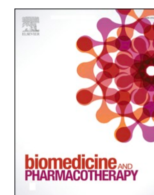
Biomedicine & Pharmacotherapy 160 (2023) 114329

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha



Prandial state and biological sex modulate clinically relevant efflux transporters to different extents in Wistar and Sprague Dawley rats

Francesca K.H. Gavins [a,1], Liu Dou [b,1], Yujia Qin [b,1], Christine M. Madla [a], Sudaxshina Murdan [a], Abdul W. Basit [a], Yang Mai [b,*], Mine Orlu [a,*]

[a] Department of Pharmaceutics, UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London WC1N 1AX, UK
[b] School of Pharmaceutical Sciences (Shenzhen), Sun Yat-Sen University, Guangzhou 510275, China

ARTICLE INFO

Keywords:
Pharmacokinetics
mRNA quantification
Intestinal permeability
Animal models
Food effects on drug bioavailability
ABC transporters

ABSTRACT

P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein 2 (MRP2) are clinically relevant efflux transporters implicated in the oral absorption of many food and drug substrates. Here, we hypothesised that food intake could influence protein and mRNA intestinal expression of P-gp/*abcb1a*, BCRP/*abcg2*, and MRP2/*abcc2* differently in male and female Wistar and Sprague Dawley rats. To test this hypothesis, we used enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (PCR) to quantify the protein and mRNA intestinal expression of these transporters, respectively. Our study found food and sex differences in P-gp expression, whereby in the fed state P-gp expression decreased in male Wistar rats, but P-gp expression increased in females. In the fed state, BCRP expression increased in both male and female Wistar rats, compared with the fasted state. In contrast, no sex differences or food effect differences were seen in Sprague Dawley rats for P-gp and BCRP expression. On the other hand, in the fed state, MRP2 expression was higher in male and female Wistar and Sprague Dawley rats when compared with the fasted state. Sex differences were also observed in the fasted state. Overall, significant strain differences were reported for P-gp, BCRP and MRP2 expression. Strong to moderate positive linear correlations were found between ELISA and PCR quantification methods. ELISA may be more useful than PCR as it reports protein expression as opposed to transcript expression. Researchers must consider the influence of sex, strain and feeding status in preclinical studies of P-gp, BCRP and MRP2 drug substrates.

1. Introduction

Efflux transporters can affect the pharmacokinetics, safety, and efficacy of a wide range of drugs [1]. Most efflux transporters belong to the ATP binding cassette (ABC) family and actively pump substrates out of cells. The overexpression of ABC transporters in cancer cells can result in multidrug resistance, and this phenomenon has driven extensive mechanistic studies into the expression of these proteins [2]. To date, more than 400 membrane transporters in the ABC and solute carrier superfamilies have been discovered in the human genome, with a total of 50 ABC transporters characterised in humans [3,4]. The most researched ABC transporter is permeability-glycoprotein, also known as P-glycoprotein (P-gp) (molecular mass of 170 kDa). It was first identified in colchicine resistant Chinese hamster ovarian cells by Juliano and Ling in 1974 [5]. More than 20 years later, a structurally similar transporter termed multidrug resistance-associated protein 2 (MRP2) was characterised as a canalicular multi-specific organic anion transporter in hepatocytes in 1996 [6]. Later, breast cancer resistance protein (BCRP) was isolated in multidrug-resistant human breast cancer cells in 1998 [7].

The International Transporter Consortium (ITC) and the Food and Drug Administration (FDA) recognise P-gp and BCRP as clinically relevant transporters [1,8]. Here, MRP2 was additionally investigated as a key efflux transporter, with reportedly high levels of expression in

Abbreviations: ABC, ATP binding casette; BCRP, Breast cancer resistance protein; ELISA, Enzyme-linked immunosorbent assay; FDA, Food and Drug Administration; GI, Gastrointestinal; ITC, International Transporter Consortium; MRP2, Multidrug resistance-associated protein 2; P-gp, P-glycoprotein; PCR, Polymerase chain reaction; mRNA, Messenger ribonucleic acid.

* Corresponding authors.
E-mail addresses: maiy6@mail.sysu.edu.cn (Y. Mai), m.orlu@ucl.ac.uk (M. Orlu).

[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.biopha.2023.114329
Received 29 October 2022; Received in revised form 23 January 2023; Accepted 27 January 2023
Available online 31 January 2023
0753-3322/© 2023 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

intestinal barrier tissues [9]. These transporters are found in the blood-brain barrier and epithelia of the kidney, liver, as well as in the enterocytes of the intestinal tract. At the intestinal layer, membrane transporters are apically located and efflux endogenous and exogenous substrates, potentially limiting absorption into the systemic circulation. Physiologically, efflux transporters protects the body from toxic substances, such as tumour necrosis factor and lipopolysaccharide in cases of injury [10]. Also, P-gp was reported to be involved in cholesterol trafficking [11] and lipid homeostasis [12]. BCRP is reported to transport conjugates of steroid hormones [13], and MRP2 is found to transport glutathione, glucuronide, and sulphate conjugates. A wide range of structurally unrelated hydrophobic substances bind to these transporters and show partially overlapping substrate specificity [14]. Food components as well as drugs are reported to interact with these transporters, and can limit oral bioavailability through food-drug interactions at the transporter level [15]. The bioavailability of orally administered drugs is in part dependent on the expression and activity of these transporters. Importantly, the distribution of transporters in the GI tract is heterogenous and each GI segment (duodenum, jejunum, ileum and colon) shows different patterns of expression [16,17]. This regional heterogeneity in the drug transporter expression may impact the site of absorption for an oral drug product.

Food can interact with a co-administered drug in the gastrointestinal (GI) tract during the stages of drug absorption, distribution, metabolism and excretion [18]. The intake of food can alter the luminal pH, gastric emptying times, fluid volumes, and bile salt concentrations as well as other key physiological parameters [19]. *In vitro* studies have reported that dietary components, such as monoglycerides, can inhibit intestinal enzymes, as well as uptake and efflux transporter activity [20,21]. While many preclinical and clinical studies examine the effect of specific food components, our current study considers the overall effect of a meal. Recent analyses of oral drugs licensed between 2010 and 2017 by the FDA and the EMA found that over 40 % of drugs displayed significant changes in oral drug bioavailability in the fed state, relative to the fasted state [22]. Despite extensive research and significant progress in the field, the changes to drug bioavailability caused by food intake, termed 'the food effect', remains a problem in pharmaceutical drug development. Importantly, key mechanisms that drive the food effect are often unknown [23].

A major goal of preclinical drug development is to predict drug activity in humans [24]. *In vitro* models, computational predictive tools, and animal models are harnessed for valuable early insights. A useful animal model is the rat, which is frequently used in biomedical studies due to cost, convenience, expertise, and extensive characterisation studies [25]. Preclinical animal studies conventionally use male animals as researchers believe that female animals could introduce variability due to the female oestrous cycle, high body fat content, and increased cost and sample sizes [26]. However, the lack of female animal models in biomedical research has not accounted for potential sex differences in pharmacological responses [27]. Numerous drugs show distinct pharmacokinetic differences between the sexes in preclinical animal models, explored in a recent review [27]. For example, female rats showed a 20-fold increase in the drug bioavailability compared to males for schizandrin, a P-gp inhibitor [28].

Multiple quantification techniques are available in biomedical research. One example is real time-polymerase chain reaction (PCR), which is considered the 'gold' standard for the amplification and quantification of mRNA due to its high sensitivity and sequence-specificity [29]. However, post-translational modifications may take place that might not necessarily correlate with the respective protein expression [30]. Enzyme-linked immunosorbent assay (ELISA), on the other hand, quantifies protein expression by complexing antibodies and antigens. Commercial ELISA kits are readily available, and the test is highly sensitive and robust. One subtype of ELISA is sandwich ELISA, whereby well-plates are pre-coated with primary antibodies, which allows specific antigen binding on the ELISA plate [31]. The antibodies used in ELISA can specifically measure the protein in a biological sample, whereas the primers employed in PCR will bind to a specific mRNA sequence within a biological sample. Comparisons of ELISA and PCR quantification can yield complementary information on the respective protein and mRNA expression.

In the reported literature, there is heterogeneity in the choice of preclinical models for investigations into efflux transporters. Commonly used cell line models are derived from different species and sexes, for example, Caco-2 cells are derived from male human colonic epithelial, whereas MDCK cells are derived from female dog kidney epithelial [32]. Therefore, findings from these different models should not be directly compared. Our research group previously reported significant differences in P-gp expression between the sexes, strains of rats and feeding status [33–36]. Here, we hypothesise that the feeding status, sex, and strain of the rat animal model will differently affect the expression of key efflux transporters P-gp/*abcb1a,* BCRP/*abcg2* and MRP2/*abcc2* to different magnitudes. The study is also the first to compare PCR and ELISA methods for intestinal mRNA and protein transporter expression.

2. Materials and methods

2.1. Materials

Krebs-bicarbonate Ringer's solution (KBR), pH 7.4, was freshly prepared before the experiment at room temperature and was kept at 37 °C. KBR was composed of 10 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 115 mM NaCl, 25 mM $NaHCO_3$, 0.4 mM KH_2PO_4 and 2.4 mM K_2HPO_4 [13]. Lysis buffer was freshly prepared with 50 mM Tris, 250 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 % Nonidet P40 and protease inhibitor cocktail from Sigma (Darmstadt, Germany) in a phosphate-buffered saline (PBS) solution and stored at 4 °C. All other chemicals and kits are mentioned individually in the following methods.

2.2. Animals

Five male and five female Wistar and Sprague Dawley rats (healthy, 8–13 weeks old) were used as the animal models. The rats were housed at room temperature (25 °C) in a light–dark cycle of 12 h were provided with free access to food and water. The rats acclimatised in the animal unit for at least 7 days. On the evening before the experiments, the fasted group of rats were fasted overnight with free access to water (for 12 h) and housed individually in metabolic cages until the following morning at 8:30 am. For the fed group of rats, no fasting took place. The animal diet was a maintenance diet purchased from JiangSu XieTong Ltd. (Cat No. SWS9102). The protocols were agreed by the Administrative Committee on Animal Research in Sun Yat-sen University. Full compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were practiced and followed.

2.3. Intestinal tissue collection

Following sacrifice by CO_2 asphyxiation, their intestines were immediately excised and stored in an ice-cold KBR solution. Roughly 2 cm pieces of the intestine; duodenum (1 cm from the ligament of Treitz), jejunum (10 cm from the ligament of Treitz), ileum (1 cm from the caecum), and colon (descending) were opened along their mesenteric border. The tissues were gently washed with KBR solution to remove the intestinal contents.

2.4. Initial total protein quantification

The mucosal tissues (approximately 50 mg) were cut into small pieces and homogenized in 0.5 mL RIPA lysis buffer at 30 Hz for 30 s with a TissueLyser (QIAGEN, Hilden, Germany)., The process was repeated twice at intervals of 30 s for complete homogenisation. The

tissue homogenates were incubated at 4 °C for 2 h, and then centrifuged at 12,000g for 5 min. The total tissue protein was collected in the supernatants, and its concentration was subsequently determined with the Pierce™ BCA protein assay kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions.

2.5. Measurement of P-gp, BCRP, and MRP2 protein levels in by quantitative enzyme linked immunosorbent assay (ELISA)

ELISA kits were purchased from MEIMAN Biotech (Guangzhou, China). The ELISA kits were as follows: rat P-gp ELISA Kit (MM-0604R2), rat BCRP ELISA kit (MM-0606R2) and rat MRP2 ELISA kit (MM-0607R2). The ELISA kits (Meimian Biotech, Guangzhou, China) were used based on the manufacturer's description and a volume of the supernatant (from Section 2.4) containing a mass of 50 µg of total protein lysate was used as the samples. The units of protein expression were ng of transporter per mg of protein.

2.6. Measurement of P-gp, BCRP, and MRP2 mRNA levels in by PCR

Following collection (as described in Sections 2.3 and 2.4), the mucosal tissues were kept in RNAlater Stabilization Solution (Thermofisher). Total RNA in each intestinal sample was isolated and purified with the PureLink RNA Mini Kit (Thermofisher) and RNA concentration was measured with a Nanodrop 2000 (Thermofisher) according to the manufacturer's instructions.

Subsequently, the quantification of the target RNA was conducted as follows: 1 mg of total RNA of each sample was reverse transcribed using the iScript cDNA Synthesis Kit (BioRad). To quantify the level of P-gp mRNA (*abcb1a*), BCRP mRNA (*abcg2*) and MRP2 mRNA (*abcc2*), real time–PCR was performed with the 7500 Real-Time PCR System (Applied Biosystems, Thermofisher) using the method described in a study by MacLean et al. [37]. Briefly, 50 µL of PCR reaction contained 25 µL of PowerUp SYBR Green PCR Master Mix (Thermofisher), 500 nM each of forward and reverse primers, and 1 µg of cDNA. β-actin (*ACTB*) was used for normalization and amplification of 1 µg of cDNA. Real time-PCR was carried out in 96-well PCR plates (Thermofisher). The amplification program for all genes consisted of one preincubation cycle at 95 °C with a 10 min hold, followed by 45 amplification cycles with denaturation at 95 °C with a 10 s hold, an annealing temperature of 50 °C with a 10 s hold and an extension at 72 °C with a 10 s hold. Amplification was followed by a melting curve analysis which ran for one cycle with denaturation at 95 °C with a 1 s hold, annealing at 65 °C with a 15 s hold and melting at 95 °C with a 1 s hold. Distilled water was included as a negative control in each run to determine the specificity of primers and possible contaminants.

Primers (shown in Table 1) were designed by primer-BLAST searching with publicly available sequence information from GeneBank, National Center for Biotechnology Information (NCBI) and purchased from Eurofins (Eurofins Genomics, Germany).

Relative expressions of *abcb1a*, *abcg2,* and *abcc2* mRNA in different intestinal segments were calculated using 7500 software (version 2.0.6, Thermofisher). The averages of the threshold cycle (Ct) values for tested genes and the internal control (β-actin, *ACTB*) were taken, and then the differences between the Ct values for tested genes and internal control (ΔCt) were calculated for all the experimental samples.

2.7. Statistical analysis

The data was tested for normality using the Shapiro-Wilk test. As this was true, significant differences among experimental groups were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis using Python (version 3.9.0, Dover, DE, USA). A significance value of $p < 0.05$ was used for all tests. The correlations between the ELISA and PCR methods for protein (P-gp, BCRP, and MRP2) and mRNA (*abcb1a*, *abcg2,* and *abcc2*) expression, respectively, were assessed by the Spearman's rank coefficient method in Python (version 3.9.0, Dover, DE, USA).

2.8. Data presentation

Most of the figures were constructed as box plots consisting of a central line indicative of the median, a box indicative of the interquartile range (IQR), where the whiskers were 1.5 times the 25th and 75th percentile, respectively and the diamond shapes represented the outliers. Any value that was 1.5 x IQR greater than the third quartile was classified as an outlier and any value that was 1.5 x IQR less than the first quartile was also as an outlier. No outliers were removed. Correlation plots were represented as line and scatter plots. Jupyter Notebook version 6.0.3 (San Diego, CA, USA) was used with the Matplotlib package version 3.4.3 [38].

3. Results

3.1. Intestinal P-gp and abcb1a quantification in Wistar rats

Fig. 1 and Table S1 show the P-gp expression in Wistar rats, comparing the feeding states. The P-gp expression in the fasted and fed states were statistically different between the sexes ($p < 0.05$). In the male rat, the P-gp expression was decreased in the duodenum (−32 %), jejunum (−42 %), ileum (−29 %), and colon (−35 %) in the fed state, compared with the fasted state. On the other hand, in the female rat, the P-gp expression increased in the jejunum (+28 %), ileum (+70 %), and colon (+140 %) in the fed state. Regional differences were also observed, where the fasted male rat showed regional differences in the expression from the duodenum to the jejunum, the jejunum to the ileum, and the ileum to the colon. The fasted female P-gp expression were comparable between the regions; although a statistically significant difference was seen from the ileum to the colon (10.219 ± 1.246 ng/mg to 8.500 ± 1.059 ng/mg, respectively). A moderately strong positive correlation ($r = 0.615$) was seen for the Wistar rat between the ELISA protein quantification method and PCR mRNA quantification method

Table 1
Primers used for the analysis of P-gp, BCRP, and MRP2 in rat intestines by PCR.

Gene		Primer (5′−3′)	Amplicon (bp)	Genebank accession
P-gp				
abcb1a	forward	CACCATCCAGAACGCAGACT	139	NM_133401
	reverse	ACATCTCGCATGGTCACAGTT		
BCRP				
abcg2	forward	GTAGGTCGGTGTGCGAGTCA	717	NM_181381
	reverse	AACCAGTTGTGGGCTCATCC		
MRP2				
abcc2	forward	GACGACGATGATGGGCTGAT	883	NM_012833
	reverse	AGGCACGGATAATGGGCAAA		
Beta-actin				
ACTB	forward	GCAGGAGTACGATGAGTCCG	74	NM_031144
	reverse	ACGCAGCTCAGTAACAGTCC		

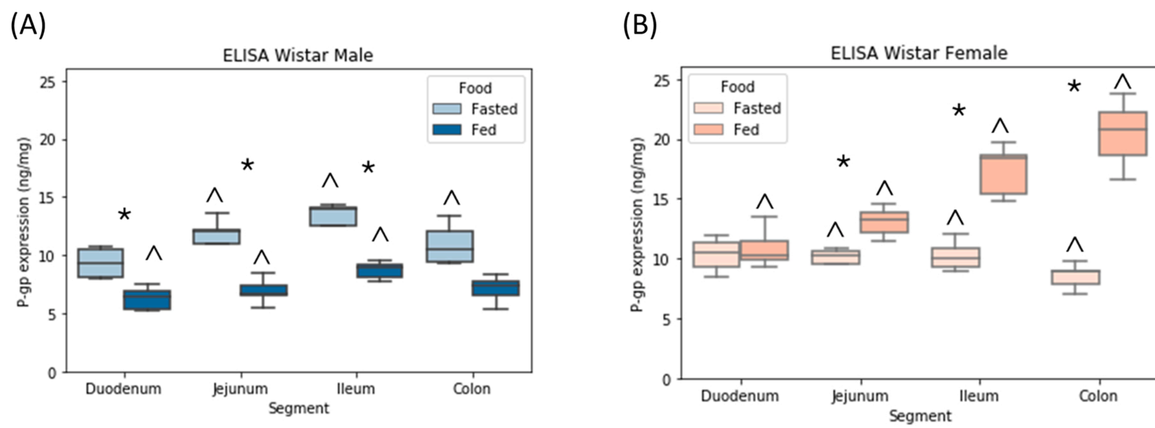


Fig. 1. – P-gp expression in fasted and fed (A) male and (B) female Wistar rats quantified by ELISA (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

(Fig. S1).

In the Wistar rat, the PCR quantification showed similar trends to the protein quantification in the male rat; the *abcb1a* expression decreased by −55 % in both jejunum and ileum in the fed state (Fig. 2 and Table S2). In contrast, *abcb1a* quantification showed differing trends in the female jejunum and ileum. *Abcb1a* expression decreased in the jejunum from 7.530 ± 0.524 ng/mg to 5.736 ± 0.347 ng/mg and the ileum from 7.093 ± 0.638 ng/mg to 6.604 ± 0.505 ng/mg in the fed state, compared with the fasted state. Sex differences were seen in the jejunum, ileum and colon in both prandial states.

3.2. Intestinal P-gp and abcb1a quantification in Sprague Dawley rats

Fig. 3 and Table S3 display the P-gp expression in Sprague Dawley rats. In the intestinal tract, P-gp increased across the small intestine (from the duodenum to the ileum) then decreased in the colon. In the male rats, the ileal P-gp expression decreased by −20 % in the fed state, compared with the fasted state. In the female rat, the colonic P-gp expression increased from 11.88 ± 1.66 ng/mg to 17.24 ± 2.56 ng/mg, in the fed state, compared with the fasted state. Sex differences were observed in the ileum in both prandial states. The fasted female ileal P-gp was −11 % lower than the male ileal P-gp. On the other hand, the fed female ileal P-gp was +23 % higher. The largest inter-individual variability was seen in the fed state, with the largest standard deviation of ± 3.61 in the fed male jejunum.

Fig. 4 and table S4 show the *abcb1a* expression in Sprague Dawley rats. Sex differences were seen in the fed state. The fed state female *abcb1a* expression was +21 %, +17 %, and +11 % higher than their male counterparts, in the duodenum, jejunum, and colon, respectively. In the female Sprague Dawley rat, the *abcb1a* expression increased by +19 % and +28 % in the duodenum and colon in the fed state, compared with the fasted state. Regional differences were seen between the jejunum and ileum. A moderately strong positive correlation (r = 0.741) was found for the Sprague Dawley rat between ELISA P-gp and PCR *abcb1a* expression (Fig. S1).

Strain differences were found in the P-gp protein and *abcb1a* mRNA expression between the Wistar and Sprague Dawley rats ($p < 0.05$) (Figs. S3–10). To be precise in male rats, strain differences were seen in the *abcb1a* expression in both prandial states, and P-gp expression in the fed state and the fasted jejunum and ileum.

In the female rats, strain differences were seen in the fasted jejunum, ileum, and colon, as well as the fed ileum for the P-gp expression measured by ELISA. For the mRNA levels, strain differences were observed in the jejunum and colon in both prandial states. Overall, Sprague Dawley rats showed higher P-gp and *abcb1a* expression than Wistar rats by ELISA and PCR, respectively.

3.3. Intestinal BCRP and abcg2 quantification in Wistar rats

The intestinal BCRP expression in male and female Wistar rats, in the fasted and fed state, quantified by ELISA is reported in Fig. 5 and Table S5. The BCRP expression increased in both sexes in the fed state. In male Wistar rats, the BCRP expression increased by +77 %, +172 %, and +206 % in the male jejunum, ileum and colon, respectively, in the fed

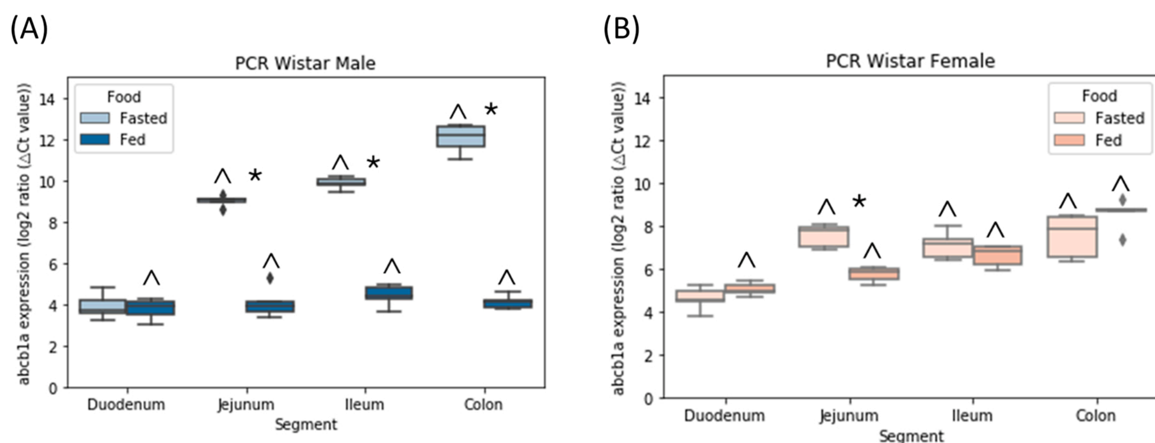


Fig. 2. – *abcb1a* expression in fasted and fed (A) male and (B) female Wistar rats quantified by PCR (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

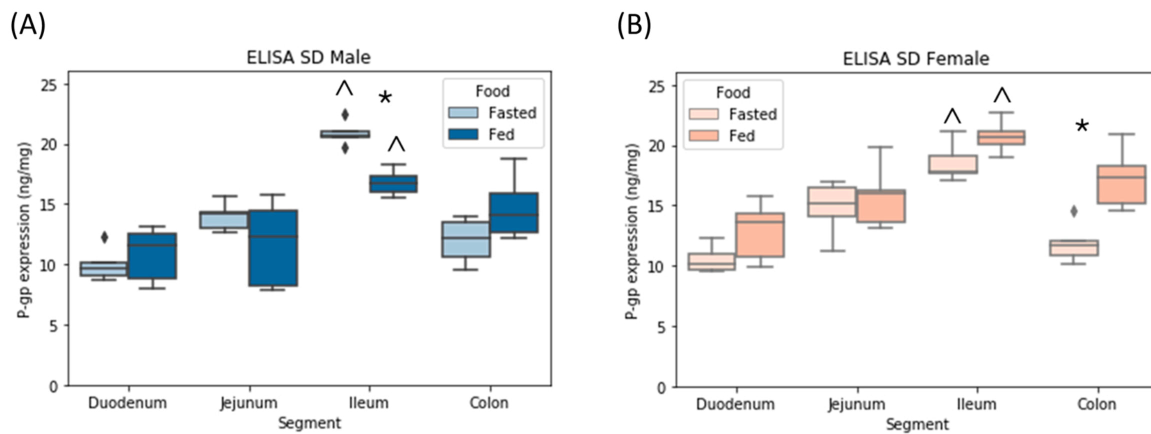


Fig. 3. – P-gp expression in fasted and fed (A) male and (B) female Sprague Dawley rats quantified by ELISA (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

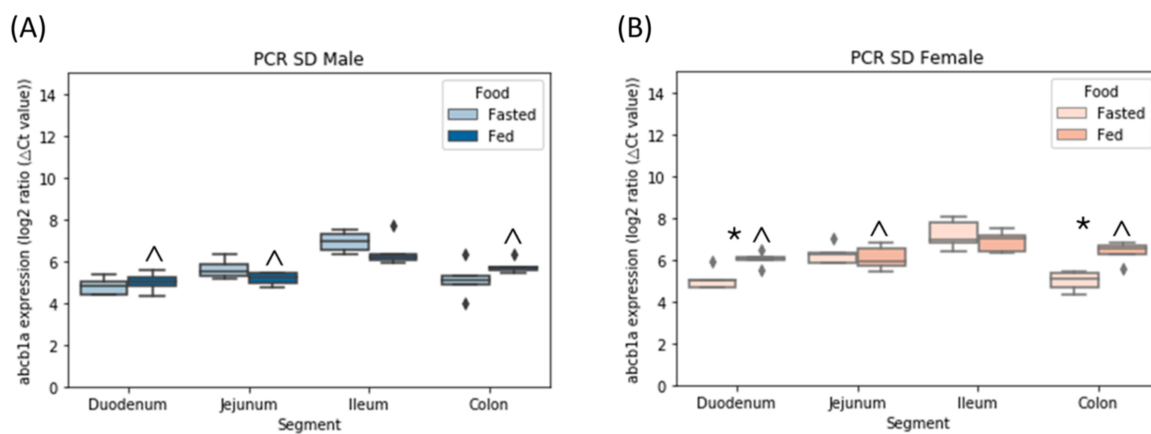


Fig. 4. – *abcb1a* expression in fasted and fed (A) male and (B) female Sprague Dawley rats quantified by PCR (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

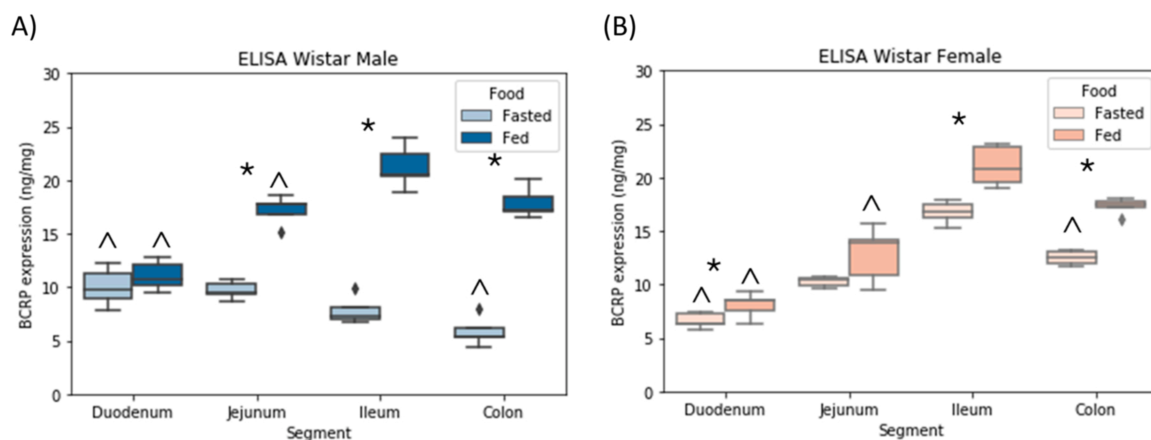


Fig. 5. – BCRP expression in fasted and fed (A) male and (B) female Wistar rats quantified by ELISA (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

state compared with the fasted state. A similar trend was observed in the female Wistar rat, with an increase in BCRP expression by +22 %, +26 % and +39 % in the female duodenum, ileum, and colon, respectively, in the fed state compared with the fasted state. Regional differences were observed across the intestine. In the fed state, BCRP expression significantly increased from the duodenum to the ileum, then dropped in the colon. However, in the male fasted state, BCRP decreased from the jejunum to the ileum (9.711 ± 0.798 ng/mg to 7.813 ± 1.292 ng/mg) and the ileum to the colon (7.813 ± 1.292 ng/mg to 5.857 ± 1.357 ng/mg). In the female fasted state, BCRP significantly increased from the duodenum to the ileum by 2.5-times, then decreased from the ileum to the colon (16.748 ± 1.033 ng/mg to 12.470 ± 0.699 ng/mg).

The *abcg2* expression in Wistar rats quantified by PCR showed a similar trend to the BCRP expression quantified by ELISA, reflected in

the good positive correlation between the methods (r = 0.816) (Fig. S11). A sex difference was observed in the *abcg2* expression of the fasted duodenum, ileum, and colon. The fasted male duodenal *abcg2* was higher than in the female (3.141 ± 0.575 ng/mg vs 2.232 ± 0.429 ng/mg, respectively). Whereas the ileal *abcg2* was higher in the female fasted ileum and colon (3.639 ± 0.295 ng/mg vs 2.483 ± 0.567 ng/mg and 2.995 ± 0.358 ng/mg vs 2.057 ± 0.178 ng/mg). In addition, for the male rat the *abcg2* expression increased by +24 %, +62 %, and +58 % in the jejunum, ileum, and colon, respectively, in the fed state compared with the fasted state. (Fig. 6).

3.4. Intestinal BCRP and abcg2 quantification in Sprague Dawley rats

The BCRP expression in Sprague Dawley rats is reported in Fig. 7 and Table S7. A comparable pattern was observed between the sexes in the fed state, except in the ileum. In the male rats, the BCRP expression gradually rose from the duodenum to the ileum; by +103 % in the fasted state and +128 % in the fed state. A similar trend was seen in the female rat from the duodenum to the ileum, where the BCRP expression was +107 % higher in the fasted state and +29 % higher in the fed state. Sex differences were seen in the ileum, where the BCRP in the male rat increased by +13 % but decreased the BCRP expression by −23 % in the female rat in the fed state, compared with the fasted state.

Fig. 8 and Table S8 report the *abcg2* expression in the Sprague Dawley rats. A comparable profile was seen with the BCRP expression reported in Fig. 7, which is reflected in the good positive correlation seen between the ELISA and PCR methods (r = 0.773) (Fig. S11). The *abcg2* expression decreased in females by −23 % in the fed state, compared with the fasted state.

Figs. S13-S20 display the BCRP and *abcg2* expression across the intestinal tract, comparing Wistar and Sprague Dawley rats. Higher BCRP expression was seen in the male Sprague Dawley rat compared with the male Wistar rats; 1.7- and 2.8-times higher in the fasted jejunum and ileum and 1.2-times higher in the fed ileum. In the female Sprague Dawley rat, higher BCRP was also seen in the female Wistar rat in the fasted duodenum, jejunum, and fed jejunum. For the PCR quantification, higher *abcg2* expression was observed in the Sprague Dawley rat in the male fasted jejunum (+25 %), ileum (+75 %) and colon (+92 %), female fasted ileum (+30 %) and colon (+28 %) and male fed ileum (+22 %), than in the Wistar rat counterpart.

3.5. Intestinal MRP2 and abcc2 quantification in Wistar rats

Fig. 9 and Table S10 display the MRP2 expression in male and female Wistar rats in the fasted and fed states. A significant increase in MRP2 expression in both sexes was seen in the fed state, compared with the fasted state. Specifically, in the male rats an increase in the MRP2 expression was seen duodenum (+50 %), jejunum (+136 %), ileum (+50 %), and colon (132 %) in the fed state, compared with the fasted state. An increase in the MRP2 expression was seen female rat in the fed state compared with the fasted state; jejunum (+101 %), ileum (+156 %) and colon (+157 %). Sex differences were observed in the fasted duodenum, jejunum, and colon.

The *abcc2* expression levels quantified by PCR (Fig. 10 & Table S10) are comparable with the MRP2 protein levels quantified by ELISA. In fact, a good positive correlation of r = 0.881 was found between MRP2 measured by ELISA and *abcc2* measured by PCR (Fig. S21). In the male rat, a significant rise in the *abcc2* levels was seen in the fed state compared with the fasted state, with a 1.5-, 2.1-, 2.1- and 1.8-fold increase in the duodenum, jejunum, ileum and colon. In the female rat, a change in the *abcc2* expression was also seen in the fed state compared with the fasted state, with a 1.7-, 2.3- and 2.4-fold increase in the jejunum, ileum, and colon, respectively. Sex differences were observed in both prandial states in the duodenum and the jejunum, with the greatest difference in the jejunum; a 1.3-times increase in the fasted state and 1.0-times increase in the fed state, between the male and female Wistar rats.

3.6. Intestinal MRP2 and abcc2 quantification in Sprague Dawley rats

The MRP2 quantification levels in the Sprague Dawley rats are shown in Fig. 11 and Table S11. In the fed state, the MRP2 expression significantly increased in both sexes, compared with the fasted state. The male Sprague Dawley rat showed a +134 %, +121 %, +80 %, and +113 % increase between the prandial states in the duodenum, jejunum, ileum and colon, respectively. A similar trend was observed in the female rat, where a + 108 %, +128 %, +78 % and +107 % increase was seen in the fed state, compared with the fasted state in the duodenum, jejunum, ileum and colon, respectively. A sex difference was found in the jejunum in both prandial states; a +30 % and +26 % higher in the male rat compared with the female rat, in fasted and fed states, respectively.

The *abcc2* expression in male and female Sprague Dawley rats is reported in Fig. 12 and Table S12. Importantly, similar trends were shown between the MRP2 protein levels and the *abcc2* mRNA levels. In fact, a strong positive correlation of r = 0.935 was calculated between the MRP2 and the *abcc2* (Fig. S22). Here, an increase of 2.4-, 2.2-, 1.7-, and 2.0-fold was seen in the male rats across the intestine (duodenum, jejunum, ileum, and colon) in the fed state, compared with the fasted state. An increase was seen in the *abcc2* expression –2.0-, 2.3-, 1.5- and 1.9-times higher in the duodenum, jejunum, ileum and colon, respectively, in the fed state compared with the fasted state. Sex differences were also shown in the main site of absorption – the jejunum. To be

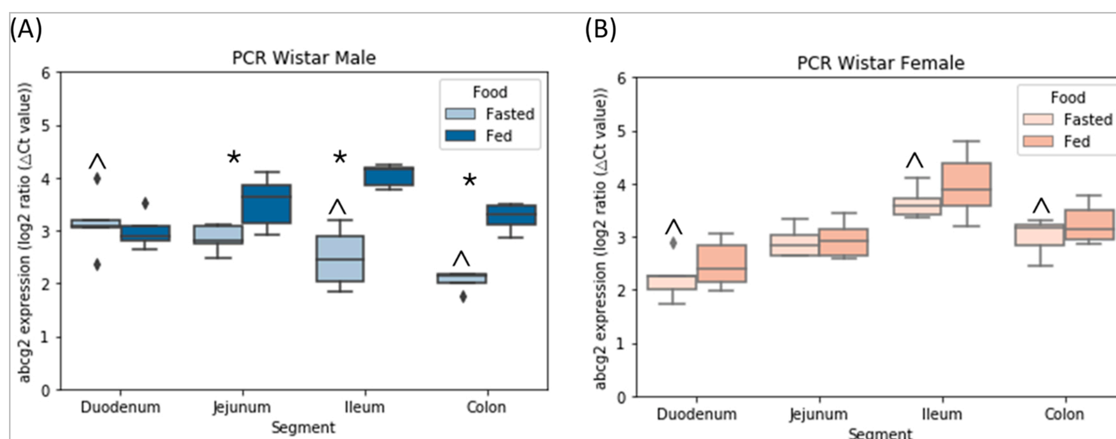


Fig. 6. – *abcg2* expression in fasted and fed (A) male and (B) female Wistar rats quantified by ELISA (n = 5). The * symbol denotes statistical significance between the feeding state and ^ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

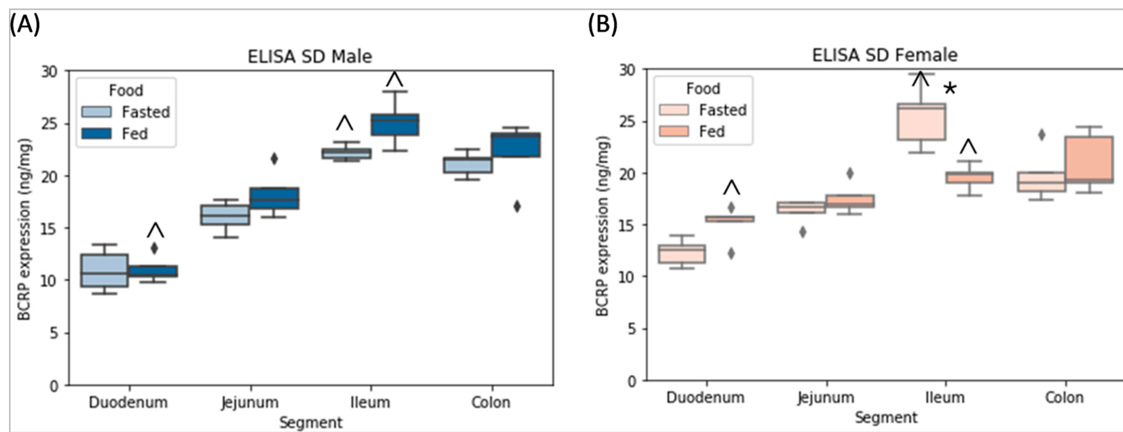


Fig. 7. – BCRP expression in fasted and fed (A) male and (B) female Sprague Dawley rats quantified by ELISA (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

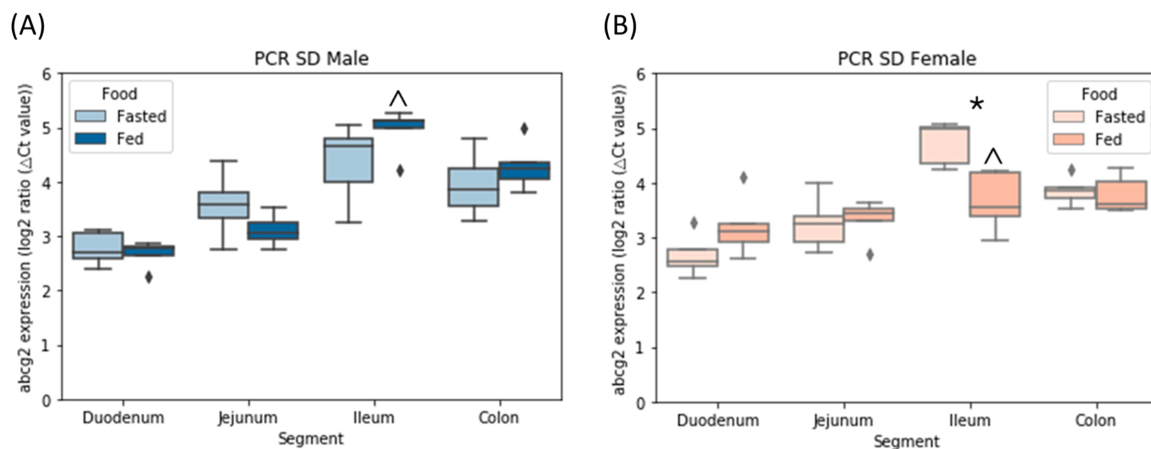


Fig. 8. – *abcg2* expression in fasted and fed (A) male and (B) female Sprague Dawley rats quantified by PCR (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

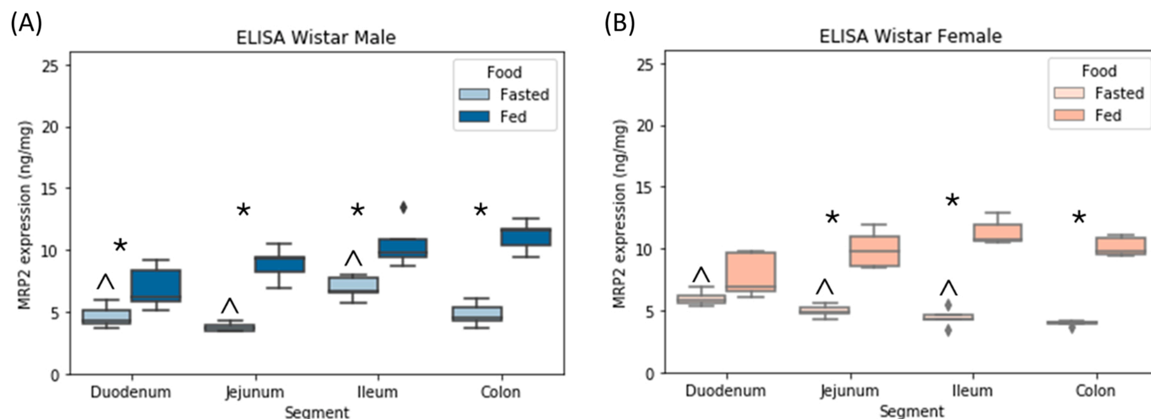


Fig. 9. – MRP2 expression in fasted and fed (A) male and (B) female Wistar rats quantified by ELISA (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

specific, the *abcc2* level was 1.2- and 1.2-times higher in the fasted and fed male jejunum.

Figs. S23 - 30 present the comparative plots of the MRP2 and *abcc2* levels between the Wistar and Sprague Dawley rats. Strain differences were found in the fasted female rat for the MRP2 and *abcc2* levels (Fig. S24 and Fig. S28). Here, in the duodenum and jejunum, MRP2 was 15 % and 6 % higher in the Wistar rat, respectively. Whereas, in the ileum and colon, the MRP2 was 3.5 % and 4.7 % higher in the Sprague Dawley rat. No strain differences were seen in the fed female rats. In the male rats, strain differences were seen for MRP2 in the fasted jejunum and fed duodenum and jejunum. The PCR quantification showed strain differences in the male fasted jejunum and ileum and the male fed small intestine.

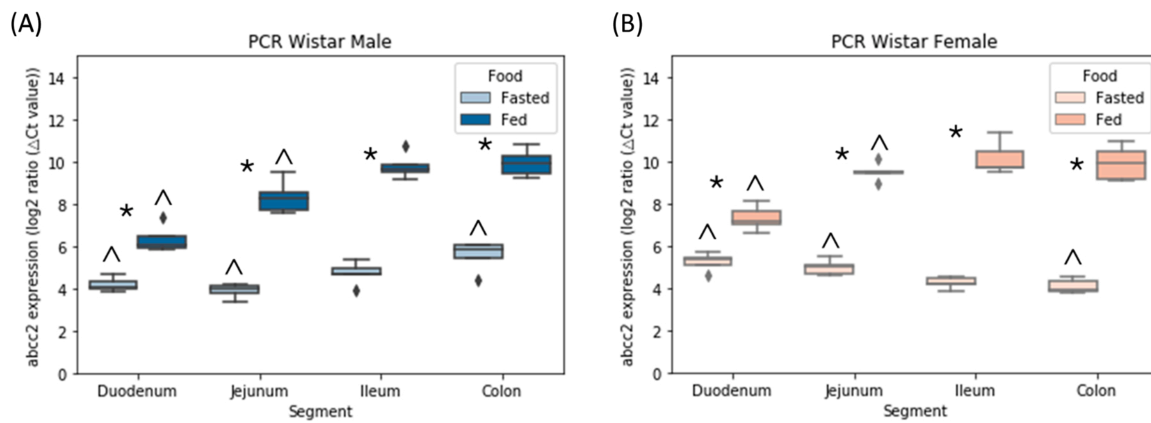


Fig. 10. – *abcc2* expression in fasted and fed (A) male and (B) female Wistar rats quantified by PCR (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

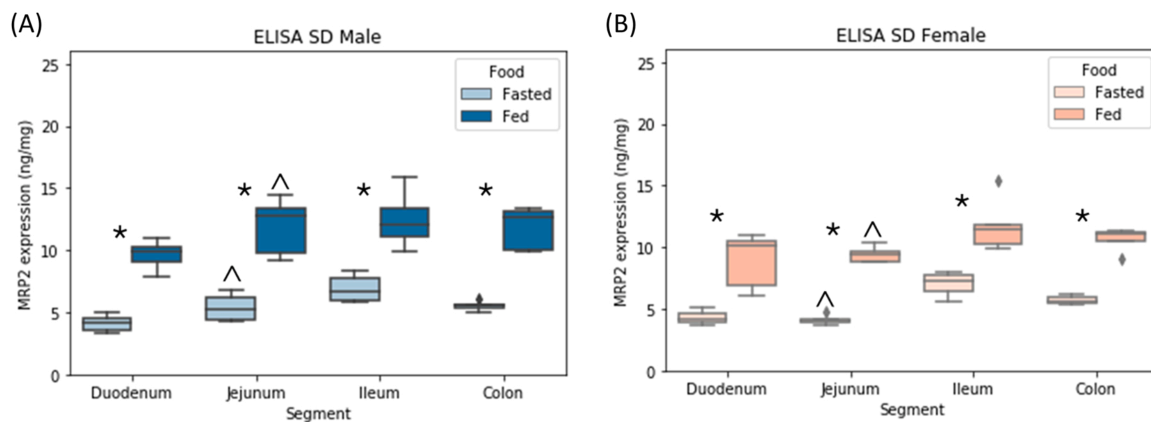


Fig. 11. – MRP2 expression in fasted and fed (A) male and (B) female Sprague Dawley rats quantified by ELISA (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

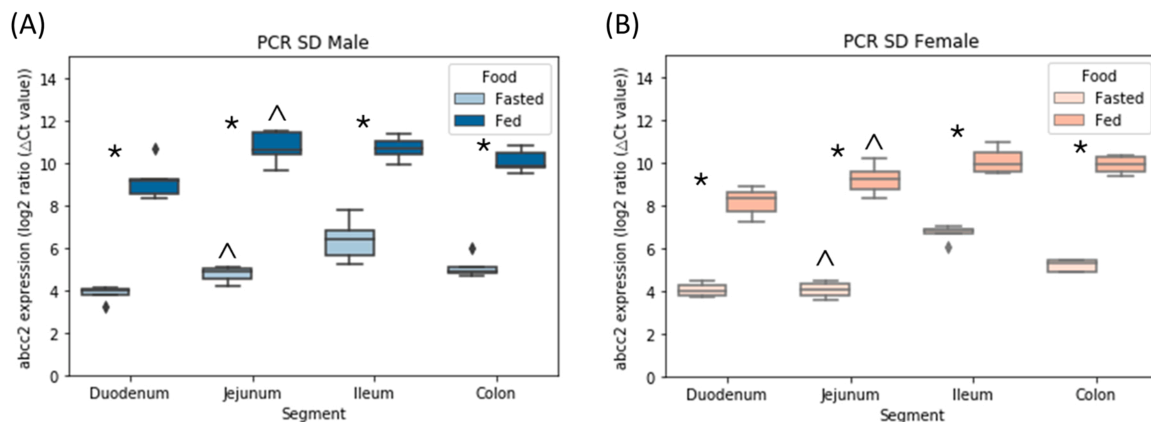


Fig. 12. – *abcc2* expression in fasted and fed (A) male and (B) female Sprague Dawley rats quantified by PCR (n = 5). The * symbol denotes statistical significance between the feeding state and ˆ denotes a statistical significance between the sexes in an intestinal region ($p < 0.05$).

4. Discussion

Efflux transporters provide protection from xenobiotics at biological barriers, which includes the intestinal tract. Knowledge of the intricacies of GI tract, such as the expression of efflux transporters, should be understood to choose the most appropriate animal model for preclinical testing. While there are numerous investigations on ABC transporter expression in rodent models, due to the heterogeneity in the methods and animal models used in these studies, it is difficult to find a conclusive message [39,40]. Here, food, sex, and strain were shown to alter the expression of P-gp, BCRP, and MRP2 in contrasting ways.

Drug molecules and food products use the same biochemical pathways to permeate through the GI tract. Therefore, food-drug interactions at the level of the intestinal monolayer are expected, although not fully understood [19]. Experimental testing using several *in vitro* models, such as the Caco-2 cell line, have shown that food-derived compounds are

capable of inhibiting efflux transporters [41]. However, clinical studies are in this area are limited. It is rational that food intake would increase the expression of efflux transporters as a protective mechanism to shield the body from xenobiotics. Furthermore, the increase in female P-gp expression may be attributed to innate protection that females may have for successful reproduction. For instance, P-gp, BCRP, and MRP2 are found in the maternal-foetus barrier [42]. However, it is less clear why food intake causes P-gp expression levels to decrease in the male Wistar rat. Differences in protein expression may be due to signalling by nuclear receptors, which are induced by a variety of xenobiotics and physiological cues [43,44]. The observed two- to three-fold differences in transporter expression between the experimental groups may increase or decrease drug absorption and total drug exposure, the disposition of the parent drug, and its metabolites, and the excretion of parent drug and its metabolites by therapeutically relevant concentrations. Although, it is also important to remember that transporter expression may not be representative of transport activity [43].

Our results showed that rodent P-gp expression increased from the proximal small intestine to the distal small intestine, and then decreased in the colon. This reflects our groups' previous findings, that of the literature, and the profile seen in humans [35,37,39,45]. Studies in the literature have reported that BCRP increases from the duodenum to the ileum, and then decreases in the colon in humans [16]. Our study reflected this, except in the fasted male Wistar rat where the BCRP/*abcg2* levels decreased along the intestinal tract. For MRP2, studies have reported that intestinal expression in male Wistar rats was highest in the duodenum then decreases directionally from the jejunum to the ileum and the colon [37,46,47]. Although, it was not clear if the rats were fasted or fed in those studies. In contrast, Drozdzik and colleagues found in human intestinal tissue that the protein abundance of MRP2 peaked in the jejunum [16]. Our findings reported a decrease in MRP2 between the jejunum and ileum in the female fasted Wistar rats, however in the male fasted rats, an increase was reported between the jejunum and ileum. In the fed state, an increase in MRP2 across the intestinal tract was seen in both strains and sexes, compared with the fasted state. Our study also found that P-gp/*abcb1a* and BCRP/*abcg2* expression levels were higher than the MRP2/*abcc2* levels. Sprague Dawley rats showed innately higher P-gp levels, in corroboration with one of our previous studies [33], this study also found higher BCRP and MRP2 levels than in Wistar rats. These differences in P-gp, BCRP, and MRP2 transcript and protein expression levels in the different experimental groups highlight that the sex, strain, feeding status, and quantification method should be clearly stated in the reporting of transporter expression abundances. The absorption of key drug substrates and physiological substrates may be altered between the fasted and fed states., An example from the clinic; sex differences were reported in the bioavailability of cyclosporine A after a fat-rich meal, where decreased bioavailability was found in females, and increased bioavailability was found in male humans [48]. The authors are not aware of previous studies in rats where the oral bioavailability of efflux transporter substrates are studies between the fasted and fed states.

PCR and ELISA are analytical techniques used to quantify mRNA and protein expression, respectively. Here, PCR appears to be more sensitive than ELISA to variability, as the significant differences between the sexes and prandial states are more pronounced with PCR. A limitation of PCR is that it measures the relative expression of mRNA, using the housekeeping protein, beta-actin, as a control. Therefore, as no optimum method exists, we propose that ELISA and PCR methods should both be used to assess mRNA and protein transporter expression. The use of another technique, liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based quantitative proteomics, has be used to quantify transporter abundance [24–26]. Our recent comparison of ELISA and LC-MS/MS concluded that the methods produced similar trends in P-gp expression.

Strong to moderate positive correlations were found between ELISA and PCR; $r = 0.615$ and $r = 0.741$ for P-gp/*abcb1a*, $r = 0.816$ and $r = 0.773$ for BCRP/*abcg2*, and $r = 0.881$ and $r = 0.935$ for MRP2/*abcc2* in Wistar and Sprague Dawley rats (Supplementary materials Fig. S1, S2, S11, S12, S21 and S22). Drozdzik et al. also found significant positive correlation in human small intestinal tissue between mRNA level and protein abundance by P-gp/*abcb1a*, BCRP/*abcg2*, and MRP2/*abcc2* [16]. The differing correlations between mRNA/protein expression suggest that predictive power of transcript analysis should be examined on a gene-by-gene basis [49]. The lowest correlation between the methods was for P-gp/*abcb1a*, suggesting downstream processes could affect the P-gp expression. We propose that PCR and ELISA should be used together for comprehensive insights into transcript and protein expression.

The use of *in vivo* animal models serve as primary tools to guide development into novel active pharmaceutical ingredients (APIs) [50]. We suggest that for investigations into P-gp, BCRP, and MRP2 substrates as the drugs of interest, these fundamental differences in transporter expression should be used to guide the choice of the animal model. There are limited studies in human intestinal tissue examining the BCRP and MRP2 expression [16], where the results were pooled rather than stratified by sex. Therefore, further studies are needed where analyses are separated by sex to assess the most appropriate animal model to understand the oral absorption of substrates. Furthermore, the influence of feeding on these key efflux transporters should be assessed at the human level. In addition, the authors are not aware of studies investigating how different diets, other than the high-fat diet, alter transporter expression, which will be an area of interest. Another avenue of further interest is the use of transporter-knockout animal models to evaluate the impact of a single transporter on the absorption, distribution, metabolism, and excretion of a drug substrate. However, while male P-gp transporter-knockout rat models can be sourced from suppliers, no female models was found. When appropriate models become available, the consequences of prandial state, sex, and species differences on the mechanisms of transporter in question could be investigated. The intra- and inter-variability in drug substrate response seen in the clinic may be attributed to differing efflux transporter expression seen between the sexes, ethnicities, and food intake.

5. Conclusion

The intake of food was found to modulate the expression of P-gp/*abcb1a*, BCRP/*abcg2*, and MRP2/*abcc2* differently in frequently used Wistar and Sprague Dawley animal models. Sex differences were reported in the P-gp/*abcb1a*, BCRP/*abcg2,* and MRP2/*abcc2* expression in Wistar rats, but not Sprague Dawley rats. Similar trends were seen between the protein and transcript expression, quantified by ELISA and PCR, respectively. We suggest that ELISA may be more useful as protein quantification expression data, the functional unit, are yielded. The comprehensive characterisation offered by this study can be used in the extrapolation of preclinical studies to the design of clinical trials and can inputted into physiologically based pharmacokinetic models for early predictions.

Funding

This research was funded by the Engineering and Physical Sciences Research Council (EPSRC) UK, grant number EP/L01646X and The National Natural Science Foundation of China (Grant no. 82003672).

CRediT authorship contribution statement

Francesca K.H. Gavins. – conceptualisation, investigation, data curation, software, formal analysis, original draft preparation, writing review and editing, visualisation, project administration, Liu Dou. – investigation, data curation and data validation, Yujia Qin. – investigation, data curation and data validation, Christine M. Madla – conceptualization, writing—original draft preparation, writing—review

and editing, Murdan, S. – review and editing, funding acquisition, supervision, Abdul W. Basit. – conceptualisation, writing review and editing, funding acquisition, supervision, Yang Mai. – conceptualisation, investigation, data curation, data validation, formal analysis, Mine Orlu. – conceptualisation, writing review and editing, funding acquisition, supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available in the Supplementary material.

Acknowledgements

The graphical abstract was created with BioRender.com.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114329.

References

[1] K.M. Giacomini, S.-M. Huang, D.J. Tweedie, L.Z. Benet, K.L.R. Brouwer, X. Chu, A. Dahlin, R. Evers, V. Fischer, K.M. Hillgren, K.A. Hoffmaster, T. Ishikawa, D. Keppler, R.B. Kim, C.A. Lee, M. Niemi, J.W. Polli, Y. Sugiyama, P.W. Swaan, J. A. Ware, S.H. Wright, S. Wah Yee, M.J. Zamek-Gliszczynski, L. Zhang, C. The International Transporter, Membrane transporters in drug development, Nat. Rev. Drug Discov. 9 (3) (2010) 215–236.

[2] A. Seelig, P-Glycoprotein: one mechanism, many tasks and the consequences for pharmacotherapy of cancers, Front. Oncol. 10 (2020).

[3] E.J. Tarling, T.Q. de Aguiar Vallim, P.A. Edwards, Role of ABC transporters in lipid transport and human disease, Trends Endocrinol. Metab.: TEM 24 (7) (2013) 342–350.

[4] A.H. Schinkel, J.W. Jonker, Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview, Adv. Drug Deliv. Rev. 55 (1) (2003) 3–29.

[5] V. Ling, L.H. Thompson, Reduced permeability in CHO cells as a mechanism of resistance to colchicine, J. Cell. Physiol. 83 (1) (1974) 103–116.

[6] M. Buchler, J. Konig, M. Brom, J. Kartenbeck, H. SPRING, cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats, J. Biol. Chem. 271 (1996) 15091–15098.

[7] L.A. Doyle, W. Yang, L.V. Abruzzo, T. Krogmann, Y. Gao, A.K. Rishi, D.D. Ross, A multidrug resistance transporter from human MCF-7 breast cancer cells, Proc. Natl. Acad. Sci. 95 (26) (1998) 15665–15670.

[8] FDA, In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry, 2020.

[9] G. Fricker, D.S. Miller, Relevance of multidrug resistance proteins for intestinal drug absorption in vitro and in vivo, Pharmacol. Toxicol. 90 (1) (2002) 5–13.

[10] S. Heemskerk, J.G. Peters, J. Louisse, S. Sagar, F.G. Russel, R. Masereeuw, Regulation of P-glycoprotein in renal proximal tubule epithelial cells by LPS and TNF-α, J. Biomed. Biotechnol. 2010 (2010).

[11] G.D. Luker, K.R. Nilsson, D.F. Covey, D. Piwnica-Worms, Multidrug resistance (MDR1) P-glycoprotein enhances esterification of plasma membrane cholesterol, J. Biol. Chem. 274 (11) (1999) 6979–6991.

[12] M. Foucaud-Vignault, Z. Soayfane, C. Ménez, J. Bertrand-Michel, P.G.P. Martin, H. Guillou, X. Collet, A. Lespine, P-glycoprotein dysfunction contributes to hepatic steatosis and obesity in mice, PLoS One 6 (9) (2011), e23614.

[13] T. Nakanishi, D.D. Ross, Breast cancer resistance protein (BCRP/ABCG2): its role in multidrug resistance and regulation of its gene expression, Chin. J. Cancer 31 (2) (2012) 73–99.

[14] E. Egido, R. Muller, X. Li-Blatter, G. Merino, A. Seelig, Predicting activators and inhibitors of the breast cancer resistance protein (ABCG2) and P-glycoprotein (ABCB1) based on mechanistic considerations, Mol. Pharm. 12 (11) (2015) 4026–4037.

[15] S. Deferme, P. Augustijns, The effect of food components on the absorption of P-gp substrates: a review, J. Pharm. Pharm. 55 (2) (2003) 153–162.

[16] M. Drozdzik, D. Busch, J. Lapczuk, J. Muller, M. Ostrowski, M. Kurzawski, S. Oswald, Protein abundance of clinically relevant drug-metabolizing enzymes in the human liver and intestine: a comparative analysis in paired tissue specimens, Clin. Pharmacol. Ther. 104 (3) (2018) 515–524.

[17] M.D. Harwood, M. Zhang, S.M. Pathak, S. Neuhoff, The regional-specific relative and absolute expression of gut transporters in adult caucasians: a meta-analysis, Drug Metab. Dispos. 47 (8) (2019) 854–864.

[18] F.J.O. Varum, G.B. Hatton, A.W. Basit, Food, physiology and drug delivery, Int. J. Pharm. 457 (2) (2013) 446–460.

[19] M. Koziolek, S. Alcaro, P. Augustijns, A.W. Basit, M. Grimm, B. Hens, C.L. Hoad, P. Jedamzik, C.M. Madla, M. Maliepaard, L. Marciani, A. Maruca, N. Parrott, P. Pávek, C.J.H. Porter, C. Reppas, D. van Riet-Nales, J. Rubbens, M. Statelova, N. L. Trevaskis, K. Valentová, M. Vertzoni, D.V. Čepo, M. Corsetti, The mechanisms of pharmacokinetic food-drug interactions – a perspective from the UNGAP group, Eur. J. Pharm. Sci. 134 (2019) 31–59.

[20] J.M. Custodio, C.-Y. Wu, L.Z. Benet, Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption, Adv. Drug Deliv. Rev. 60 (6) (2008) 717–733.

[21] C.S. Won, N.H. Oberlies, M.F. Paine, Mechanisms underlying food–drug interactions: inhibition of intestinal metabolism and transport, Pharmacol. Ther. 136 (2) (2012) 186–201.

[22] J.P. O'Shea, R. Holm, C.M. O'Driscoll, B.T. Griffin, Food for thought: formulating away the food effect – a PEARRL review, J. Pharm. Pharmacol. 71 (4) (2019) 510–535.

[23] Z. Vinarov, B. Abrahamsson, P. Artursson, H. Batchelor, P. Berben, A. Bernkop-Schnürch, J. Butler, J. Ceulemans, N. Davies, D. Dupont, G.E. Flaten, N. Fotaki, B. T. Griffin, V. Jannin, J. Keemink, F. Kesisoglou, M. Koziolek, M. Kuentz, A. Mackie, A.J. Meléndez-Martínez, M. McAllister, A. Müllertz, C.M. O'Driscoll, N. Parrott, J. Paszkowska, P. Pavek, C.J.H. Porter, C. Reppas, C. Stillhart, K. Sugano, E. Toader, K. Valentová, M. Vertzoni, S.N. De Wildt, C.G. Wilson, P. Augustijns, Current challenges and future perspectives in oral absorption research: an opinion of the UNGAP network, Adv. Drug Deliv. Rev. 171 (2021) 289–331.

[24] G.B. Hatton, V. Yadav, A.W. Basit, H.A. Merchant, Animal farm: considerations in animal gastrointestinal physiology and relevance to drug delivery in humans, J. Pharm. Sci. 104 (9) (2015) 2747–2776.

[25] E. Sjögren, B. Abrahamsson, P. Augustijns, D. Becker, M.B. Bolger, M. Brewster, J. Brouwers, T. Flanagan, M. Harwood, C. Heinen, R. Holm, H.-P. Juretschke, M. Kubbinga, A. Lindahl, V. Lukacova, U. Münster, S. Neuhoff, M.A. Nguyen, Av Peer, C. Reppas, A.R. Hodjegan, C. Tannergren, W. Weitschies, C. Wilson, P. Zane, H. Lennernäs, P. Langguth, In vivo methods for drug absorption – Comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects, Eur. J. Pharm. Sci. 57 (2014) 99–151.

[26] A.K. Beery, Inclusion of females does not increase variability in rodent research studies, Curr. Opin. Behav. Sci. 23 (2018) 143–149.

[27] C.M. Madla, F.K.H. Gavins, H. Merchant, M. Orlu, S. Murdan, A.W. Basit, Let's talk about sex: differences in drug therapy in males and females, Adv. Drug Deliv. Rev. (2021).

[28] M.-J. Xu, G.-J. Wang, H.-T. Xie, H. Li, Q. Huang, R. Wang, Y.-W. Jia, T. Lv, Gender difference regarding schizandrin pharmacokinetics in rats, Eur. J. Drug Metab. Pharmacokinet. 33 (2) (2008) 65–68.

[29] T. Nolan, R.E. Hands, S.A. Bustin, Quantification of mRNA using real-time RT-PCR, Nat. Protoc. 1 (3) (2006) 1559–1582.

[30] S. Ramazi, J. Zahiri, Post-translational modifications in proteins: resources, tools and prediction methods, Database 2021 (2021).

[31] M.S. Tabatabaei, M. Ahmed, Enzyme-linked immunosorbent assay (ELISA), in: S. L. Christian (Ed.), Cancer Cell Biology: Methods and Protocols, Springer, US, New York, NY, 2022, pp. 115–134.

[32] A. Braun, S. Hämmerle, K. Suda, B. Rothen-Rutishauser, M. Günthert, S.D. Krämer, H. Wunderli-Allenspach, Cell cultures as tools in biopharmacy, Eur. J. Pharm. Sci. 11 (Suppl 2) (2000) S51–S60.

[33] C.M. Madla, Y. Qin, F.K.H. Gavins, J. Liu, L. Dou, M. Orlu, S. Murdan, Y. Mai, A. W. Basit, Sex differences in intestinal p-glycoprotein expression in wistar versus Sprague Dawley rats, Pharmaceutics 14 (5) (2022) 1030.

[34] L. Dou, F.K.H. Gavins, Y. Mai, C.M. Madla, F. Taherali, M. Orlu, S. Murdan, A. W. Basit, Effect of food and an animal's sex on P-glycoprotein expression and luminal fluids in the gastrointestinal tract of Wistar rats, Pharmaceutics 12 (4) (2020).

[35] Y. Mai, L. Dou, Z. Yao, C.M. Madla, F.K.H. Gavins, F. Taherali, H. Yin, M. Orlu, S. Murdan, A.W. Basit, Quantification of P-glycoprotein in the gastrointestinal tract of humans and rodents: methodology, gut region, sex, and species matter, Mol. Pharm. 18 (5) (2021) 1895–1904.

[36] Y. Mai, F.K.H. Gavins, L. Dou, J. Liu, F. Taherali, M.E. Alkahtani, S. Murdan, A. W. Basit, M. Orlu, A non-nutritive feeding intervention alters the expression of efflux transporters in the gastrointestinal tract, Pharmaceutics 13 (11) (2021) 1789.

[37] C. MacLean, U. Moenning, A. Reichel, G. Fricker, Closing the gaps: a full scan of the intestinal expression of P-glycoprotein, breast cancer resistance protein, and multidrug resistance-associated protein 2 in male and female rats, Drug Metab. Dispos. 36 (7) (2008) 1249.

[38] J.D. Hunter, Matplotlib: a 2D graphics environment, Comput. Sci. Eng. 9 (2007) 90–95.

[39] C. Zimmermann, H. Gutmann, P. Hruz, J.P. Gutzwiller, C. Beglinger, J. Drewe, Mapping of multidrug resistance gene 1 and multidrug resistance-associated protein isoform 1 to 5 mRNA expression along the human intestinal tract, Drug Metab. Dispos. 33 (2) (2005) 219–224.

[40] B.M. Johnson, P. Zhang, J.D. Schuetz, K.L. Brouwer, Characterization of transport protein expression in multidrug resistance-associated protein (Mrp) 2-deficient rats, Drug Metab. Dispos. 34 (4) (2006) 556–562.
[41] N. Sjöstedt, K. Holvikari, P. Tammela, H. Kidron, Inhibition of breast cancer resistance protein and multidrug resistance associated protein 2 by natural compounds and their derivatives, Mol. Pharm. 14 (1) (2017) 135–146.
[42] E.M. Leslie, R.G. Deeley, S.P.C. Cole, Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense, Toxicol. Appl. Pharmacol. 204 (3) (2005) 216–237.
[43] C.G. Dietrich, A. Geier, R.P. Oude Elferink, ABC of oral bioavailability: transporters as gatekeepers in the gut, Gut 52 (12) (2003) 1788–1795.
[44] C. Wenzel, L. Gödtke, A. Reichstein, M. Keiser, D. Busch, M. Drozdzik, S. Oswald, Gene expression and protein abundance of nuclear receptors in human intestine and liver: a new application for mass spectrometry-based targeted proteomics, Molecules 27 (14) (2022).
[45] H. Suzuki, Y. Sugiyama, Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine, Eur. J. Pharm. Sci. 12 (1) (2000) 3–12.
[46] A.D. Mottino, T. Hoffman, L. Jennes, M. Vore, Expression and localization of multidrug resistant protein mrp2 in rat small intestine, J. Pharmacol. Exp. Ther. 293 (3) (2000) 717.
[47] D. Rost, S. Mahner, Y. Sugiyama, W. Stremmel, Expression and localization of the multidrug resistance-associated protein 3 in rat small and large intestine, Am. J. Physiol. -Gastrointest. Liver Physiol. 282 (4) (2002) G720–G726.
[48] F. Kees, M. Bucher, F. Schweda, H. Gschaidmeier, L. Faerber, R. Seifert, Neoimmun versus Neoral: a bioequivalence study in healthy volunteers and influence of a fat-rich meal on the bioavailability of Neoimmun, Naunyn-Schmiede 'S. Arch. Pharmacol. 375 (6) (2007) 393–399.
[49] J. Taipalensuu, H. Törnblom, G. Lindberg, C. Einarsson, F. Sjöqvist, H. Melhus, P. Garberg, B. Sjöström, B. Lundgren, P. Artursson, Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers, J. Pharmacol. Exp. Ther. 299 (1) (2001) 164–170.
[50] H. Lennernäs, L. Aarons, P. Augustijns, S. Beato, M. Bolger, K. Box, M. Brewster, J. Butler, J. Dressman, R. Holm, K. Julia Frank, R. Kendall, P. Langguth, J. Sydor, A. Lindahl, M. McAllister, U. Muenster, A. Müllertz, K. Ojala, X. Pepin, C. Reppas, A. Rostami-Hodjegan, M. Verwei, W. Weitschies, C. Wilson, C. Karlsson, B. Abrahamsson, Oral biopharmaceutics tools – time for a new initiative – an introduction to the IMI project OrBiTo, Eur. J. Pharm. Sci. 57 (2014) 292–299.