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Title: P-glycoprotein expression in the gastrointestinal tract of male and female rats is influenced differently by food

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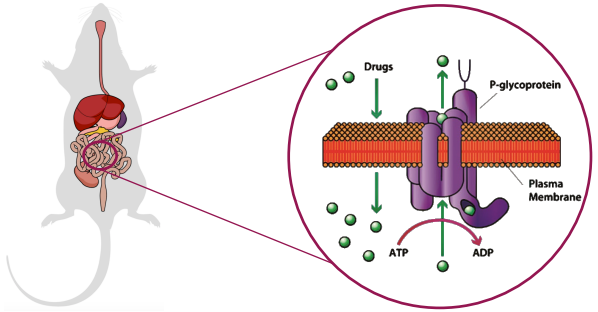
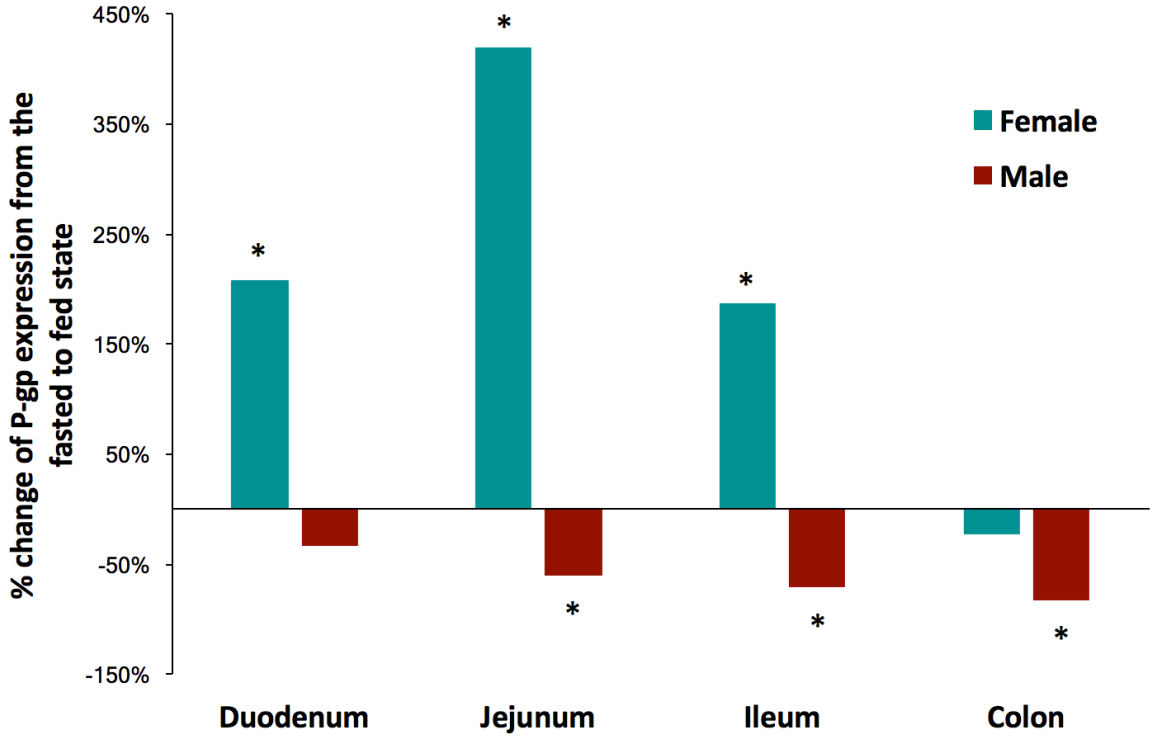
*Graphical Abstract (for review)

Food

P-gp expression





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P-glycoprotein expression in the gastrointestinal tract of male and female rats is influenced differently by food

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Abstract

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1.0 Introduction

It is well-known that males and females respond differently to medicines (Freire et al., 2011; Bigos et al., 2009; Nicolas et al., 2009). Despite this, pre-clinical research has demonstrated a tendency to focus on males in animal studies which may conceal profound sex differences. To date, no resounding guideline or standardisation practice have been proposed to consider the variations between male and female animals (Clayton, 2014). In order to advance pharmaceutical research, it is crucial to establish a better understanding of the innate differences between the sexes.

The majority of oral medicines are routinely pre-clinically tested on rats due to its inexpensiveness, ease of collection and the ability to identify compounds with promising or toxic biopharmaceutical properties (Downing, 2014). In order to determine the potential implications of drugs in a sex-dependent manner, it is firstly important to elucidate the differences in gastrointestinal (GI) physiology between male and female rats. In particular the efflux transporter intestinal P-glycoprotein (P-gp) is a putative limiting factor the absorption of drugs (Sharom, 2011; Lin et al., 2003; Murakami et al., 2008; Zakeri-Milani et al., 2014). A recent document issued by the Food and Drug Administration (FDA) suggested that the pharmaceutical industry should evaluate the effect of P-gp in early drug development (FDA, 2017). However, the expression of P-gp in laboratory rats is poorly understood. In the literature, contradicting results have been reported regarding the segmental differences in P-gp expression along the small intestine. Several investigations have demonstrated that P-gp expression increases in the lower segments of the small intestine in rats (Hatton et al., 2015, Afonso-Pereira et al., 2018; Mai et al., 2018) although others have reported that the highest expression can be determined towards the more proximal regions (Makhey et al., 1998, Yumoto et al., 1999, Stephens et al., 2002, Ho et al., 2003, Berggren et al., 2007). Assessing the methodologies used to determine P-gp in the literature, however, has revealed disparities in data collection regarding the reference protein, the strain of rat, the lack of consideration of potential sex differences and more notably, the influence of food on P-gp expression.

Food consumption induces dynamic changes in the GI tract (Varum et al., 2013; Abuhelwa et al., 2017; O'Shea et al., 2018) including luminal fluid volume and composition, and patterns of intestinal motility which ultimately affect the transit time of dosage forms (Ofutet et al., 2015; Ibekwe et al., 2008; Fadda et al., 2009; Liu et al., 2009). The understanding of the

influence of food on P-gp expression in rodents, its potential difference between sexes and its implications for oral drug delivery are still in its infancy.

This study aimed to explore the effect of food on the protein and relative mRNA expression level of intestinal P-gp. An *ex vivo* study was further conducted to evaluate the intestinal permeation of P-gp substrates ranitidine and ganciclovir, and a non-P-gp substrate metformin under the effect of food in both sexes.

2.0 Materials and Methods

2.1 Materials

Ranitidine (a P-gp and organic cation transporter (OCT) protein substrate) and ganciclovir (a P-gp substrate) were purchased from Sigma Aldrich (Dorset, UK). Metformin hydrochloride (an OCT protein substrate) was obtained from USV Ltd. (Mumbai, India). NuPAGE LDS Sample Buffer, Tris Buffered Saline, 10 X Solution, NuPAGE MOPS SDS Running Buffer (20X), NuPAGE Transfer Buffer (20X) and SuperSignal West Pico Chemiluminescent Substrate were purchased from Thermo Scientific (Paisley, UK). Tween 20, Bovine Serum Albumin and Monoclonal Anti-β-Actin were obtained from Sigma Aldrich (Dorset, UK). TBE Running Buffer (5X) and 10X TBE Electrophoresis Buffer were bought from Thermo Scientific (Paisley, UK). All other chemicals and kits are noted individually in the following methods.

2.2 Preparation of intestinal tissues from male and female rats

12 male and 12 female Wistar rats (10 weeks old weighing approximately 250 g and 200 g respectively) were purchased from Harlan UK Ltd. (Oxfordshire, UK) and housed at room temperature of 25°C in a light-dark cycle for 12 h. Fed state rats (6 male and 6 female) were provided with free access to food (EURodent Diet 22%) and water. Fasted state rats (6 male and 6 female) were subject to an overnight fasting of 12 h prior to the experiment. On the day of experiment at approximately 8:30 am, rats were sacrificed by a CO_2 euthanasia chamber. The whole intestinal tract was then rapidly removed and kept in an ice-bath filled with Krebs-Bicarbonate Ringer's solution (KBR) at pH 7.4. The intestine was then cut into four segments; the duodenum (1 cm from the ligament of Treitz); jejunum (10 cm from the ligament of Treitz); ileum (1 cm from the cecum) and colon. Tissue pieces from the mid part of the duodenum, the proximal part of the jejunum, the distal to mid part of ileum and the

descending colon were separated. 1 cm of tissue was used for permeation study and protein determination, and 2 cm for mRNA determination. The separated tissue was then opened along the mesenteric border and the mucosal layer was obtained by gently squeezing the serosal-side of tissue with a cover slip on ice-cold glass plate. The prepared tissue with the mucosal layer was then freshly used for the following studies.

2.3 Intestinal P-gp protein quantification via Western-blotting

The prepared tissue from section 2.2 was placed into a glass vial containing 3 ml of freshly prepared lysis buffer (50 mM Tris, 250 mM NaCl, 5 mM EDTA, 1 mM Na_3VO_4, 1 mM PMSF, 1% Nonidet P40 and a protease inhibitor cocktail) and homogenised for 20 s at 10,000 rpm with a T18 digital ULTRA-TURRAX® (IKA). The homogenised tissue solution was then incubated in a 4°C fridge for 2 h for protein extraction. Two hours later, the solution was transferred to a 1.5 ml Eppendorf tube and centrifuged with 10,000 rpm at 4°C for 10 min. The supernatant was transferred to micro-tubes and stored at –20°C until used for analysis (stable for 6 months). The total extracted protein was quantified according to the instruction adapted from the Pierce BCA Protein Assay Kit (ThermoFisher, UK).

25 µg protein sample was suspended in NuPAGE® LDS Sample Buffer (Invitrogen, Carlsbad, CA) and kept in a 70°C incubator for 10 min to induce denaturation. The denatured protein sample was then loaded on a NuPAGETM NovexTM 4 – 12% Bis-Tris gel (Invitrogen). 5 µl Sharp Pre-Stained protein standard (Invitrogen) was also loaded as molecular weight marker. Gel electrophoresis was then applied according to the instructions from the manufacturer. The separated protein samples in the gel were then transferred to a nitrocellulose membrane with an XCell SureLock™ Mini-Cell Electrophoresis System (Invitrogen). Membranes were blocked with 3% bovine serum albumin (BSA) in tris-buffered saline with tween (TBS-T) and incubated for 1 h at room temperature (25°C). For the detection of P-gp and reference protein (β-actin) blots were incubated for 1 h at room temperature with the respective primary antibodies, diluted in a 3% bovine serum albumin (BSA) in TBS-T: mouse monoclonal anti-P-gp (C-219 3:200; Enzo Life Science, Exeter, UK) and anti-β-actin mouse monoclonal antibody (1:2000; Sigma-Aldrich, Poole, UK). The detection of bound antibodies was completed with affinity-purified rabbit anti-mouse immunoglobulin G (IgG) coupled to peroxidase (secondary antibody; Sigma) and diluted to 1:5000 in 3% BSA in TBS-T. After 1 h incubation with the secondary antibody conjugated with horseradish peroxidase, P-gp and β-actin protein bands were visualised by a chem-

iluminescence detection method with Pierce™ ECL Western Blotting Substrate (ThermoFisher). The blots were then photographed with a ChemiDoc XRS camera (Bio-Rad). The relative expression of P-gp was calculated using the Image Lab™ software linked to the camera (Bio-Rad).

2.4 Intestinal P-gp mRNA quantification via Reverse-Transcription Polymerase Chain Reaction

The prepared tissues from section 2.2 were kept in an RNAlater buffer (Thermo Scientific). The total mRNA from the tissues were then extracted following the instruction from Pure Link RNA Mini Kit and On-column PureLink® DNase Treatment protocol. The extracted mRNA samples were stored in a –80°C freezer until analysis. The purification and quantification of the extracted mRNA was evaluated prior to experiment. The frozen mRNA samples were firstly thawed on ice and 2 µl mRNA solution was then transferred to a NanoDrop 2000c Spectrophotometer (Therma Scientific) for the evaluation.

Two-step real time PCR was applied for mRNA quantification. The first step was the preparation of cDNA. 1 µg of extracted mRNA from each sample was reverse transcribed to cDNA by following the instruction from iScriptTM cDNA Sythesis Kit (Bio-Rad). The second step was Real Time-qPCR (RT-qPCR). A 7500 Real Time PCR System (Thermofisher) was applied and the method was adapted from the user guide of SYBR® Green PCR Master Mix and SYBR® Green RT-PCR Reagents Kit. The experiment was conducted in a microAmp optical 96-well reaction plate with each well containing a 50 µl reaction system. This included a 1 ng transcribed cDNA, 25 µl SYBR Mix solution, 5 µl forward primer, 5 µl reverse primer and 10 µl RNAse-free water. The sequences of the primers were shown in Table 1. The relative quantification of mRNA, *mdr1a*, *mdr1b* and β-actin (internal standard) was carried out with the programme as follows; The amplification program consisted of one pre-incubation cycle at 95°C with a 12 min hold, followed by 40 amplification cycles with denaturation at 95°C with a 15 s hold, an annealing temperature of 60°C with a 10 s hold and an extension at 60°C and a 1 min hold. Amplification was followed by a melting curve analysis. This ran for one cycle with denaturation at 95°C with a 15 s hold, annealing at 60°C with a 1 min hold and melting at 95°C with a 30 s hold. A negative control was included for each analysed sample by adding deionised water instead of primers. The control group which contained deionised water instead of cDNA was also included in each run. The relative expression of *mdr1a* and *mdr1b* mRNA in different

samples were obtained by designing the programme on 7500 Real Time PCR System based on the principal of previous studies (MacLean et al., 2008). The relative expression of P-gp mRNA, *mdr1a* and *mdr1b* in fasted-state and fed-state rats were calculated using a 7500 software (version 2.0.6, Thermofisher).

2.5 Ex-vivo permeation studies

2.5.1 Evaluation of drug permeation via Ussing chamber

Drug solutions (3 mM ranitidine, 3 mM metformin and 1.96 mM ganciclovir) were freshly prepared in a KBR solution and stored in a 37°C incubator for the experiment. Intestinal tissues from the jejunum and ileum were obtained following section 2.2 and the luminal content was gently washed with KBR solution. The well-prepared mucosal tissues were then mounted in the vertical Ussing Chamber (Harvard Apparatus Inc., Holliston, MA, U.S.A) as flat sheets on a 0.28 cm^2 segment holder with needles for stability purposes. The chambers were tightly screwed with high spring-tension retaining rings and the entire assembly was maintained at 37°C with a circulating water bath for a 30 min equilibrium period. Tissue integrity was evaluated every 30 min during the experiment by measuring tissue transepithelial electrical resistance (TEER) with an EVOMX meter (World Precision Instruments Inc., WPI, Hertfordshire, United Kingdom). Any duodenal, jejunal, ileal and colonic segments that presented a value of TEER lower than 20 Ω•cm^2, 40 Ω•cm^2, 50 Ω•cm^2 and 70 Ω•cm^2 respectively at the beginning of experiment was regarded as poorly viable and excluded immediately. The tissue was not considered viable whenever TEER values decreased more than 15% from the value measured at the end of equilibration period.

The study began with a 20 – 30 min equilibrium period. 5 mL KBR solution was added to both the apical (mucosal surface) and basolateral (endothelial surface) chambers, gassed with an O_2/CO_2 (95%/5%) gas mixture. Following the emptying of the chamber, 5 ml of fresh KBR solution was added in the basolateral chamber whilst 5 ml of the drug solution was added in the apical chamber. During the experiment, 100 µL solution from basolateral chamber was withdrawn every 30 min. The experiment lasted for 2 h and the intestinal permeation was evaluated by analysing the drug amount in the withdrawn samples (mucosa to serosa, M – S). An equal volume of fresh KBR solution was replaced immediately.

2.5.2 Chromatographic analysis

Chromatographic analysis was performed with a high performance liquid chromatography (HPLC) system (Agilent Technologies, 1260 Infinity) equipped with a pump (model G1311C), an auto-sampler (model G1329B) and a diode array UV detector (model G1314B). The methods were summarised in Table 2. Ranitidine and metformin samples were subjected to HPLC-UV analysis using previously validated methods (Ashiru et al., 2007, Mai et al., 2017). The evaluation of ranitidine was achieved by using a 5 μm Luna SCX (Phenomenex, UK) column and a mobile phase mixture of 20:80 (acetonitrile):(0.1 M sodium acetate pH = 5.0) with a flow rate of 2 mL/min. In the case of metformin, a Luna C18 (250 mm × 4.6 mm I.D./5 μm) column (Phenomenex, UK) was applied with a flow rate of 1 mL/min. The ganciclovir samples were quantified by HPLC using a Luna C18 (250 mm × 4.6 mm I.D./5 μm) column (Phenomenex, UK) with a flow rate of 1 mL/min. The mobile phase consisted of 0.5% formic acid water and acetonitrile (95:5, v/v). The UV detector was set at 275 nm. A linear calibration curve was obtained at concentration ranges of 0.5 – 50 μg/ml.

2.6 Data analysis

The apparent permeability coefficient (P_{app}) was calculated for the evaluation of ranitidine, ganciclovir and metformin permeation study by using the following equation:

$$Papp(\mathrm{cm/s}) = \frac{Q}{C \cdot A \cdot t}$$

where Q (μmol) is the total amount of drug that permeated to the basolateral chamber throughout the incubation time, C (μmol/mL) is the initial drug concentration in the apical chamber, A (cm^2) is the diffusion area of the Ussing Chamber and t (s) is the time of experiment.

2.7 Statistical analysis

All results are expressed as mean ± SD (n = 6) and were analysed by one-way ANOVA and followed by a Tukey post-hoc analysis with a 95% confidence interval using IBM SPSS Statistics 16 (SPSS Inc., Illinois, USA).

3.0 Results

3.1 P-gp expression along the intestine in fasted-state and fed-state rats

As shown in Figures 1 and 2, the relative expression pattern of intestinal P-gp demonstrated a stark difference in both sexes following food intake. In male rats, the relative expression level of P-gp significantly decreased in the jejunum, ileum and colon following food intake whilst

the duodenum segment exhibited no significant change. The largest reduction in P-gp intestinal expression occurred in the colon where levels decreased by approximately 84% from 1.73 ± 0.36 to 0.31 ± 0.15 (Supplementary Table A). Interestingly, in female rats, an entirely contrasting result was observed. Food intake enhanced P-gp expression level along the intestinal tract except in the colon which remained unchanged; P-gp expression increased by approximately 200% after food intake in the duodenum (from 0.39 ± 0.18 to 1.21 ± 0.77) and in the ileum (from 0.32 ± 0.14 to 0.90 ± 0.37). The largest change was observed in the jejunum region where P-gp expression in the fed-state was 1.76 ± 0.95 which demonstrated a 6-fold increase over the fasted-state at 0.34 ± 0.13.

The unexpected food effect on intestinal P-gp was also demonstrated at an mRNA level as the results of the real time PCR experiment in the current study supported the same effect following food intake. As shown in Figure 3, male rats in the fed-state demonstrated a significant decrease in expression of the *mdr1a* gene along the whole intestine. The *mdr1b* gene, however, maintained a stable expression compared to that of the fasted-state. In female rats, both the *mdr1a* and *mdr1b* gene achieved a statistically significant increase along the intestine following food intake. This was consistent with the increase in P-gp expression in female rats after food consumption.

3.2 Intestinal permeation of ranitidine, ganciclovir and metformin

The permeability of ranitidine and ganciclovir exhibited a sex difference in the fasted state of the rat intestine (Figure 4 and supplementary table C). Ranitidine P_{app} in the jejunal and ileal regions of female rats was higher than males ($8.24 \pm 1.29*10^{-6}$ cm/s versus $6.78 \pm 1.24*10^{-6}$ cm/s; and $9.97 \pm 0.52*10^{-6}$ cm/s versus $6.67 \pm 0.10*10^{-6}$ cm/s respectively). Similarly, in the case of ganciclovir, the permeability in the jejunum and ileum of female rats were $10.11 \pm 1.51*10^{-6}$ cm/s and $7.87 \pm 0.53*10^{-6}$ cm/s which were higher than males at $7.64 \pm 1.48*10^{-6}$ cm/s and $5.17 \pm 0.63*10^{-6}$ cm/s respectively. However, no significant sex difference was identified in the intestinal permeability of metformin as it is a non-P-gp substrate. Interestingly, in the case of ranitidine permeability from the fasted to fed state in males, a 22.7% and a 27.3% increase was observed in the jejunum and ileum respectively whilst that of females achieved a 25.8% and a 41.3% decrease. Ganciclovir permeability decreased in the female rat jejunum (36.2%) and ileum (37.8%) from the fasted-state to fed-state. In male rats, however, an 83.6% and 97.3% increase was observed in the jejunum and ileum

respectively. The permeability of metformin remained consistent in the different sexes in both fasted and fed states.

4.0 Discussion

Attempts have previously been made using Western blot analysis to investigate the distribution of P-gp in the rat intestinal tract, for instance; male Sprague-Dawley rats (Brady, 2002); fasted male Wistar rats (Valenzuela et al., 2004, Johnson et al., 2006, Wada et al., 2013); fed male and female Han-Wistar rats (MacLean et al., 2008); and fed male and female Wistar rats (Afonso-Pereira et al., 2018). The results of these studies, however, are inconsistent and further complicated by the differences in the reference proteins used to normalise transporter abundance. In this study, β-actin was used as the reference protein to normalise P-gp in male and female rats. As the same amount of total protein was loaded for each sample analyzation, the variability of β-actin protein bands intensity therefore can be used to understand the variability of β-actin protein expression in male and female rats. As shown in Supplementary Table B, there was no significant difference in the intensity of colour of β-actin protein bands between male and female rats, which demonstrated that β-actin was consistently expressed, and therefore the relative P-gp expression data are reliable. In addition, the agreement between the Western blot data and the PCR as well as Ussing chamber data further support the consistent characteristic of β-actin as a reference protein in the Western blot analysis. Moreover, no definitive study has considered the effect of food in P-gp expression between the sexes. This, however, is the first study to report that general food consumption itself can affect intestinal P-gp expression to different extents in males and females.

The *in vivo* relative expression of P-gp protein following food intake highly correlated to the *in vitro* relative expression of genes coding for P-gp in male and female rats. In male rats, the significant decrease of *mdr1a* gene highly correlated to the decrease in protein expression with food intake when compared to the expression in the fasted state. In female rats, a significant increase in P-gp expression in all intestinal regions was observed after food intake which correlates to the increase in *mdr1a* and *mdr1b* gene expression. Unlike in male rats, the *mdr1b* gene in female rats demonstrated a greater enhancement with food when compared with the *mdr1a* gene. *Mdr1b* may, therefore, be a more important gene for the production of P-gp in female rats than that of their male counterparts.

The results of the *ex vivo* study demonstrated that the change in intestinal P-gp following food intake in male and female rats significantly influenced the intestinal permeation of P-gp drug substrates. Ranitidine and metformin share the same OCT protein absorption mechanism, however, ranitidine is also a P-gp substrate (Konig et al., 2013, Leibach and Ganapathy, 1996, Muller et al., 2005, Bourdet and Thakker, 2006, Collett et al., 1999, Liang et al., 1995). As a result, the change of permeability of ranitidine highly correlated with the change of intestinal P-gp protein and mRNA level factoring in food and sex whilst metformin remained constant in all conditions. Although ganciclovir and ranitidine share the same P-gp absorption mechanism, ganciclovir experienced a greater modification in intestinal permeability following food intake. The fact that ranitidine is also an OCT protein substrate, therefore, may have contributed towards its lower permeation (Collett et al., 1999, Bourdet and Thakker, 2006, Muller et al., 2005, Li et al., 2011, Shah et al., 2007).

The potential reasons for this sex-dependent food effect are multifactorial. Firstly, the food ingredients themselves may contribute to this observed phenomenon. According to the manufacturer's document, the food supplied for the rats in the current study (EURodent Diet 22%) contained 32 different ingredients (LabDiet, US). A study demonstrated that certain dietary components including capsaicin, curcumin, [6]-gingerol, and resveratrol was able to inhibit the activity of P-gp in human multidrug-resistant carcinoma KB-C2 cells (Nabekura et al., 2005). Moreover, the oral administration of 60 mg/kg curcumin, a common food ingredient, can result in a decrease of intestinal P-gp expression in male rats (Zhang et al., 2007). Secondly, physiological changes in the intestinal luminal environment during food consumption may further contribute to the sex difference. Luminal fluid composition is normally altered from food consumption by the modification in production of bile salts, cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1). In addition, food-stimulated sex hormones may also influence the sex-dependent food effect on intestinal P-gp expression. A study proved that a diet containing menhaden oil n-3 PUFA increased serum oestradiol concentration levels from 90 pg/ml to approximately 130 pg/ml in pregnant rats (Davis et al., 2013). With the distribution of receptors in rodent intestine, sex hormones have been demonstrated to regulate P-gp expression; testosterone, the primary sex hormone in males, has been shown to induce an inhibitory effect on P-gp (Wessler et al., 2013). Conversely, another study reported that P-gp expression significantly increased after the incubation with

progesterone and β-estradiol at the concentration of or greater than 10 nM and 100 nM respectively (Coles et al., 2009).

The most interesting phenomenon identified in the current study was the notable increase of P-gp in the small intestine of female rats in the fed state. It can be suggested that this may occur due to the innate protection required for successful reproduction. In the fasted-state, female rats exhibited low levels of P-gp expression. In the fed-state, however, as food contains multiple components of which some may be harmful, the body can protect itself by increasing the expression of the efflux transporter as a barrier function to hinder the absorption of potentially toxic food compounds. This mechanism could be a complex interplay of the modulation of P-gp expression, enzyme reaction and the defence ability of epithelial cells which may be further influenced by sex hormones. It was reported that oestrone and oestradiol both increase intestinal enzyme activity in female rats. By administrating b.i.d. 1 mg/kg oestrone and oestradiol for two days in female rats, the intestinal CYP-450 enzyme concentration was enhanced from 0.03 ± 0.01 nmol/mg in the control group to 0.16 ± 0.01 and 0.09 ± 0.01 nmol/mg in the oestrone and oestradiol treated groups respectively Brady (2002). A study conducted investigated ileum tissues obtained from both male and female rats that were exposed to harsh conditions (such hypoxia for 40 min and acidosis at pH 6.8) and normal conditions (normoxia at a normal pH of pH 7.3) via an Ussing chamber experiment. Cytokine and nitric oxide concentration levels in the Ussing chamber were subsequently measured to evaluate the immune-inflammatory response. Fluorescein Isothiocyanate-dextran (FITC-dextran, molecular weight of 4,300 Da) was checked to assess the barrier function of the intestinal lumen. As a result, female intestinal tissue showed a higher anti-inflammatory response and an enhanced intestinal barrier function when compared with males. More interestingly, the addition of oestradiol in male rats relieved the intestinal injury and enhanced their anti-inflammatory ability (Homma et al., 2005).

5.0 Conclusion

The current study is the first to report that relative intestinal P-gp expression was drastically affected by food and to different extents in male and female rats. In males, intestinal P-gp decreased at both protein and mRNA level following food intake, however, an increase in

expression was observed in female rats. In addition, P-gp expression in both fasted and fed conditions exhibited a sex difference in the intestinal permeability of P-gp substrates ranitidine and ganciclovir. Therefore, the influence of food and sex should be acknowledged and implemented when using animal models for the early stage development of oral pharmaceutical products that are known or identified to be P-gp substrates.

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Figure 1
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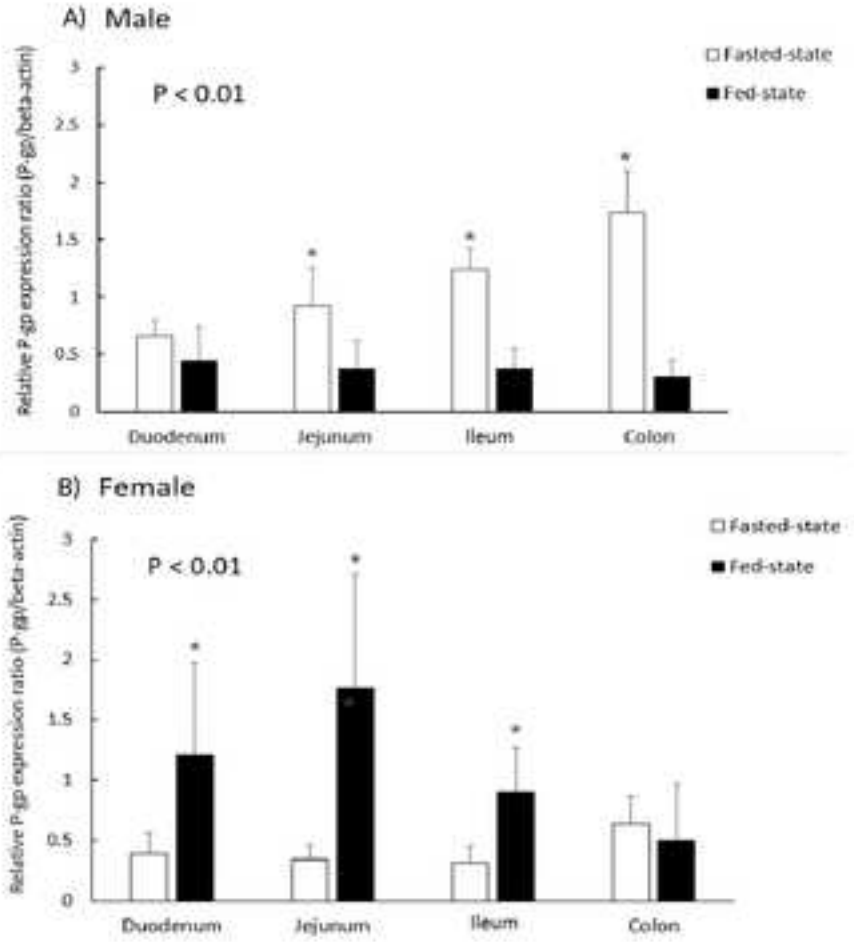

Figure 1. Relative intestinal P-gp protein expression in fasted-state vs. fed-state rats in A) males and B) females (n = 6).

Figure 2
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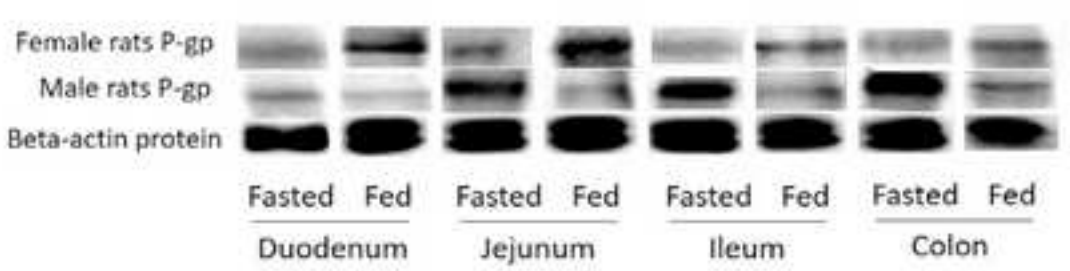

Figure 2. Western-blotting results of intestinal P-gp expression in fasted vs. fed conditions in male and female rats.

Figure 3

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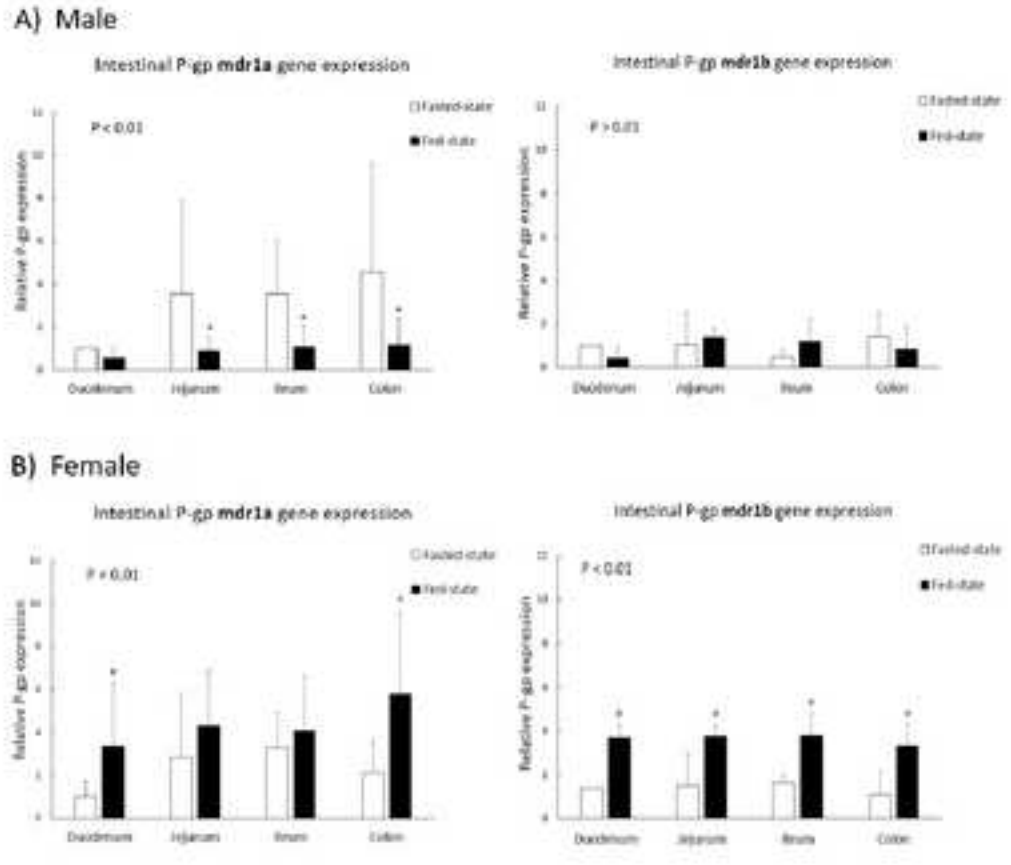

Figure 3. Relative expression of intestinal P-gp mRNA *mdr1a* and *mdr1b* gene in the fasted or fed state of A) male and B) female rats (n = 6).

Figure 4

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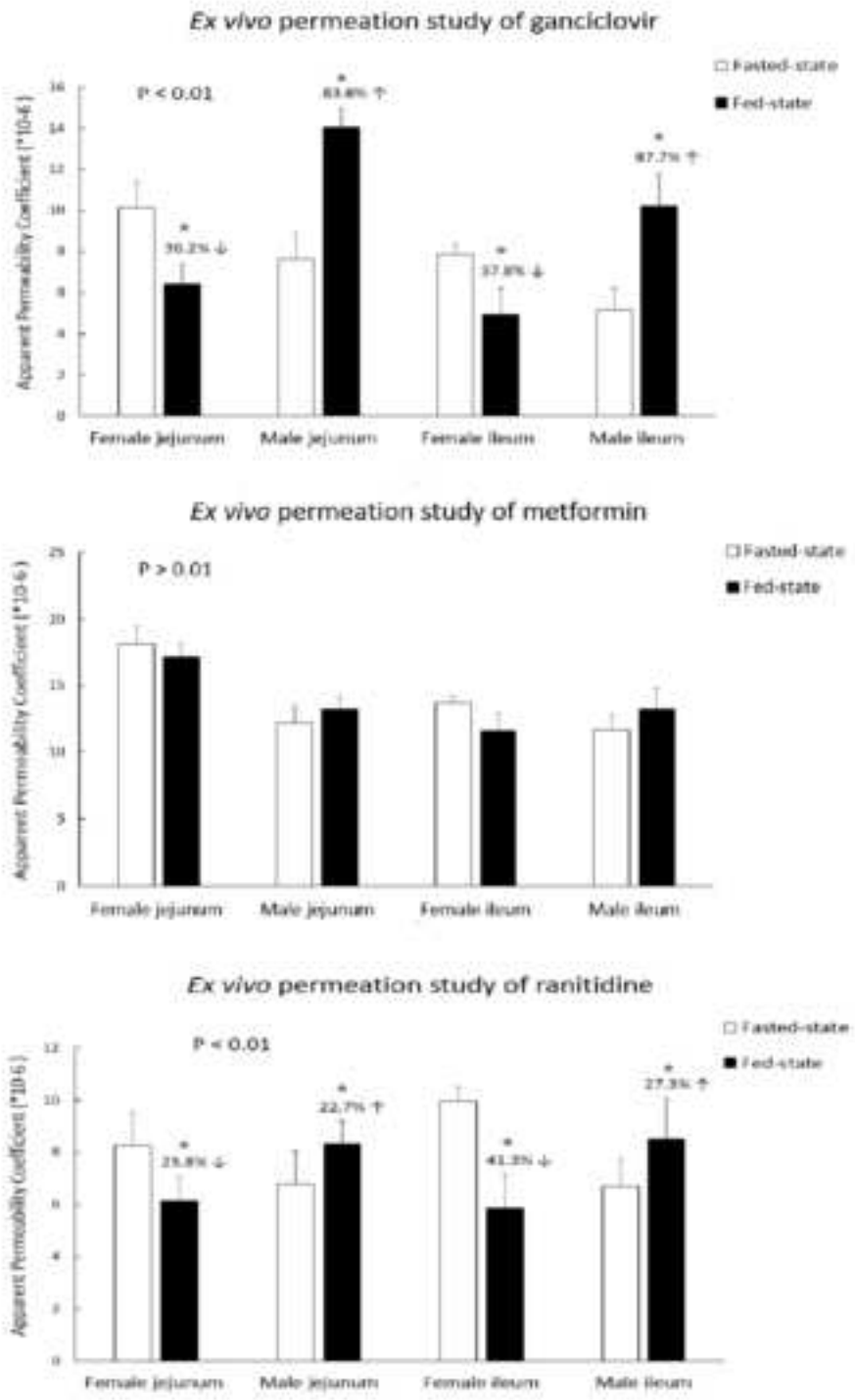

Figure 4. Permeation of ranitidine, ganciclovir and metformin in the jejunal and ileal regions of fasted vs. fed state male and female rats (n = 6).

Table 1. Sequences of designed primers used in the real-time qPCR experiment

Primers	Sense and Antisense	PCR product (*bq*)	Reference
mdr1a	Forward 5'-CACCATCCAGAACGCAGACT -3'	159	This paper
	Reverse 5'-ACATCTCGCATGGTCACAGTT-3'		
mdr1b	Forward 5'-AACGCAGACTTGATCGTGGT-3'	144	This paper
	Reverse 5'-AGCACCTCAAATACTCCCAGC-3'		
β-actin	Forward 5'-GCAGGAGTACGATGAGTCCG-3'	74	This paper
	Reverse 5'-ACGCAGCTCAGTAACAGTCC-3'		

Table 2. Summary of the HPLC methods for ranitidine, ganciclovir and metformin quantification

Drug	Column	Temperature (°C)	Mobile phase	Flow rate (ml/min)	UV detection wavelength (nm)	Injection (µl)	Reference
Ranitidine	SCX (250 mm × 4.6 mm I.D./5 µm)	50	0.1 M Sodium Acetate Buffer (pH 5.0, 80%); Acetonitrile (20%)	2	320	40	Ashiru et al., 2007
Ganciclovir	C18 (250 mm × 4.6 mm I.D./5 µm)	40	0.5% Formic acid water (95%); Acetonitrile (5%)	1	275	20	This paper
Metformin	C18 (250 mm × 4.6 mm I.D./5 µm)	25	10 mM Sodium Dihydrogen Phosphate Buffer with 10 mM Sodium Dodecyl Sulfonate (pH 7.0, 60%); Acetonitrile (40%)	1	234	50	Mai et al., 2017

Supplementary Material A

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Supplementary Material B

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