute protein expression levels of BCRP, BSEP, GLUT1, MCT1, MCT5, MDR1, MRPI, MRP2, MRP3, NTCP, OATP4A1, OATP1B1, OATP1B3, OATP2B1, OATP1C1, organic cation transporter (OCT) 1, OCT3, OCTN2, peptide transporter 1 (PEPT1) at the plasma membrane along the pig intestinal tract and compared these data to expression levels in Caco-2 cells and ex vivo human intestinal tissue. Moreover, mRNA expression profiles of several metabolizing enzymes of important cytochrome P450 and uridine 5'-diphospho-glucuronosyltransferase (UGT) families were studied. In future studies, these abundance data will be integrated in in silico models to be able to better predict human oral bioavailability based on in vitro absorption studies.
during 5 minutes with a flow of 600 μL/min followed by a 2 minute wash-out with 100% mobile phase B. Peptides were ionized with electrospray, and quantification was performed with a 6500 QTrap (ABSciex) using a scheduled MRM-mode. Cone voltage and collision energy were optimized for each compound individually. For each peptide 3 transitions were chosen (Q3–1, Q3–2, and Q3–3) for quantitation and confirmation (Supplemental Table 1). In case no suitable prototypic peptide could be selected for the human and porcine transporter proteins, two separate peptides were selected and synthesized (Supplemental Table 1). Peptides labeled with 15N and 13C (AQUA peptide) were synthesized (Sigma Aldrich Chemie, Steinheim, Germany) and used as an internal standard.

**Fig. 1.** Expression of various uptake and efflux transporter proteins within the plasma membrane of different regions of the pig intestinal tract presented as absolute expression (A) and expression relative to villin (B), as well as the relative protein expression in, respectively, duodenum (C), jejenum (D), and ileum (E) ranked in increasing order according to protein expression levels. Data are presented as mean ± S.E.M. (n = 4, samples processed in duplicate). *P < 0.05; **P < 0.01; ***P < 0.001 compared with expression level in duodenum.
for quantification. For each peptide, a calibration curve of 0.01–50 ng/ml and quality controls were included in every run. Peak identification and quantification was performed using Analyst software version 1.6.

RNA Isolation and cDNA Synthesis. Total RNA was isolated from flash frozen pig jejunum tissue (5 pigs, 10 mg of tissue), human jejunum tissue (9 donors, 10 mg tissue), and from Caco-2 cells after a 21-day differentiation in transwells (10 independent 12-well incubations from same passage) using the Quick-RNA miniprep kit (Zymo Research, Irvine, CA), which includes a DNAse step to ensure complete removal of genomic DNA. Total RNA was transcribed to cDNA using the iScript cDNA synthesis kit (BioRad, Hercules, CA) and used directly for qPCR analysis after a fivefold dilution.

RT-PCR Measurements. Primer pairs (see Supplemental Table 2) were designed within 1 exon, allowing absolute copy quantification using a genomic DNA standard curve thus enabling the comparison of results between different gene targets and between different tissues, i.e., pig jejunum, human jejunum, and Caco-2 cells. qPCR analysis was performed on a StepOnePlus system (Applied Biosystems, Waltham, MA) using Fast SYBR Green Master mix (Applied Biosystems) according to the protocol provided. Absolute gene copy numbers were calculated using standard curves constructed with human or porcine genomic DNA (Novagen, Merck Millipore, Billerica, MA) as described by Yun et al. (2006). Calculated gene copy numbers were corrected for beta-actin and villin copy numbers to correct for material input.

Data Analysis. One-way analysis of variance followed by Tukey’s multiple comparison test was used throughout the study to assess the statistical significance of differences between multiple datasets (GraphPad Prism 4.1 software was used for this). Differences were considered to be statistically significant when $P < 0.05$.

Results

Absolute Transporter Protein Expression along the Pig Gastrointestinal Tract. The abundance of a set of transporter proteins along the pig intestinal tract (duodenum, jejunum, and ileum) was determined using quantitative mass spectrometry (Fig. 1). We isolated the plasma membrane fractions of duodenum, jejunum, and ileum mucosal tissue samples derived from four individual domestic pigs. Transporter proteins are functionally active when expressed at the outer plasma membrane. Absolute expression levels of the various transporter proteins ranged between 0.01 and 2 pmol/mg tissue (Fig. 1A). The expression of BSEP, NTCP, OATP1B1, OATP1B3, OATP1C1, and OCT3 transporter proteins in pig intestine was below the lower limit of quantification (i.e., ≤0.01 pmol/mg, equivalent to approximately 0.01 pmol/g tissue) in all tissue samples (Fig. 2). Of the detectable transporter

![Fig. 2.](image-url)
proteins, MDR1 and OATP4A1 were most abundantly and almost equally expressed in pig samples of duodenum, jejunum, and ileum, followed by MRP3 and BCRP in pig duodenum and ileum and PEPT1 and BCRP in pig jejunum. Overall, GLUT1, MCT1, MRP2, and MRP3 were among the lowest expressed transporters in pig samples, but their absolute numbers were significantly lower in segments from pig jejunum and ileum compared with duodenum. This could mainly be explained by decreases in epithelial cell numbers in the more distal parts of the gastrointestinal tract, indicated by decreasing amounts of villin, a known epithelial marker (West et al., 1988) (Supplemental Fig. 1). After correction for the amount of villin, only the expression of MR2 remained significantly lower in jejunum and ileum compared with duodenum (Fig. 1, B–E). Interestingly, an opposite tendency was observed for the transporters MDR1, OATP4A1, and MCT5, with slightly increasing expression levels when comparing the duodenum to the ileum.

### Transporter Protein Expression in Pig and Human Jejunum in Comparison with Caco-2 Cells

Because monolayers of the human intestinal epithelial Caco-2 cells are generally used as an in vitro screening model for human intestinal permeability, we compared the expression of several transporter proteins in human Caco-2 cells with ex vivo human or pig jejunal tissue samples (Fig. 2A). By correcting for the amount of villin (epithelial marker), we enabled direct comparison of transporter protein abundance in human or pig jejunum with Caco-2 cells and also enabled direct comparison of human and porcine intestinal tissue, which were processed slightly differently (mucosal layer versus whole tissue) resulting in possible differences in the amount of epithelial cells included in the sample (absolute expression of villin in Caco-2 cells compared with pig intestine is presented in Supplemental Fig. 1). Villin expression was not significantly different between human and pig jejunum. Comparable to human and pig jejunum, expression of BSEP, NTCP, OATP1B1, OATP1B3, OATP1C1, and OCT3 in Caco-2 cells was below the lower limit of quantification (i.e., ≤0.01 ng/ml, comparable to ~4 × 10⁻⁵ pmol/10⁶ cells). Relative expression of GLUT1, OATP4A1, MRP2, MRP1, and OATP2B1 was significantly higher (3- to 130-fold) in Caco-2 cells compared with human jejunum, whereas expression levels of BCRP, MCT1, MCT5, MDR1, MRP3, OCT1, OCTN2, and PEPT1 did not differ significantly between differentiated Caco-2 cells and human or pig jejunum samples. Expression of BCRP, MCT5, MDR1, MRP1, MRP3, and OATP4A1 appeared to be slightly (although significantly) higher in pig jejunum compared with human jejunum (Fig. 2A). Absolute expression levels of transporter proteins in pig and human jejunum are presented in Fig. 2B.

### Intestinal Gene Expression of Metabolizing Enzymes

To compare gene expression of metabolizing enzymes between human jejunal tissue (n = 9), pig jejunal tissue (n = 5), and human Caco-2 cells (n = 10), we determined gene expression of a selected panel of CYPs and UGTs. Based on data in the literature, the most abundantly expressed and therefore likely the most relevant human CYPs and UGTs were selected, i.e., for CYPs: CYP2C9, CYP2C12, CYP3A4, and CYP3A5 and for UGTs: UGT1A1, UGT1A6, UGT1A10, UGT2A3, and UGT2B7 (Paine et al., 2006; Bieche et al., 2007; Pavek and Dvorak, 2008). In addition, four different CYP genes and one UGT gene were included with lower intestinal (protein) expression levels, but with known relevance for human drug metabolism, i.e., CYP1A1, CYP2C18, CYP2D6, CYP2E1, and UGT1A8. For every human CYP and UGT gene we attempted to include at least one pig homolog (see Tables 1 and 2). The identified pig homologs shared on average 75% amino acid homology with their human counterparts. By using a genomic DNA calibration curve in every qPCR analysis allowed for the absolute quantification of copy numbers and therefore the possibility of comparing results of different gene targets and between sample types. Relative CYP gene expression (corrected for actin-beta and villin expression) in the three models, i.e., human jejunal tissue, human Caco-2 cells and pig jejunal tissue, are presented in Fig. 3. As expected for human jejunal tissue, CYP3A enzymes showed the highest expression, whereas CYP1A1 and CYP2E1 were of very low expression. All examined CYPs showed at least a fivefold lower gene expression in Caco-2 cells compared with ex vivo human jejunal tissue, with the smallest differences for CYP1A1 and CYP3A5 and the largest difference for CYP3A4, i.e., 871-fold higher expression in human jejunal compared with Caco-2 cells. In general, pig CYP homologs in pig jejenum showed expression levels more comparable to human jejenum than to Caco-2 cells. However, whereas the expression level of pig CYP2C42 was comparable to CYP2C enzymes in human jejenum, the expression levels of the pig CYP2C33 and CYP2C49 enzymes were more comparable to expression levels of CYP2C enzymes in Caco-2 cells. Figure 4 shows the gene expression levels of UGTs. All examined UGTs, except for UGT1A6, showed higher expression levels in human jejunal tissue compared with Caco-2 cells. Expression of pig homologs was for some UGTs comparable to expression in human tissue, i.e., UGT1A10 and to a lesser extent UGT1A1 and UGT1A6, but pig UGT2B enzymes were hardly expressed, whereas human UGT2B7 showed significant expression both in human ex vivo jejunal tissue and in Caco-2 cells.

### Discussion

In this study, we provide a comprehensive dataset for the expression of transporter proteins and metabolic enzymes along the pig intestinal tract and compared these expression profiles with human intestinal tissue and Caco-2 cells. To the best of our knowledge, this is the first study that...
directly compares expression of a set of active transporter proteins and metabolizing enzymes between porcine intestinal tissue, human intestinal tissue, and Caco-2 cells. Here, we also describe for the first time regional differences in expression of a set of 19 transporter proteins in the pig gastrointestinal tract. In this study, we determined transporter protein expression at the outer plasma membrane of cells, which is the most pure fraction only containing the outer plasma membranes where the transporter proteins are actively expressed. We previously successfully set up and used a method for plasma membrane isolation (Bosgra et al., 2014) and used this method in the current study. Various protein isolation protocols have been applied and described in literature for transporter protein abundance using LC-MS/MS, and recent insights show that the loss of proteins during various centrifugations steps is significant (Ohtsuki et al., 2012; Harwood et al., 2014). It is therefore very important to use the same protein isolation method for comparison between cell lines and (human) tissue levels, and for application in in vitro-in vivo extrapolation (IVIVE). In the current study we also used villin expression to normalize for the amount of epithelial cells to enable direct comparison between tissue types and cell lines.

Regional differences in expression of transporter proteins in human intestinal tissue have been observed before by Western blot analysis for single transporter proteins (Englund et al., 2006; Meier et al., 2007). Interestingly, a recent paper describes the protein abundance of relevant drug transporters in differential regions of the human gastrointestinal tract (Drozdzik et al., 2014) using a crude membrane extraction procedure. Because this crude membrane preparation differs from the plasma membrane preparation used in the current study, direct and quantitative comparison between the studies is difficult. Nevertheless, Drozdzik and coworkers observed regional-dependent differences in the level of protein expression for MDR1, which appeared to be higher toward the more distal parts of the small intestine. A similar pattern was observed in the current study for MDR1 in pig intestinal tissue, as was the case for OATP4A1 and MCT5 (these latter 2 proteins were not included in the study by Drozdik et al.). For all other transporter proteins, no regional differences in absolute expression levels were detected in the current study. By using a slightly different detection method (QconCat, in which isotope-labeled peptides are generated by proteolytic digestion of an artificial protein constructed within *Escherichia coli*) and a crude membrane isolation method, Harwood et al. (2015) recently described the expression MDR1, BCRP, and MRP2 in human jejunal and ileum tissue samples. Relative differences in abundance between these three transporter proteins confirmed lowest expression of MRP2 compared with MDR1 and BCRP in human jejunum (and ileum), which we also found in our dataset for human jejunum. Moreover, the same trend of lowest MRP2 expression compared with MDR1 and BCRP was observed in pig intestinal tissue (duodenum, jejunum, and ileum), indicating good similarity between human and pig intestinal tissue.

Because monolayers of Caco-2 cells are generally used as an in vitro screening model for assessment of human intestinal permeability, we compared expression of a set of transporter proteins in human small intestinal tissue with Caco-2 cells. In a recent paper by Harwood et al. (2016), they describe a cross-laboratory study between two laboratories where they looked at expression of MDR1 and BCRP in human jejunum and Caco-2 cells. Both laboratories observed twofold higher absolute expression of MDR1 in Caco-2 cells compared with human jejunum, and 1.5- to 2-fold decreased expression of BCRP in Caco-2 cells compared with human jejunum. Similar trends for MDR1 and BCRP in comparing Caco-2 cells with human jejunum were observed in the

**Fig. 3.** Gene expression of different CYP enzymes in human jejunum, human Caco-2 cells, and pig jejunum. Gene copy numbers are corrected for beta-actin (ACTB) and villin (Vil) copy numbers to correct for input material. Data are presented as mean ± S.E.M. (*n* = 9 for human jejunum, *n* = 10 for Caco-2 cells, and *n* = 5 for pig jejunum), and samples were processed in duplicate. na, Not applicable (no pig homolog available).

**Fig. 4.** Gene expression of different UGT enzymes in human jejunum, human Caco-2 cells, and pig jejunum. Gene copy numbers are corrected for beta-actin (ACTB) and villin (Vil) copy numbers to correct for input material. Data are presented as mean ± S.E.M. (*n* = 9 for human jejunum, *n* = 10 for Caco-2 cells, and *n* = 5 for pig jejunum), and samples were processed in duplicate. na, Not applicable (no pig homolog available).
To study the intestinal absorption and gut health in a more physiologically relevant model using intestinal tissue, we recently developed an improved alternative for the Ussing chamber system, the InTESTine system (Westerhout et al., 2014). Because of rather limited availability of human intestinal tissue, we initially set up and evaluated the InTESTine system with porcine intestinal tissue. The application of human donor intestinal tissue in InTESTine was only added recently, and will possibly fasten the translation to the human in vivo situation and enables the study of human specific intestinal targets (unpublished data). Data from the current study will further improve our understanding of the observed differences in the intestinal absorption and metabolism of various drugs and nutrients between these different preclinical intestinal models. We revealed some important differences between Caco-2 cells, porcine intestinal tissue, and human intestinal tissue that need to be taken into account when using one of these models, for example, by scaling the differential expression of these transporter proteins and metabolizing enzymes to human tissue. We recently showed the value of absolute transporter protein expression determination for IVIVE (Bosgra et al., 2014), where we predict hepatic disposition of rosuvastatin by scaling from individually transfected cell lines by correcting for absolute transporter protein expression within the plasma membrane. Therefore, as a next step, these data will be integrated into in silico models to use of IVIVE to better predict processes that determine intestinal absorption and finally predict oral bioavailability of orally administrated compounds.

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Authorship Contributions

Participated in research design: Vaessen, Lipzig, Pieters, Krul, Wortelboer, and van de Steeg.

Conducted experiments: Vaessen and van de Steeg.

Performed data analysis: Vaessen and van de Steeg.

Wrote or contributed to the writing of the manuscript: Vaessen, Lipzig, Pieters, Krul, Wortelboer, and van de Steeg.

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