The regional-specific relative and absolute expression of gut transporters in adult Caucasians: A meta-analysis

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Healthy Adult Caucasian Gut Transporter Abundances

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NON-STANDARD ABBREVIATIONS – ABC (ATP-Binding Cassette); ADAM (Advanced Dissolution Absorption and Metabolism); ASBT (Apical Sodium-Dependent Bile Acid Transporter, also see IBAT); BCRP (Breast Cancer Resistance Protein); CYP450 (Cytochrome P450); DDI (Drug-Drug Interaction); EMA (European Medicines Agency); ET (Extensive Transporter (phenotype)); FDA (Food and Drug Administration); GM (Geometric Mean); IBAT (Ileal Bile Acid Transporter, also see ASBT); ISEF,T (Inter-System Extrapolation Factor for Transporters); ITC (International Transporter Consortium); IVIVE (In Vitro-to-In Vivo Extrapolation); LC-MS/MS (Liquid Chromatography with Tandem Mass Spectrometry); M-ADAM (Multi-layer gut wall within ADAM); MRP (Multidrug Resistance-associated Protein); NCE (New Chemical Entity); OATP (Organic Anion Transporting Polypeptide); OCT (Organic Cation Transporter); OST (Organic Solute Transporter); P-gp (P-Glycoprotein); PBPK (Physiologically-Based Pharmacokinetic); RAF (Relative Activity Factor); REF (Relative Expression Factor); RT-PCR (Reverse Transcription-Polymerase Chain Reaction); SLC (Solute Carrier Family); TM (Total Membrane Fraction); WX (weighted mean).
ABSTRACT

The aim of this study was to derive region-specific transporter expression data suitable for In Vitro-to-In Vivo Extrapolation (IVIVE) within a Physiologically-Based Pharmacokinetic (PBPK) Modelling framework. A meta-analysis was performed whereby literary sources reporting region-specific transporter expression obtained via absolute and relative quantification approaches were considered in healthy adult Caucasian individuals. Furthermore, intestinal total membrane protein yield was calculated to enable mechanistic IVIVE via absolute transporter abundances. Where required, authors were contacted for additional information. A refined database was constructed where samples were excluded based on quantification in; non-Caucasian subjects; disease tissue; subjects <18 years old; duplicated samples; non-total membrane matrix; pooled matrices or cDNA. Demographic data was collected where available. The weighted and geometric mean, coefficient of variation and between-study homogeneity was calculated in each of 8 gut segments (duodenum, 2 jejunum, 4 ileum and colon) for 16 transporters. Expression data was normalized to that in the proximal jejunum. From a total of 47 articles, the final database consisted of 2238 measurements for 16 transporters. The solute carrier PepT1 showed the highest jejunal abundance, while MRP2 was the highest abundance ATP-Binding Cassette Transporter. Transporters displaying significant region-specific expression included, IBAT, which showed 18-fold greater terminal ileum expression compared to the proximal jejunum, while MRP3, OCTN1 and OCT1 showed >2-fold higher expression in other regions compared to the proximal jejunum. This is the first systematic analysis incorporating absolute quantification methodology to determine region-specific intestinal transporter expression. It is expected to be beneficial for mechanistic transporter IVIVE in healthy adult Caucasians.
STATEMENT OF SIGNIFICANCE

Given the burgeoning reports of absolute transporter abundances in the human intestine, the incorporation of such information into mechanistic IVIVE-PBPK models could offer a distinct advantage to facilitate the robust assessment of the impact of gut transporters on drug disposition. The systematic and formal assessment via a literature meta-analysis described here, enables assignment of the regional-specific expression, absolute transporter abundances, inter-individual variability and other associated scaling factors to healthy Caucasian populations within PBPK models. The resulting values are available to incorporate into PBPK models, and offer a verifiable account describing intestinal transporter expression within PBPK models for persons wishing to utilize them. Furthermore, these data facilitate the development of appropriate IVIVE scaling strategies using absolute transporter abundances.
INTRODUCTION

There is an increasing application of employing Physiologically-Based Pharmacokinetic (PBPK) models for making key decisions on the clinical progress of New Chemical Entities (NCEs) in the drug development and regulatory spheres (Shebley et al., 2018). A growing assurance in the ability to predict the pharmacokinetics, pharmacodynamics and Drug-Drug interactions (DDI) of NCEs within virtual individuals built into PBPK models has been based on the continued dedication of allied academic, industrial and regulatory institutions to develop robust physiological parameters, that are essential to facilitate the generation of relevant individuals within virtual populations. Alongside this, the development of mechanistic strategies that harness data generated from in vitro assays routinely performed to characterize an NCE’s PK liability, via In Vitro-to-In Vitro Extrapolation (IVIVE) strategies, is critical to enable insightful judgements on clinical progress to be reached. For several years now the capacity to scale the cytochrome P450 (CYP450) activities via recombinant in vitro systems and absolute protein expression, has been demonstrated in the intestine (Gertz et al., 2010). Historically, the availability of protein standards employed within assays to quantify CYP450 absolute protein abundances in both, in vitro systems and mammalian tissues, has facilitated the development of such strategies. These approaches are underpinned by incorporating scaling factors that act to bridge any mechanistic gaps between the in vitro and in vivo milieu, and are typically based on determining the relative expression (or activity), or the difference in functional protein abundance between the in vivo and in vitro systems (Proctor et al., 2004). An expanding body of evidence has meant that in particular for certain CYP450-mediated DDIs, judgements on a NCEs clinical progress can be reached by harnessing PBPK modelling strategies which predict its’ PK/DDI liabilities (Jones et al., 2015; Wagner et al., 2015; de Zwart et al., 2016; Shebley et al., 2018). Furthermore, regulatory authorities are developing guidance to ensure rigorous quality assurance is applied to NCE submissions that harness mechanistic PBPK modelling (CDER, 2016; CHMP, 2016).
As protein standards have historically not been routinely available for membrane transporter expression absolute quantification, the capacity to scale transporter activity by IVIVE in order to predict the impact transporter-mediated drug clearance within specific organs of a PBPK model has relied upon transporter expression data from relative quantification approaches, i.e. from mRNA expression quantification via Reverse Transcription-Polymerase Chain Reaction (RT-PCR) or protein expression from immunoblotting to derive and apply relevant scaling strategies (Harwood et al., 2013; Neuhoff et al., 2013), while other related transporter IVIVE-PBPK models have required additional empirical scalars to ensure the model captures the clinical observations (Jones et al., 2012; Varma et al., 2012; Jamei et al., 2014). Recently, we undertook and reported on an extensive literature meta-analysis to establish 19 transporter protein abundances in the healthy Caucasian liver (Burt et al., 2016). Accompanying the meta-analysis is the development of an IVIVE strategy to harness the inter-individual variability in the hepatic absolute transporter abundances in picomoles determined via the meta-analysis, using a unit-less Inter System Extrapolation Factor for Transporters (ISEF,T) within IVIVE. The drive to develop this strategy was thus; (1) A desire within industry to develop more mechanistic scaling factors to facilitate model development for transporter IVIVE (Jones et al., 2015; Pan et al., 2016; Guo et al., 2018), and (2) The literary reporting of absolute transporter protein abundances in human livers utilising burgeoning proteomics techniques (Heikkinen et al., 2015).

The capacity to scale transporter activity data obtained in relevant in vitro cell monolayers, within PBPK models, that describe the region-specific intestinal transporter expression levels based on relative expression approaches, has been demonstrated for intestinal efflux transporters like P-gp (Neuhoff et al., 2013; Yamazaki et al., 2018). However, building on the incorporation of absolute transporter abundance scaling of hepatic transporter activity (i.e., the ISEF,T approach), our aim was to perform an extensive meta-analysis of the expanding human intestinal transporter absolute abundance quantification literature to facilitate the development of an ISEF,T approach to scale
transporter activity data in and along the gut. Furthermore, an appraisal of the literature utilizing ‘relative’ quantification approaches was undertaken to determine region-specific expression of gut transporters and assimilated with that of the ‘absolute’ quantification-based studies. A similar rigor was applied to the gut transporter abundance meta-analysis as was done for that of the liver in terms of study exclusion criteria (Burt et al., 2016). We provide a meta-analysis of quantitative intestinal transport abundance data to employ the ISEF,T approach in IVIVE-PBPK.
METHODS

Priming the Database: Identifying and Prioritizing Intestinal Transporters for Analysis

There are greater than 400 transporter genes identified in the human genome (Cesar-Razquin et al., 2015). Therefore, prior to undertaking a systematic meta-analysis to establish region-specific transporter expression in the human intestine, it was important to primarily identify and prioritize transporter isoforms that show demonstrable expression in the human small and large intestine, and that possess the capacity interact with drugs to potentially influence drug disposition. Based on analysis of the literature (via searching the electronic database PubMed), a database comprising 52 transporters relevant to the human intestine was collated, in which evidence was gathered and recorded on the transporter isoform(s) human intestinal expression and the methodology employed to quantify expression; membrane localization (apical, basolateral or both); transporter function (i.e., uptake, efflux or both); in vivo (human clinical) and in vitro evidence (cell monolayer studies) of interaction with drugs (substrate/inhibitor moieties), regulatory requirement/interest (i.e., the US Food and Drugs Administration Agency (FDA), European Medicines Agency (EMA)), and focussed groups such as the International Transporter Consortium (ITC). Given the available evidence, the transporters were ranked based on criteria such as; robust evidence of mRNA transcription, protein expression, or are known to be involved in intestinal drug absorption/disposition. Ultimately, the 16 highest ranked transporters are summarized in Table 1, including 10 transporters from the Solute Carrier family (SLC) and 6 transporters from the ATP-dependent Binding Cassette superfamily (ABC), and were prioritized for subsequent electronic literature searches and integration into an abundance database for meta-analysis.

Transporter Abundance Database

A single overarching ‘complete’ database was collated to contain published abundance data for the 16 prioritized transporters quantified in human intestinal tissue. The complete database included separate datasets where the quantification of transporter abundances was performed using...
either ‘absolute’ or ‘relative’ approaches. Absolute transporter abundance data was typically quantified against a standard curve of a verified surrogate peptide(s), using Quantitative Targeted Proteomics via LC-MS/MS or a quantitative Western blot approach. In these assays the transporter protein abundance is expressed in moles per mass of protein. For the relative transporter abundance data, quantification was typically performed using PCR or immunoblotting technology, where the abundance of the transporter was expressed relative to a ‘housekeeper’ gene or protein.

Original research articles were retrieved via searching the electronic database PubMed using the following keyword combinations: <Human>, <Intestinal>, <Transporter>, <Absolute>, <Relative>, <Protein>, <Expression>, <Abundance>, <Proteomics>. The database including all available measurements was established (final literature search June 2017), with background information on the methods as well as donor demographics collated where provided. In cases where individual data were not directly reported, data were extracted via GetData Graph Digitizer (version 2.22, http://getdata-graph-digitizer.com) or authors were contacted directly to request individual donor data. A refined sub-database was created through the use of various exclusion criteria. First, study methodologies were reviewed to ensure that absolute abundances were quantified using LC-MS/MS or quantitative Western blot in Total Membrane (TM) fractions. For relative expression studies the same stringency for quantification in a TM fraction was not appropriate as numerous studies were included that required mRNA extraction and subsequent reverse transcription to cDNA for PCR-based expression quantification. Next, data in which it was stated that the human intestinal tissue was not from adult (aged < 18 years), healthy (or macroscopically normal after histological assessment) or Caucasian individuals were excluded. Any study in which pooling of sample matrices took place (mRNA, cDNA (Herrera-Ruiz et al., 2001; Seward et al., 2003) or microsomal samples for protein absolute abundance analysis (Nakamura et al., 2016)) was not included as inter-individual variability is lost when pooling as only mean with experimental error/deviation is therefore available. However, such datasets can be used for comparing to the results of meta-analysed expression data for relevant transporters. For
the relative expression studies, any study which did not normalize their target (i.e. transporter) gene or protein against a reference (or ‘housekeeper’ gene/protein) within the same assay was excluded (Landowski et al., 2003; Kim et al., 2007). Exclusion occurred in instances where there was 'relative' quantification of a transporter in a single region of the intestine (Hilgendorf et al., 2007), in which quantification only took place in the Jejunum, hence normalization to other segments could not take place (see procedural aspects of Meta-Analysis Section below). Finally, the source of data was identified to ensure that duplicate measurements from the same tissue sample were not included in the refined-database. Meta-analysis was then used to characterize the region-specific abundance of intestinal transporters in the refined-database.

**Data Analysis: Determining Region-Specific Intestinal Transporter Expression via Meta-Analysis**

Within the Simcyp Simulator the Advanced Dissolution Absorption and Metabolism (ADAM) and Multi-layer-ADAM (M-ADAM) model, which constitutes 7 small intestinal (duodenum, 2 jejunum, and 4 ileum segments) segments and a single segment representing the colon, contains the transporter expression specific to each intestinal segment (Jamei et al., 2009). The region-specific transporter expression is normalized relative to the proximal jejunum segment ‘Jejunum I’, as was previously described for *ABCB1* (P-Glycoprotein (P-gp)); *ABCC2* (Multidrug Resistance-associated Protein 2 (MRP2)) and *ABCG2* (Breast Cancer Resistance Protein (BCRP)) using relative quantification approaches (Harwood et al., 2013). Hence, the meta-analysis was structured to account for quantification of transporter abundance in each segment of the ADAM and M-ADAM models. Where there was insufficient information described in the study, for example where samples were described as from the ileum, and not described with greater precision to a specific region of the ileum, the expression data for that study was assigned with those expression values to each of the four ileum segments constituting ADAM and M-ADAM.
The meta-analysis comprised three datasets: (1) region-specific abundances determined from absolute abundance data; (2) region-specific abundances determined from relative expression data and (3) region-specific abundances determined from a combination of absolute and relative data.

For the meta-analysis the region-specific abundances were determined after normalization to the weighted mean (WX) abundances in the reference segment the proximal jejunum (i.e., Jejunum I) (Harwood et al., 2013). For the relative abundance measurements that did not contain a jejunum sample, the values were initially scaled to colon or ileum and the relative average value was later used to combine all data relative to Jejunum I.

In line with the previous meta-analysis (Harwood et al., 2013), where studies differentiated between colonic regions, transporter expression data were incorporated into the final analysis from those samples originating from the ascending colon.

Where no suitable absolute abundance quantification data was available for a transporter, a Jejunum I absolute abundance value of zero (in pmol/mg TM protein) were assigned, and the meta-analysis proceeded to establish the region-specific abundance levels of that transporter based on relative transporter quantification methodology, where quantification was undertaken in the jejunum, therefore normalization to the Jejunum I could be performed. In instances where abundance data generated via absolute or relative quantification methods was available for a transporter, the databases were combined within the meta-analysis framework.

After applying the exclusion criteria to the complete database, the collated abundance values for the healthy, Caucasian adult sub-database were combined for a given transporter to generate WX, geometric mean (GM), standard deviation (S.D.) and coefficient of variation (CV) for the Jejunum I segment for the Extensive Transporter (ET, representing the wild type) phenotype based on the equations described previously for metabolising enzymes (Perrett et al., 2007). The abundance values were further tested for between-study heterogeneity using the Cochran $X^2$-based Q test.
(Perrett et al., 2007), whereby heterogeneity was apparent when a probability of \( p < 0.05 \) was returned.

The assigned CV was preferentially based on absolute abundance data, where this was available for a transporter isoform, while for the majority of the other transporters analysed, a CV based on relative expression data was required as absolute data was either not available, or was excluded based on the aforementioned criteria. For \( SLC51A/B \) (OST-α/β) and \( SLC10A2 \) (Ileal Bile Acid Transporter (IBAT)) additional considerations for CV determination were required. The rationale for their derivation are described in the “Results: Absolute abundance data analysis” section.

Where abundance values in picomoles per milligram of TM protein, the mean is provided in the text as mean ± standard deviation, unless otherwise stated.

**Total Membrane Protein Yield in the Small intestine and Colon**

To facilitate the scaling of in vitro activity data per picomole of transporter to the entire small intestine and colon, requires that the human intestinal abundances, as determined in the aforementioned meta-analysis, are converted to picomoles per intestinal segment, thus enabling the calculation of segmental transporter activity (clearance). As the meta-analysis of human intestinal abundances incorporated values reported as picomoles per milligram of TM protein, we sought to determine the small intestinal and colonic total membrane protein yield in milligrams.

Literature sources that specifically reported Total Membrane Protein Per Intestine (TMePPI; related to small intestinal yield) and Total Membrane Protein Per Colon (TMePPC) were sought. For each study the TM protein yield for the specific intestinal segment were scaled to the duodenum, jejunum and ileum dependent on the procedure i.e., mucosal scraping (Tucker et al., 2012), mucosal crushing (Drozdzik et al., 2014) or enterocyte elution (Harwood, 2015) from which the TM fractions were obtained. In instances where procedural losses during preparation of TM protein from tissue homogenates were available, these were accounted for in the final
segmental yield calculations. The weighted mean of the TMePPI and TMePPC are given in milligrams. The specific methodology to obtain segmental TM protein yield from each study are provided in the Supplemental Section ‘Total Membrane Protein Yield’.

RESULTS

Abundance database

In this study, a total of 47 articles were recorded in the complete database of which 30 were accepted into the meta-analysis after exclusion criteria were applied. The complete database consisted of 3374 absolute and relative quantification measurements of transporter expression (See Supplemental Table 2A and 2B for studies and sample quantification information). Of this complete database, 2238 relative- and absolute-based quantification measurements across all intestinal regions for 16 transporters matched our inclusion criteria and were thus included in the final dataset for adult healthy Caucasians (Figure 1). The final absolute database consisted of 5 independent studies (Tucker et al., 2012; Groer et al., 2013; Oswald et al., 2013; Drozdzik et al., 2014; Harwood et al., 2015), and data from a PhD program published in a Thesis (Harwood, 2015), with the data linked to that published in Harwood et al., 2015. The most common reason for the exclusion of absolute abundance data was the use of samples from individuals with underlying disease conditions (40%), whereas for the relative abundance it was data from non-Caucasian samples (12%). Other reasons for exclusion of absolute abundance data was the reporting of data from duplicate samples (Bruck et al., 2017), quantification in samples other than TM fractions (9% of complete absolute database) (Wisniewski et al., 2015; Vaessen et al., 2017), while the other criteria for exclusion in both the absolute and relative dataset constituted a relatively minor component (Figure 1 & Supplemental Table 1). There was limited information available on an individual’s phenotype status, therefore no studies were excluded for possessing non-extensive-transporter phenotype samples. The samples quantifying absolute abundances and excluded due to underlying disease, were due to individuals who were morbidly obese with a Body Mass Index
> 30 (Miyauchi et al., 2016), and those samples classified as possessing adenoma or cancer (Wisniewski et al., 2015), while for the relative abundance quantifications, Crohns and Ulcerative colitis samples (Thibault et al., 2007), required that these were excluded from the refined database. The non-Caucasian ethnicity samples were excluded as they incorporated abundance data from Japanese individuals (Hinoshita et al., 2000; Terada et al., 2005).

Of the 16 transporters on which the final meta-analysis was based (Table 1), suitable absolute data could not be obtained for 6 transporters at the time of this meta-analysis due to one or more of the criteria outlined in Figure 1. These transporters were, SLC16A1 (Mono-Carboxylate Transporter 1 (MCT1)), SLC22A4 (Organic Cation Transporter, Type 1, (OCTN1)), SLC2A2 (Glucose Transporter 2 (GLUT2)), ABCC1 and ABCC4 (Multidrug Resistance-associated Protein 1 and 4, (MRP1 and MRP4), respectively), and SLCO4C1 (Organic Anion Transporting Polypeptide 4C1, (OATP4C1)). Where absolute abundance data was not available for any transporter, the relative quantification data was used to determine the region-specific abundance in the intestine.

**Absolute abundance data analysis**

A summary of the absolute abundances for the 10 transporters in the healthy Caucasian adult proximal jejunum, assigned as ‘Jejunum I’ in the ADAM model, is provided in Table 2. The SLC apical uptake carrier PepT1 showed the highest abundance in the human Jejunum I samples with a weighted mean abundance of $3.69 \pm 1.5$ pmol/mg TM protein (n=11). The highest abundance ABC transporter was MRP2 with $0.86 \pm 0.58$ pmol/mg TM protein (n=11). In this analysis the mean Jejunum I abundance for OST-α/β is taken from analysis of distal, rather than proximal jejunum, due to the lack of quantification in proximal jejunum regions. OST-α/β is a dimeric protein conferring functionality when both the alpha and beta subunits coalesce (Seward et al., 2003). However, absolute quantification methods typically endeavour to quantify the abundance of each subunit separately (Harwood, 2015). For this analysis, the alpha subunit was used for OST-α/β abundance in Jejunum I, as the alpha subunit is considered to be the limiting component for
conferring activity for this dimer (Sun et al., 2007). With the translation of in vitro transporter activity through the ADAM and M-ADAM models, the variability (CV) for a given transporter is assigned for the Jejunum I only, and this variability is applied and propagated through all the segments of the gastrointestinal tract (Neuhoff et al., 2013). The highest variability was also associated with OST-α/β, having CV values of 99% (Table 2), which due to the limited number of samples from the absolute analysis (i.e., n=1 in Jejunum II), was derived from the combination of the relative and absolute data analysis for the jejunum samples. The levels for IBAT are negligible in the proximal small intestine (Groer et al., 2013; Drozdzik et al., 2014), which may give rise to an exaggerated inter-individual variability due to analytical imprecision at such low levels of abundance. Hence for this analysis the CV values were assigned from PCR-based jejunum mRNA analysis (Hilgendorf et al., 2007). In the final database, heterogeneity in absolute abundance values was found for MRP2 in the Duodenum (p = 0.049) and Ileum II (p = 0.047) segments. There was no between-study heterogeneity found for the other transporters within the absolute abundance database. The region-specific abundance based on the absolute abundance dataset, once normalized to the Jejunum I is provided as Figure 2A & B with values given in Supp. Table 3. Figure 3 shows the relative proportion of the abundance for each transporter in the final database after performing a simulation in 2000 North European Caucasians (values provided in Supplemental Table 4).

**Relative Abundance and combining relative and absolute abundance data-analysis**

A summary of the studies recorded and the region-specific abundances based on analysis utilising relative abundance quantification methodology is provided for 16 transporters in the healthy Caucasian adult is shown in Figure 2C & D and Supplemental Table 2B and Table 3. With the exception of IBAT there is good consistency when comparing the region-specific abundances determined from absolute or relative quantification methodologies (Supplemental Table S3 and Figure 2A & D). The differences observed for this protein between methodologies may be due to
the ability of the highly sensitive targeted proteomics analysis to derive low abundances for this protein in the proximal jejunum (≈ 0.01 pmol/mg total membrane protein (Groer et al., 2013; Drozdzik et al., 2014)). Nevertheless, an increasing gradient of expression peaking in the terminal ileum is expected for this protein. For GLUT2 relative quantification data was only available in the duodenum, thus the normalization to Jejunum I was not possible and a relative expression value of 1 was assigned across regions. Although not shown specifically here, for the studies excluded due to ethnicity, i.e. Japanese samples (Hinoshita et al., 2000; Terada et al., 2005), the mRNA expression normalized to GAPDH in the colon was ranked MRP3 > MRP1 = MDR1 > MRP2, and in the final Caucasian analysis (Table 3) the ranking is similar once normalised to Jejunum I with the exception of MRPI and MDR1 showing a more pronounced difference in the Japanese data set (MRP3>MRP1>MDR1>MRP2) (Hinoshita et al., 2000). While along the region-specific mRNA expression the Japanese data is fairly similar for MDR1, OCTN1 and OCT1, for PepT1 there is a distinct decrease in expression more distally as compared the Caucasian analysis (Table 3) in which there is a more uniform distribution along the small intestinal segments (Terada et al., 2005). In Japanese samples, OCT3 generally showed lower regional mRNA expression than Caucasians, yet its region-specific expression showed similar trends to Caucasians (Table 3) (Terada et al., 2005).

To enhance the rigor of the meta-analysis and facilitate the incorporation of an increasing number of relevant and new transporters into the ADAM and M-ADAM models, the absolute and relative quantification for transporters was combined to obtain region-specific abundances and inter-individual variability specifically for 7 transporters (Table 1 and Table 3). Furthermore, the final relative abundances incorporated into the ADAM and M-ADAM models are provided in Table 3. Irrespective of whether the meta-analysis for a transporter used a combined approach or where only relative quantification methodologies were available, the transporters displaying significant region-specific expression compared to the proximal jejunum (Table 3) include: IBAT which
showed a 100-fold or more greater expression in the terminal ileum; ABCC3 (MRP3) showed > 5-fold higher expression in the colon and > 2-fold higher in the duodenum; OCTN1 and SLC22A1 (OCT1) showed > 2-fold lower expression in the colon. For all other evaluated transporters, the region-specific expression was relatively uniform across the segments with no transporter displaying expression greater or less than 2-fold higher than in proximal jejunum.

**Intestinal and Colon Total Membrane Protein Yield**

The yield of TM protein in the intestine (TMePPI) and colon (TMePPC) was determined from 3 studies (Tucker et al., 2012; Drozdzik et al., 2014; Harwood, 2015). The yields based on the study of Drozdzik et al., 2014 were obtained via personal Communication from Dr Stefan Oswald, The University of Greifswald, Germany. The dataset consisted of 35 sample measurements, \( n = 14 \) duodenal (Tucker et al., 2012), \( n = 5 \) jejunum and \( n = 3 \) ileum (Harwood, 2015) and \( n = 7 \) for colon (Drozdzik et al., 2014; Harwood, 2015) where the age and gender distribution (where known) was 24 – 72 years, with a minimum of 2 females. The small intestinal TM protein yield \((n = 6)\) from Drozdzik et al., 2014 was provided as a lumped value covering the entire small intestinal region as was the colon \((n = 6)\) from the same study. The 5 jejunum samples measured from Harwood, 2015 consisted of 1 sample from the proximal jejunum with the remainder from the distal jejunum (1 female, 41-62 years). As each study did not contain specific TM protein yield data for each segment, the capacity to determine TMePPI and TMePPC required several conversions and assumptions to estimate yields in regions that were not measured experimentally (see Supplemental Data: Section – ‘Total Membrane Protein Yield’). The weighted mean \((\pm \text{ S.D.})\) TMePPI from the 3 studies was \(2737 \pm 1807 \text{mg}\) and TMePPC was \(112 \pm 37 \text{mg}\) which is used to calculate the absolute abundance of protein in pmol/mg TM protein from the meta-analysis to pmol concentrations.
DISCUSSION

The heightened recognition that PBPK models play to evaluate the mechanisms responsible for drug pharmacokinetics at industrial and regulatory levels, has driven the demand to precisely and accurately quantify key drug and physiological elements in PBPK models. The Relative Expression Factor (REF) approach provided a means to scale transporter activity from cell monolayers to various regions of the intestine (Neuhoff et al., 2013). Although practical, these models are not as sophisticated as those for CYP450’s, for which the ISEF scalar corrects for activity differences per unit of enzymes in the liver versus recombinant systems (Proctor et al., 2004). The increasing utilisation of methodologies to quantitatively determine a protein’s absolute abundance within a biological system, has led to efforts from developers of PBPK model platforms to harness these data within a physiological framework. The ISEF,T approach permits transporter-specific scaling of in vitro kinetics, based on a protein’s molar concentration within an individual’s organ (Burt et al., 2016). Given the increasing availability of region-specific intestinal transporter expression data from ‘relative’ and ‘absolute’ quantification approaches, a rigorous meta-analysis is provided to obtain region-specific transporter abundances and variability in healthy adult Caucasians.

Literary evidence was evaluated to prioritize intestinal transporters involved in drug disposition for inclusion into the meta-analysis. To accurately construct PBPK models that can assess the impact of transporter proteins, substantiating their intestinal expression, function and localization on the enterocyte plasma membrane is imperative. In vitro studies were required to assess the plasma membrane localization and functional aspects for certain transporters; while, intestinal transporter expression was confirmed primarily via immunoblotting, quantitative proteomics, immunohistochemistry and mRNA expression. The clinical pharmacokinetic relevance was also considered (CHMP, 2012; CDER, 2017; Zamek-Gliszczynski et al., 2018). For the majority of transporters (Table 1), intestinal protein expression was unequivocal (P-gp; MRP2, MRP3, BCRP;
OATP2B1; IBAT; PepT1; MCT1; OCT1; OCT3). For MRP1, OCTN1, MRP4 and OST-α/β where proteomics data was limited to a single study or intestinal region (Harwood, 2015; Wisniewski et al., 2015; Nakamura et al., 2016), supporting evidence relating to other protein quantification methods or mRNA expression was sought prior to ranking for inclusion. For OATP4C1, its capacity to transport the P-gp probe digoxin across the basolateral membrane of the renal proximal tubule cell is implicated (Mikkaichi et al., 2004), while transcriptional information supports potential expression in the small intestine, and immunohistochemistry demonstrates basolateral membrane expression in colon enterocytes (Hilgendorf et al., 2007; Bourgine et al., 2012; Kleberg et al., 2012). OATP1A2 (one of the initial 52 transporters evaluated) is of potential pharmacokinetic relevance, yet several studies show intestinal mRNA and protein levels are absent or negligible (Supplemental Table 1). Hence this transporter is not included in the final meta-analysis. The glucose transporter GLUT2 is considered in the model as a potential drug-target for obesity and diabetes, as jejunal GLUT2 is highly abundant in morbidly obese individuals (Miyauchi et al., 2016). In obesity GLUT2, translocates from the enterocytes’ basolateral membrane in healthy individuals to the apical membrane (Ait-Omar et al., 2011).

Understanding a transporters location and function is crucial to constructing the appropriate model structure and algorithms to accurately scale transporter activity. There is conflicting information as to the enterocyte localization of OCT1, where earlier studies implicated lateral/basolateral membrane localization (Muller et al., 2005; Giacomini et al., 2010), yet a later study which combined immunocytochemistry and functional transporter assays, using the OCT1-specific substrate pentamidine, concluded that apical uptake predominated (Han et al., 2013). Hence, OCT1 is assigned as an apical membrane uptake transporter (Table 1). The enterocyte localization of OATP2B1 has also been under scrutiny recently (Keiser et al., 2017). Early studies implicate apical localization on human intestinal sections and Caco-2 cells (Kobayashi et al., 2003; Sai et al., 2006). However, recent proteomic investigations demonstrate markedly higher OATP2B1 expression in basolateral compared to apical membrane fractions, yet accompanying
immunostaining revealed less emphasis on basolateral localization (Keiser et al., 2017). Given the findings, OATP2B1 expression in both apical and basolateral membranes is plausible, OATP2B1 is assigned as an apical uptake transporter (Table 1). However, the switching of transporter localization and function in the model is possible, thus OATP2B1 could represent a basolateral uptake transporter.

To ascertain region-specific absolute transporter abundances via meta-analysis for the prioritized 16 transporters, a complete database was compiled cataloguing 3374 measurements across all intestinal regions. To define the healthy Caucasian adult intestinal transporter expression a refined database was curated where several exclusion criteria were applied, where the largest proportion of measurements excluded were that of non-healthy samples (Figure 1). Disease can affect transporter expression (Evers et al., 2018), so distinguishing between those samples which are directly affected by disease is critical. However, the challenge with curating such a dataset is that a routine means of obtaining intestinal samples to quantify protein expression is from individuals undergoing surgical intervention for an array of complications. In such cases, if studies classified their samples as ‘macroscopically’ normal it was assumed that the tissues’ protein expression was unaffected by the disease, thus these measurements were incorporated into the refined database.

The ADAM and M-ADAM models scale intestinal transporter activity in a region-specific manner after normalization against the ‘reference’ Jejunum I segment for both the ISEF, T and REF approach (Neuhoff et al., 2013). Therefore, the primary goal is to determine the Jejunum I absolute transporter protein abundance and its associated inter-individual variability (Table 2). For certain transporters, it was necessary that CVs were obtained from absolute and relative datasets, as there were insufficient jejunum-based absolute abundance quantifications (OST-α/β), or where jejunum-mRNA quantification was considered (IBAT). For all other intestinal segments where data was available, the WX transporter abundances were determined and normalization to the Jejunum I abundance (pmol/mg TM protein) was performed. Between-study heterogeneity was
not as evident as for the healthy Caucasian adult liver absolute abundance meta-analysis (Burt et al., 2016), with MRP2 the only transporter displaying this tendency in two segments. This may be due to less biological variability between the studies or lower sample numbers available to distinguish heterogeneity than for the liver. Due to the limited availability of measurements that can be directly attributed to a given donor it was not possible to perform any correlation analysis in respect to age and gender.

Although performing a region-specific meta-analysis using studies quantifying transporter protein or mRNA expression using relative quantification techniques, cannot directly inform us as to the absolute levels of transporter abundances within the sample, it provides a relatively large number of measurements, which when combined with the absolute dataset, provide robust region-specific expression information for each transporter after normalization to the Jejunum I. Except for IBAT, the ‘relative’ or ‘absolute’ quantification techniques showed limited region-specific transporter differences. This provided the confidence to utilize both absolute and relative datasets to obtain the final region-specific abundances for incorporation into a healthy Caucasian population. Alone, the relative expression dataset was instrumental in providing region-specific expression data for 6 transporters (Table 1 & Table 3) allowing the REF approach to be employed, even if absolute abundances were not available.

Absolute abundance data were available from whole tissue homogenate, TM and plasma membrane fractions. However, only data obtained from TM fractions were included in the final database, as corresponding protein yield values enabling the conversion of abundance values to pmol transporter/intestinal segment are reported here for the first time in the literature. Derivation of the intestinal (TMePPI: 2737 ± 1807 mg) and colonic (TMePPC: 112 ± 37 mg) total membrane protein yield was based on 3 studies in which the primary focus of each study was not the determination of these parameters (Tucker et al., 2012; Drozdzik et al., 2014; Harwood, 2015). Therefore, the application of several assumptions based on determination of intestinal/mucosal
tissue yields (Paine et al., 1997) and intestinal mucosal cylindrical surface areas, facilitated the
determination of the yields dependent on whether the mucosa or enterocytes specifically, were
processed (Supplemental Section – ‘Total Membrane Protein Yield’). The TMePPI is in
reasonable agreement with the small intestine microsomal protein yield of 2978 mg which is
anticipated given the methodological similarity in obtaining microsomes (Paine et al., 1997), or
TM fractions (Tucker et al., 2012) using differential centrifugation. It is difficult to gauge the
physiological plausibility of the TMePPC as there are no equivalent colonic microsomal data
published for comparative purposes, however a lower TMePPC is expected than TMePPI, given
the highly folded structure of the small intestinal mucosa compared to the colon. Further dedicated
studies are sought to investigate intestinal membrane protein yield.

This is the first in-depth systematic analysis of intestinal region-specific transporter expression
based on absolute abundance quantification methods. The expression data derived herein provides
the additional flexibility to model the region-specific active transport processes for 16 transporters
expressed in the enterocyte utilising both a relative (REF) and absolute abundance (ISEF,T)
scaling approach. In order to utilize the ISEF,T approach, an increasing focus on measuring in
vitro transporter kinetics combined with absolute transporter abundances (Meng et al., 2017a;
Meng et al., 2017b) is required. Routine updates to the database are required when applicable data
become available (Drozdzik et al., 2018) and further studies are warranted to enhance our ability
to translate absolute abundance quantification data into mechanistic models, while similar
analyses are required for other ethnicities and disease models.
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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Exclusion criteria applied to the complete database for the Absolute (left) and Relative (right) abundance data. Percentages for each exclusion criteria refer to the fraction of the samples in the complete database that were excluded on its’ basis.

Figure 2. Absolute abundance quantification of ABC transporters normalized to the Jejunum I segment (A) and SLC transporters (B) in all intestinal segments representing the ADAM model. D = Duodenum; J1 & J2 = Jejunum I & II segments; I1-to-I4 = Ileum I-to4 segments and C = Colon. Relative abundance quantification of ABC transporters normalized to the Jejunum I (C) and SLC transporters (D) in all intestinal segments representing the ADAM and M-ADAM models. The bars represent weighted mean normalized abundance of each transporter. Representative values depicted in this figure are provided in Supplemental Table 3. Where no abundance data was available a zero value was assigned, this is represented in; histogram B for SLC51A/B in all segments except Jejunum II and Ileum IV; histogram D for SLC2A2 all segments except duodenum, and SLCO4C1 Duodenum, Jejunum I – II. The values provided above the bars for SLC10A2 in histogram B, represent the scaled up expression relative to the break point ((//), i.e. 5) executed for these values.

Figure 3. Intestinal drug transporter pies. Proximal jejunum (Jejunum I) ATP-dependent (ABC) (A) and Solute Carrier (SLC) and Organic Anion Transporting Polypeptide (SLCO) (B). Distal ileum (Ileum IV) ATP-dependent (ABC) (C) and Solute Carrier (SLC) and Organic Anion Transporting Polypeptide (SLCO) (D). Protein abundance in the final sub-database for each transporter family transporters as a percentage of the total abundance (pmol) of the region shown after performing a simulation with 2000 North European Caucasians.
### Table 1. The Sixteen Transporters Selected for Inclusion into the Meta-Analysis after exclusion criteria were applied, to Determine Region-Specific Transporter Expression in the ADAM and M-ADAM models.

| Protein          | Membrane Localisation | Functionality | Quantification (Relative/Absolute) | Notes                                                                 |
|------------------|------------------------|---------------|-----------------------------------|-----------------------------------------------------------------------|
| SLC10A2 (IBAT)   | Apical                 | Uptake        | Relative & Absolute               |                                                                       |
| SLC15A1 (PEPT1)  | Apical                 | Uptake        | Relative & Absolute               |                                                                       |
| SLC16A1 (MCT1)   | Apical                 | Uptake        | Relative                          |                                                                       |
| SLCO2B1 (OATP2B1)| Apical                 | Uptake        | Relative & Absolute               |                                                                       |
| SLC22A1 (OCT1)   | Apical                 | Uptake        | Relative & Absolute               |                                                                       |
| SLC22A3 (OCT3)   | Apical                 | Uptake        | Relative & Absolute               |                                                                       |
| SLC22A4 (OCTN1)  | Apical                 | Uptake        | Relative                          |                                                                       |
| ABCB1 (P-gp)     | Apical                 | Efflux        | Relative & Absolute               |                                                                       |
| ABCC2 (MRP2)     | Apical                 | Efflux        | Relative & Absolute               |                                                                       |
| ABCG2 (BCRP)     | Apical                 | Efflux        | Relative & Absolute               |                                                                       |
| SLC2A2 (GLUT2)   | Basolateral             | Uptake        | Relative                          |                                                                       |
| SLCO4C1 (OATP4C1)| Basolateral             | Uptake        | Relative                          |                                                                       |
| SLC51A/B (OST-α/β)| Basolateral            | Efflux        | Relative & Absolute               |                                                                       |
| ABCC1 (MRP1)     | Basolateral             | Efflux        | Relative                          |                                                                       |
| ABCC3 (MRP3)     | Basolateral             | Efflux        | Relative & Absolute               |                                                                       |
| ABCC4 (MRP4)     | Basolateral             | Efflux        | Relative                          |                                                                       |

*Note that M-ADAM model requires selection to enable activation of the basolateral membrane localised transporters; \(^b\) Relates to data available and collated in the final database (i.e., without...*
exclusion criteria applied, Figure 1) for a transporter; c a substantial meta-analysis for relative expression of P-gp, MRP2 and BCRP had already been performed and results are published (Harwood et al., 2013); d data collated distinctly for alpha (SLC51A) and beta (SLC51B) subunits, hence counted as individual transporters in Figure 1 count of transporter data collated.
Table 2 – The weighted mean, coefficient of variation and geometric mean of total membrane protein abundance of drug transporters in the proximal jejunum ‘Jejunum I’ obtained from meta-analysis of measurements in tissue of healthy Caucasian adults.

| Transporter | Mean | CV (%) | Geometric Mean | No. of Samples | No. of Studies | Heterogeneity | References |
|-------------|------|--------|----------------|----------------|---------------|--------------|------------|
| ABCB1 (P-gp) | 0.4  | 44     | 0.37           | 11             | 3             | 0.98         | No (Groer et al., 2013; Oswald et al., 2013; Drozdzik et al., 2014) |
| ABCC2 (MRP2) | 0.86 | 68     | 0.71           | 11             | 3             | 0.82         | No (Groer et al., 2013; Oswald et al., 2013; Drozdzik et al., 2014) |
| ABCC3 (MRP3) | 0.58 | 64     | 0.49           | 7              | 2             | N/A          | N/A (Groer et al., 2013; Drozdzik et al., 2014) |
| ABCG2 (BCRP) | 0.34 | 62     | 0.29           | 11             | 3             | 0.93         | No (Groer et al., 2013; Oswald et al., 2013; Drozdzik et al., 2014) |
| SLC10A2 (ASBT/IBAT) | 0.01 | 43⁵ | 0.01           | 6              | 1             | N/A          | N/A (Drozdzik et al., 2014) |
| SLC15A1 (PepT1) | 3.69 | 41     | 3.41           | 11             | 3             | 0.92         | N/A (Groer et al., 2013; Oswald et al., 2013; Drozdzik et al., 2014) |
| Protein     | Value (pmol/mg membrane protein) | n | S.D. | n/a | CV (%) |
|-------------|----------------------------------|---|------|-----|--------|
| SLCO2B1     | 0.4                              | 74| 0.32 | 11  | 0.57   |
| (OATP2B1)   |                                  |   |      | 3   |        |
|             | (Groer et al., 2013; Oswald et al., 2013; Drozdzik et al., 2014) |   |      | 0.57|        |
| SLC22A1     | 0.65                             | 49| 0.58 | 6   | 1      |
| (OCT1)      |                                  |   |      | 1   | N/A<sup>b</sup> |
|             | (Drozdzik et al., 2014)          |   |      | 1   | N/A<sup>b</sup> |
| SLC22A3     | 0.06                             | 74| 0.05 | 6   | 1      |
| (OCT3)      |                                  |   |      | 1   | N/A<sup>b</sup> |
|             | (Drozdzik et al., 2014)          |   |      | 1   | N/A<sup>b</sup> |
| SLC51A/B    | 0.47<sup>d</sup>                 | 99| 0.47 | 1   | 1      |
| (OST-α/β)   |                                  |   |      | 1   | N/A<sup>b</sup> |
|             | (Harwood, 2015)                  |   |      | 1   | N/A<sup>b</sup> |

<sup>a</sup> Values are given as picomoles per mg of total membrane protein; <sup>b</sup> heterogeneity reporting is Not Applicable (N/A) with only 1 or 2 studies, when considering subtracting the degree of freedom component (i.e. n minus 1 study); <sup>c</sup> The final CV value for Jejunum I SLC10A2 was from Hilgendorf et al., 2007, based on Jejunum mRNA data as it was determined that low abundance levels could give rise to an inflated inter-individual variability due to analytical imprecision at such low levels of expression; <sup>d</sup> for OST-α/β, the mean abundance is from a distal jejunum sample and is based on the alpha subunit data considered the rate limiting component for conferring OST-α/β activity (Sun et al., 2007), also only a single sample available so CV was from a combined analysis of Jejunum samples from relative and absolute data.
Table 3. Final region-specific abundances along the gastrointestinal tract normalized to the Jejunum I based on relative abundance quantification or by combining abundance data obtained from relative and absolute quantification methodology.

| Protein | ADAM model segment | Duodenum | Jejunum I<sup>c</sup> (fixed value of 1) | Jejunum II | Ileum I | Ileum II | Ileum III | Ileum IV | Colon |
|---------|--------------------|----------|------------------------------------------|------------|---------|---------|---------|---------|-------|
| ABCB1<sup>a,b</sup> | (P-gp) | 0.51 (31) | 1 (9) | 1.46 (8) | 1.50 (42) | 1.51 (42) | 1.52 (42) | 1.51 (42) | 0.57 (27) |
| ABCC1<sup>a</sup> | (MRP1) | 0.45 (37) | 1 (9) | 0.88 (9) | 0.86 (37) | 0.86 (37) | 0.89 (37) | 0.89 (37) | 0.93 (50) |
| ABCC2<sup>a,b</sup> | (MRP2) | 1.41 (71) | 1 (4) | 1 (4) | 0.60 (41) | 0.60 (41) | 0.60 (41) | 0.60 (41) | 0.02 (26) |

See Table 2 in (Harwood et al., 2013) for references (Fromm et al., 2000; Albermann et al., 2005; Zimmermann et al., 2005; Berggren et al., 2007; Blokzijl et al., 2007; Bourgine et al., 2012; Drozdzik et al., 2014).
| Gene        | Expression Level | Reference 1 | Reference 2 | Reference 3 | Reference 4 | Reference 5 | Reference 6 |
|------------|------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| ABCC3 (MRP3) | 2.15 (29)        | 1 (16)      | 0.89 (16)   | 1.54 (35)   | 1.60 (35)   | 1.60 (35)   | 5.95 (35)   |
| ABCC4 (MRP4) | 1.02 (15)        | 1 (6)       | 1.22 (6)    | 1.71 (19)   | 1.20 (19)   | 1.80 (19)   | 1.76 (19)   |
| ABCG2 (BCRP) | 0.47 (45)        | 1 (14)      | 1 (45)      | 0.59 (45)   | 0.59 (45)   | 0.59 (45)   | 0.13 (35)   |
| SLC2A2 (GLUT2) | 1 (15)          | 1 (0)      | 1 (0)       | 1 (0)       | 1 (0)       | 1 (0)       | 1 (0)       |
| SLC10A2 (ASBT/IBAT) | 16.49 (30)   | 1 (6)      | 4 (6)       | 98.44 (59)  | 109.18 (59) | 108.00 (59) | 1.10 (56)   |

See Table 2 in (Harwood et al., 2013) for references

(Zimmermann et al., 2005; Englund et al., 2006; Seithel et al., 2006; Bourgine et al., 2012; Groer et al., 2013; Drozdzik et al., 2014)

See Table 2 in (Harwood et al., 2013) for references

(Zimmermann et al., 2005; Bourgine et al., 2012; Drozdzik et al., 2014)

See Table 2 in (Harwood et al., 2013) for references

(Wilder-Smith et al., 2014)

(Hruz et al., 2006; Meier et al., 2007; Wojtal et al., 2009; Bourgine et al., 2012; Groer et al., 2013)
| Gene      | Expression (Ref) | Expression (Sample) |
|-----------|------------------|---------------------|
| SLC15A1 (PEPT1) | 0.94 (35)         | 1 (20)              |
|           | 1.06 (20)        | 1.23 (67)           |
|           | 1.23 (67)        | 1.24 (67)           |
|           | 1.23 (67)        | 1.24 (67)           |
|           | 0.03 (63)        |                     |
| SLC16A1a (MCT1) | 1.25 (17)         | 1 (13)              |
|           | 1 (13)           | 1.29 (20)           |
|           | 1.29 (20)        | 1.29 (20)           |
|           | 1.29 (20)        | 1.29 (20)           |
|           | 4.72 (26)        |                     |
| SLC02B1 (OATP2B1) | 0.73 (30)         | 1 (20)              |
|           | 0.94 (20)        | 1.28 (76)           |
|           | 1.28 (76)        | 1.28 (76)           |
|           | 1.28 (76)        | 1.06 (58)           |
| Gene       | Symbol | Value 1 (N) | Value 2 (N) | Value 3 (N) | Value 4 (N) | Value 5 (N) | Value 6 (N) |
|------------|--------|-------------|-------------|-------------|-------------|-------------|-------------|
| SLC4C1a    | OATP4C1| 1 (0)d      | 1 (0)d      | 1 (3)e      | 1 (3)e      | 1 (3)e      | 1 (3)e      |
| SLC22A1    | OCT1   | 1.03 (6)    | 1 (6)       | 0.87 (6)    | 1.29 (35)   | 1.29 (35)   | 1.30 (35)   |
| SLC22A3    | OCT3   | 1.19 (6)    | 1 (6)       | 1.11 (6)    | 1.08 (12)   | 1.08 (12)   | 1.23 (12)   |
| SLC22A4a   | OCTN1  | 0.46 (16)   | 1 (6)       | 0.63 (6)    | 0.78 (51)   | 0.78 (51)   | 0.80 (51)   |
| SLC51A/B   | OSTα/β | 0.56 (6)    | 1 (6)       | 1.89 (7)    | 1.08 (40)   | 1.08 (40)   | 0.93 (40)   |

References:
- Bourgine et al., 2012
- Wojtal et al., 2009; Bourgine et al., 2012; Groer et al., 2013; Drozdzik et al., 2014
- Girardin et al., 2012; Drozdzik et al., 2014
SLC51B<sup>f</sup> (OST-β) 0.6 (6) 1.19 (6) 1.16 (9) 1.01 (40) 1.01 (40) 1 (40) (41) 0.33 (6) (Renner et al., 2008; Drozdzik et al., 2014; Harwood, 2015)

<sup>a</sup> Where only relative abundance data was available; <sup>b</sup> Final values not updated from those previously reported (Harwood et al., 2013) as the region-specific absolute abundance values in Supplemental Table 3, were similar; <sup>c</sup> Jejunum I fixed to 1 as final value, however prior to normalisation a weighted mean relative expression is calculated across samples, with potential for CV generation, hence the sample number provided; <sup>d</sup> Where samples were not quantified within a given region a relative abundance value of 1 was assumed; <sup>e</sup> As there is no jejunum I values to normalize the ileum and colon abundances to, the relative expression for these segments was set to 1; <sup>f</sup> Used OST-α values from relative and absolute combined analysis - for completeness OST-β segmental relative abundance values are given at the bottom of Table 3
FIGURE 2

(A) Absolute Abundance (normalized to Jejunum 1) vs. Condition (D, J1, J2, I1, I2, I3, I4, C).

(B) Absolute Abundance (normalized to Jejunum 1) vs. Condition (D, J1, J2, I1, I2, I3, I4, C).

(C) Relative Expression (normalized to Jejunum 1) vs. Condition (D, J1, J2, I1, I2, I3, I4, C).

(D) Relative Expression (normalized to Jejunum 1) vs. Condition (D, J1, J2, I1, I2, I3, I4, C).
