



Inter-individual and inter-regional variations in enteric drug metabolizing enzyme activities: Results with cryopreserved human intestinal mucosal epithelia (CHIM) from the small intestines of 14 donors

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Abstract

We have previously reported successful isolation and cryopreservation of human intestinal mucosa (CHIM) with retention of viability and drug metabolizing enzyme activities. Here we report the results of the quantification of drug metabolizing enzyme activities in CHIM from different regions of the small intestines from 14 individual donors. CHIM were isolated from the duodenum, jejunum, and ileum of 10 individuals, and from 10 consecutive 12-inch segments starting from the pyloric sphincter of human small intestines from four additional individuals. P450 and non-P450 drug metabolizing enzyme activities (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A, UGT, SULT, FMO, MAO, AO, NAT1, and NAT2) were quantified via incubation with pathway-selective substrates. Quantifiable activities were observed for all pathways except for CYP2A6. Comparison of the duodenum, jejunum, and ileum in 10 donors shows jejunum had higher activities for CYP2C9, CYP3A, UGT, SULT, MAO, and NAT1. Further definition of regional variations with CHIM from ten 12-inch segments of the proximal small intestine shows that the segments immediately after the first 12-inch segment (duodenum) had the highest activity for most of the drug metabolizing enzymes but with substantial differences among the four donors. Our overall results demonstrate that there are substantial individual differences in drug metabolizing enzymes and that jejunum, especially the regions immediately after the duodenum, had the highest drug metabolizing enzyme activities.

KEYWORDS

CHIM, duodenum, enteric drug metabolism, human intestine, ileum, jejunum

Abbreviations: CERM™, Cryopreserved enterocytes recovery medium; HQM™, Hepatocyte/enterocyte incubation medium; CHIM, Cryopreserved human intestinal mucosa.

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1 | INTRODUCTION

Intestinal drug metabolism is known to play an important role in the bioavailability of orally administered drugs including CYP3A substrates, such as midazolam,¹⁻⁴ cyclosporin,⁵ lovastatin,⁶ and digoxin,⁷ and the UGT substrates, raloxifene^{8,9} and lamotrigine.¹⁰ Furthermore, enteric metabolism is known to play a key role in drug toxicity via the formation of toxic and reactive metabolites¹¹ and in efficacy via metabolic inactivation as well as prodrug activation.^{12,13} It is therefore important to evaluate the role of intestinal drug metabolism in the development of orally administered drugs to assess bioavailability, toxicity, and efficacy.

Upon entrance to the intestinal lumen, an orally administered drug travels from the proximal to distal regions of the small intestine where the drug is subjected to absorption and metabolism by the intestinal mucosal epithelium. Knowledge of regional differences in drug metabolizing enzyme activities of the small intestine can aid the assessment of enteric metabolism of an orally administered drug during its transit in the intestinal tract. An important parameter of bioavailability, the fraction of drug that escapes intestinal metabolism (Fg),¹⁴ for instance, is a function of the abundance of the drug metabolizing enzymes responsible for its metabolism in various regions of the small intestine.

Human hepatic drug metabolism has been extensively defined due to the availability of *in vitro* experimental systems. Primary cultured human hepatocytes, which, because of the intact plasma membrane, uninterrupted cellular organelles, and endogenous cofactors, are generally considered as the “gold standard” experimental system for *in vitro* evaluation of human hepatic drug properties^{15,16} over cell-free systems such as human liver postmitochondrial supernatant (S9), microsomes, and cDNA-derived liver microsomes. In contrast, until recently, *in vitro* evaluation of human enteric drug metabolism has been hampered by the lack of experimental models with adequate drug metabolizing enzyme activities. Human *in vitro* enteric systems such as Caco-2 and primary enterocyte cultures derived from crypt and stem cells are known to express drug metabolizing enzymes substantially lower than that in the human small intestine *in vivo*.¹⁷⁻²¹ To overcome this major obstacle in the evaluation of enteric drug metabolism, our laboratory evaluated an approach previously found successful with human *in vitro* hepatic models, namely isolation of metabolically competent cells from the organ of interest followed by immediate cryopreservation without culturing. Our investigations led to the development of *in vitro* enteric systems with robust drug metabolizing enzyme activities, namely cryopreserved human enterocytes,²²⁻²⁴ permeabilized cofactor-supplemented (MetMax™) cryopreserved human enterocytes,^{23,24} and cryopreserved human intestinal mucosal epithelium (CHIM).²⁵ These experimental models are now being applied toward the assessment of human enteric drug properties in drug development.²⁴ The current status of *in vitro* enteric systems for the evaluation of drug metabolism and drug interactions has been recently reviewed.²⁶

We report here the results of a study evaluating potential inter-individual and inter-regional differences in drug metabolizing

enzyme activities in the human small intestine. Activities of 15 drug metabolizing enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A, UGT, SULT, FMO, MAO, AO, and NAT2) were quantified in CHIM derived from the duodenum, jejunum, and ileum of 10 donors as well as from 10 consecutive 12-inch segments of the small intestines from four donors. The purpose of our study is to provide information to complement current knowledge on the distribution of drug metabolizing enzymes in the human small intestine,²⁷⁻²⁹ furthering our understanding of the role of the metabolic fate, drug interaction potential, pharmacology, and toxicology of orally administered drugs.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Acetaminophen, astemizole, bupropion, N-acetyl-p-aminobenzoic acid, 4-aminobenzoic acid, benzydamine hydrochloride, chlorzoxazone, coumarin, dextromethorphan hydrobromide, 7-Ethoxyresorufin, 7-Hydroxycoumarin β -D-glucuronide sodium salt, 7-Hydroxycoumarin sulfate potassium salt, 6 β -hydroxy testosterone, irinotecan, midazolam, phenacetin, resorufin, SN38, and sulfamethazine were purchased from Sigma Aldrich. Dextrorphan tartrate, diclofenac sodium salt, 4-hydroxydiclofenac, hydroxy bupropion, S-mephenytoin, 4-hydroxyquinoline, paclitaxel, and testosterone were purchased from Cayman Chemical. 7-hydroxycoumarin was purchased from Chem Service. Benzydamine N-oxide, kynuramine hydrobromide, and N-acetyl sulfamethazine were obtained from Santa Cruz Biotechnology. Carbazeran, 4-hydroxycarbazeran, 6-hydroxychlorzoxazone, 6 α -hydroxy paclitaxel, 1'-hydroxymidazolam, and 4-hydroxy-S-mephenytoin were obtained from Toronto Research Chemicals.

2.2 | Human intestine

Human small intestines from 14 individuals were evaluated in this study. The tissues were obtained from the International Institute for the Advancement of Medicine (IIAM) as tissues intended but not used for transplantation. The small intestines were collected and stored in University of Wisconsin solution³⁰ and shipped to our laboratory on wet ice with a cold ischemic time (time on ice from initial placement on ice to processing of the tissue for CHIM isolation) of less than 24 hours. The demographics of the 14 donors are presented in Table 1.

2.3 | Ethics statement

All human tissues from IIAM had explicit donor/immediate family approval and Institutional Review Board approval for use in research.

TABLE 1 Demographic information of the donors of the small intestines used in the preparation of CHIM for the study

DONOR	Lot number	Region	Gender	Race	Age (years)	BMI	BT	Cause of death	Disease	Alcohol	Tobacco	Substance
1	CHIM6001D	Duodenum	M	C	20	30.62	O	CVA, Anoxia	None reported	NO	NO	YES
	CHIM6003J	Jejunum										
	CHIM6005I	Ileum										
2	CHIM6006-I	Duodenum	M	C	20	26.33	A	Anoxia	None reported	YES	YES	NO
	CHIM6007-J	Jejunum										
	CHIM6008-D	Ileum										
3	CHIM6014J	Duodenum	M	C	35	24.83	A	Head Trauma	Thrush	OCCASIONAL	YES	YES
	CHIM6021I	Jejunum										
	CHIM6022D	Ileum										
4	CHIM6010J	Duodenum	M	C	59	37.88	A	Head Trauma	HTN	NO	NO	NO
	CHIM6015D	Jejunum										
	CHIM6016I	Ileum										
5	CHIM6017J	Duodenum	F	C	57	37.5	A	CVA, Anoxia	Ovarian cancer	YES	YES	YES
	CHIM6018D	Jejunum										
	CHIM6019I	Ileum										
6	CHIM6030D	Duodenum	M	C	64	29.52	A	CVA, Anoxia	HTN	NO	NO	NO
	CHIM6028J	Jejunum										
	CHIM6029I	Ileum										
7	CHIM6036-D	Duodenum	F	C	44	24.26	O	Drug Overdose	None reported	YES	YES	YES
	CHIM6034-J	Jejunum										
	CHIM6035-I	Ileum										
8	CHIM6043-D	Duodenum	M	C	35	23.38	A	Drug Overdose	None reported	YES	YES	YES
	CHIM6044-J	Jejunum										
	CHIM6045-I	Ileum										
9	CHIM6050-D	Duodenum	F	C	35	19.67	O	CVA/Stoke, ICH	Polycystic kidney disease	YES	YES	NO
	CHIM6048-J	Jejunum										
	CHIM6049-I	Ileum										
10	HE3061-D	Duodenum	M	H	58	30.27	O	CVA/Stoke, ICH	None reported	OCCASIONAL	YES	NO
	HE3064-J	Jejunum										
	HE3048-I	Ileum										
11	CHIM6023	A to J	F	C	49	32.17	A	CVA/Stoke, ICH	Diabetes	NO	NO	NO

(Continues)

TABLE 1 (Continued)

DONOR	Lot number	Region	Gender	Race	Age (years)	BMI	BT	Cause of death	Disease	Alcohol	Tobacco	Substance
12	CHIM6037	A to J	F	AMERICAN SAMOAN	59	33.42	A	CVA, Anoxia	None reported	NO	NO	NO
13	CHIM6038	A to J	M	C	38	21.52	O	Head Trauma	Hemochromatosis	YES	YES	YES
14	CHIM6039	A to J	M	AA	57	41.19	A	CVA/Stroke, ICH	HTN	YES	YES	NO

Abbreviations: AA, African American; BMI, body mass index; C, Caucasian; CVA, cardiovascular arrest; F, female; HTN, hypertension; M, male; Substance, substance of abuse.

2.4 | CHIM isolation and cryopreservation

A schematic of the isolation of CHIM from the various regions of the human small intestine is shown in Figure 1. CHIM were prepared from the duodenum, jejunum, and ileum from donors 1-10 and from 10 consecutive 12-inch segments spanning the duodenum (segment A), jejunum (segments B to I), and proximal ileum (segment J) of donors 11-14. Isolation of mucosa was performed via enzymatic digestion of the intestinal lumen using procedures established in our laboratory,²⁵ which are based on that previously reported for porcine intestines.^{31,32}

2.5 | Recovery of CHIM

CHIM vials were removed from liquid nitrogen storage and thawed in a 37°C water bath for approximately 2 minutes. The contents of each individual vial were decanted into a 50-mL conical tube containing Cryopreserved Enterocyte Recovery Medium (CERM™, In Vitro ADMET Laboratories) that was prewarmed in a 37°C water bath. The thawed CHIM were recovered by centrifugation at 100× g for 10 minutes at room temperature. After centrifugation, the supernatant was removed by decanting. A volume of 5 mL of Hepatocyte Incubation Medium (HQM) at 4°C was added to the intact pellet of enterocytes at the bottom of the conical tube followed by briskly pipetting five times with a P1000 micropipette to create an even suspension of the intestinal mucosal fragments.

2.6 | Incubation of CHIM with drug metabolizing enzyme substrates

Substrates, concentrations, and the metabolites quantified for the multiple drug metabolism pathways evaluated are shown in Table 2 for P450 isoforms and Table 3 for non-P450 drug metabolizing enzymes. Incubations of CHIM and metabolism substrates were performed in a cell culture incubator maintained at 37°C with a humidified atmosphere of 5% CO₂. A volume of 50 µL of drug metabolizing enzyme substrates at 2× of the final desired concentrations was added into the designated wells of 96-well plates (reaction plate). The reaction plate was placed in a cell culture incubator for 15 minutes to prewarm the substrate solutions to 37°C, followed by addition of CHIM at a volume of 50 µL per well to initiate the reaction. The reaction plates were then incubated at 37°C for 30 minutes. All incubations were performed in triplicate. Metabolism was terminated in each well by the addition of 200-µL acetonitrile containing 250 nM of the internal standard tolbutamide. The incubated samples were stored at -80°C for the subsequent LC/MS-MS analysis.

2.7 | Quantification of protein concentration

As CHIM consists of multiple cell aggregates, cellular contents were quantified as protein concentrations. Determination of protein concentration

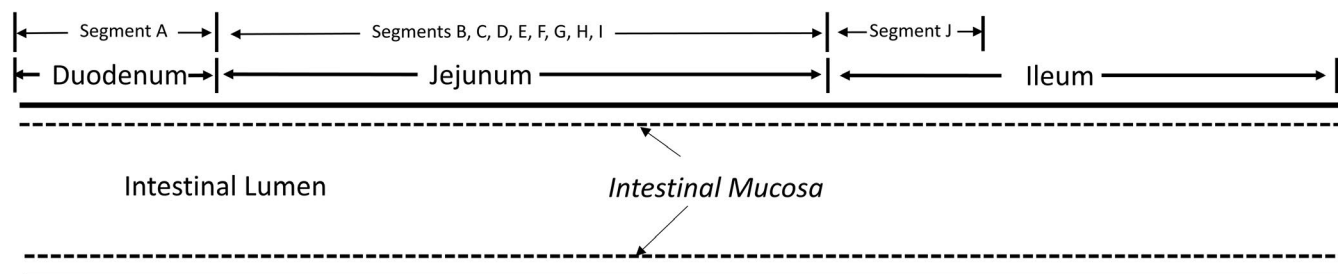


FIGURE 1 Schematic representation of the regions of the human small intestine evaluated in this study. For intestines from donors 1-10, CHIM were prepared from the duodenum, jejunum, and ileum. For donors 11-14, 10 consecutive 12-inch segments were prepared spanning the duodenum (segment A), jejunum (segments B to I), and the proximal regions of the ileum (segment J). CHIM was prepared by collagenase digestion of the intestinal lumen. The intestinal mucosa released from enzyme digestion was gently homogenized to multicellular aggregates followed by cryopreservation in liquid nitrogen as previously reported²⁵

TABLE 2 A summary of the P450 isoform-selective substrates and their respective metabolites quantified for CHIM

Isoform	Substrate	Substrate conc. (μmol/L)	Metabolites quantified
CYP1A1	7-Ethoxyresorufin	20	Resorufin
CYP1A2	Phenacetin	100	Acetaminophen
CYP2A6	Coumarin	50	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide
CYP2B6	Bupropion	500	Hydroxy bupropion
CYP2C8	Paclitaxel	20	6α-hydroxy paclitaxel
CYP2C9	Diclofenac	25	4-hydroxydiclofenac
CYP2C19	S-Mephenytoin	250	4-hydroxy S-Mephenytoin
CYP2D6	Dextromethorphan	15	Dextrorphan
CYP2E1	Chlorzoxazone	250	6-hydroxy chlorzoxazone
CYP2J2	Astemizole	50	O-Demethyl Astemizole
CYP3A-1	Midazolam	20	1'-hydroxymidazolam
CYP3A-2	Testosterone	200	6β-hydroxy testosterone

TABLE 3 A summary of the non-P450 pathway-selective substrates and their respective metabolites quantified in CHIM

DME pathway	Substrate	Substrate conc. (μmol/L)	Metabolites quantified
UGT	7-Hydroxycoumarin	100	7-Hydroxycoumarin Glucuronide
SULT	7-Hydroxycoumarin	100	7-Hydroxycoumarin Sulfate
FMO	Benzydamine HCl	250	Benzydamine-N-Oxide
MAO	Kynuramine HBr	160	4-hydroxyquinoline
AO	Carbazeran	20	4-Hydroxycarbazeran
NAT1	4-Aminobenzoic Acid	200	N-Acetyl-p-aminobenzoic acid
NAT2	Sulfamethazine	100	N-Acetyl-sulfamethazine
CES2	Irinotecan	50	SN38

was performed using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific) per instructions specified by the manufacturer.

2.8 | LC/MS/MS analysis

Quantification of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A (midazolam 1'-hydroxylation

(CYP3A-1), testosterone 6β-hydroxylation (CYP3A-2), UGT, SULT, FMO, MAO, AO, and NAT2 metabolites was performed with an API 5000 mass spectrometer with an electrospray ionization source (AB SCIEX) connected to Waters Acquity UPLC (Waters Corporation) using LC/MS/MS MRM mode, monitoring the mass transitions (parent to daughter ion) as previously described.²⁵ Data acquisition and data procession were performed with the software Analyst 1.6.2 (AB SCIEX). Metabolite formation was quantified based on metabolite/

internal standard peak ratio using standard curves with standard curve samples analyzed before, after, as well as interspersed within the experimental samples.

2.9 | Data analysis

2.9.1 | Specific activity

Specific activity (pmol/min/mg protein) of each drug metabolizing enzyme pathway was determined by dividing the total metabolite formed by the incubation time and normalized to protein contents.

2.9.2 | Relative regional activity

Drug metabolizing enzyme activities of the various regions of the human intestines were also expressed as relative regional activity for statistical evaluation of regional differences using the following equation:

$$\text{Relative regional activity} = \frac{\text{Specific activity (segment)}}{\text{Average specific activity (all segments)}}$$

where specific activity (segment) is that for each individual segment (duodenum, jejunum, or ileum; each of the A-J segments) of a specific donor, while average activity (all segments) is the mean activity of the various regions (duodenum, jejunum, and ileum or segments A to J) of the same donor.

2.9.3 | Statistical analysis

Data are presented as mean and standard errors of triplicate incubations derived using the Microsoft Excel 6.0 software and the GraphPad Prism software. Statistical analysis comparing activities of the different regions of the small intestines was performed by Tukey's multiple comparison test using the GraphPad Prism 8 software. Probability values (*P*) equal or lower than .05 are considered statistically significant.

3 | RESULTS

3.1 | Drug metabolizing enzyme activities in CHIM from duodenum, jejunum, and ileum of 10 donors

3.1.1 | Comparison of drug metabolizing enzyme pathways

The mean activities of the drug metabolizing enzyme pathways evaluated in CHIM prepared from the duodenum, jejunum, and

ileum of 10 donors are shown in Table 4. MAO was found to be the most active pathway with mean specific activities (pmol/min/mg protein) of 1582.95 ± 328.79 , 2589 ± 662.74 , and 1715 ± 457.95 for duodenum, jejunum, and ileum, respectively. Of all the P450 isoforms, CYP3A-2 activity (testosterone β -hydroxylation) is the most active, with specific activities of 112.90 ± 65.29 (duodenum), 199.40 ± 80.40 (jejunum), and 71.09 ± 33.86 (ileum). AO was the least active drug metabolizing enzyme that was quantifiable, with specific activities of 0.07 ± 0.02 (duodenum), 0.06 ± 0.01 (jejunum), and 0.13 ± 0.04 (ileum). CYP2A6 activity was not quantifiable (data not shown).

3.1.2 | Relative expression of enteric drug metabolizing enzymes

A pie chart illustrating the relative expression of the various drug metabolizing enzymes in the duodenum based on the mean activity of the 10 donors (tabulated in Tables 3 and 4) is shown in Figure 2. MAO represented the most abundant of all pathways. CYP3A was the most abundant of all P450 isoforms. Similar relative expression was observed for the jejunum and ileum (pie charts not shown).

3.1.3 | Individual variations

Drug metabolizing enzyme activities for each of the 10 donors are shown in Figure 3 (P450 isoforms) and Figure 4 (non-P450 pathways). Large individual variations, with activities differed by over two orders of magnitude, are shown for all P450 isoforms (except CYP1A2 and CYP2J2), UGT, SULT, and NAT1. FMO and MAO had the lowest individual variations, with specific activities within the same order of magnitude for the duodenum, jejunum, and ileum of the 10 donors.

3.1.4 | Regional difference

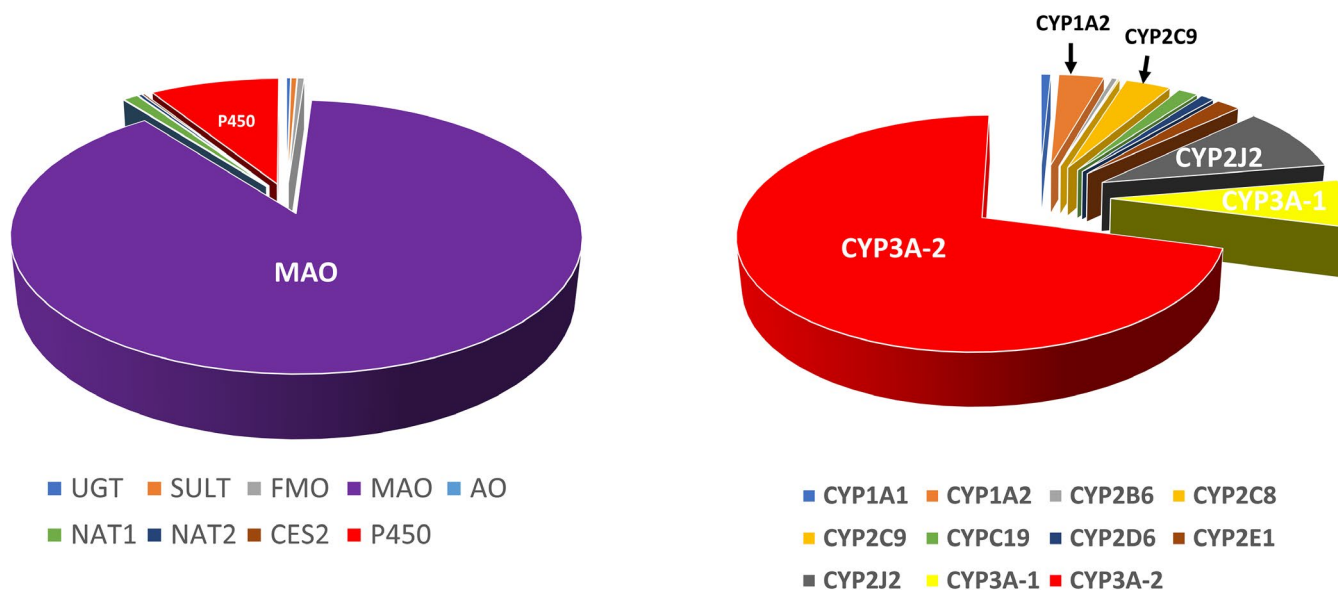
To minimize the contribution of individual variation in the evaluation of regional variations, activity from each region of the small intestine was expressed as "relative regional activity" by normalization of the activity of each of the three regions to the average activity of the three regions of each donor. Results with relative activity demonstrate statistically significant higher relative activities for jejunum than duodenum and/or ileum for the P450 isoforms CYP2C9, 3A-1, and 3A-2 (Figure 5), and for the non-P450 pathways UGT, SULT, MAO, NAT1, and NAT2 (Figure 6). A comparison of the CYP3A-1 activity (midazolam 1'-hydroxylation) in CHIM from the duodenum, jejunum, and ileum in each of the 10 donors is shown in Figure 7, with jejunum found to have statistically significant higher activities than duodenum and/or ileum in seven of the 10 donors.

TABLE 4 P450 (top) and non-P450 (bottom) drug metabolizing enzyme specific activities in CHIM from the duodenum, jejunum, and ileum of the small intestines (Mean and standard errors (sem) of 10 donors)

		Specific activity (pmol/min/mg protein)										
Region	Pathway	CYP1A1	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP2J2	CYP3A-1	CYP3A-2
Duodenum	Mean	1.21	5.72	0.71	0.19	5.75	2.53	1.68	3.07	14.68	10.77	112.90
	SEM	0.23	1.54	0.20	0.04	3.37	1.19	1.22	2.15	4.27	6.77	65.29
Jejunum	Mean	1.22	5.71	0.86	0.25	9.11	4.45	0.80	1.08	15.20	13.84	199.40
	SEM	0.38	1.25	0.25	0.07	2.92	1.77	0.37	0.44	5.69	3.92	80.40
Ileum	Mean	1.23	7.26	1.03	0.19	3.87	1.15	0.55	3.39	12.16	6.71	71.09
	SEM	0.29	1.68	0.34	0.07	1.65	0.45	0.26	2.07	3.81	2.66	33.86

		Specific activity (pmol/min/mg protein)							
Region	Pathway	UGT	SULT	FMO	MAO	AO	NAT1	NAT2	CES2
Duodenum	Mean	4.81	7.03	8.26	1582.95	0.07	17.95	5.00	3.13
	SEM	1.21	3.79	0.87	328.79	0.02	5.55	1.07	0.68
Jejunum	Mean	16.02	12.97	10.59	2589.98	0.06	70.51	7.95	2.96
	SEM	4.90	4.75	1.26	662.74	0.01	30.57	1.75	0.56
Ileum	Mean	10.98	10.36	9.10	1715.12	0.13	22.88	4.36	2.97
	SEM	4.91	5.38	1.26	457.95	0.04	7.86	0.75	0.71

Abbreviation: SEM, standard error of the mean.

**FIGURE 2** Pie charts depicting the relative contribution of the various drug metabolizing enzyme pathways in enteric drug metabolism. The pie charts were constructed based on activities quantified in CHIM from the duodenum. The distribution is similar for the jejunum and ileum. Results are from the mean specific activity of 10 donors. The relative contribution of all pathways (left) and for the individual isoforms for P450 pathways (right) are shown

3.2 | Drug metabolizing enzyme activities of CHIM from 10 consecutive 12-inch sections of the small intestines of four donors

3.2.1 | Comparison of drug metabolizing enzyme pathways

The mean activities of the drug metabolizing enzyme pathways in CHIM from the 10 consecutive 12-inch segments from

the four donors are shown in Table 5 (P450 isoforms) and Table 6 (non-P450 pathways). As observed in the duodenum, jejunum, and ileum, MAO was found to be the most active pathway, with mean specific activities (pmol/min/mg protein) ranged from 2991.06 ± 1564.21 (E) to $14\,429.44 \pm 473.95$ (segment A) (Table 6). CYP3A activity based on testosterone β -hydroxylation (CYP3A-2) was the most active, with specific activities ranged from 73.66 ± 22.65 (segment H) to 533.65 ± 230.85 (segment B). AO was the least active drug

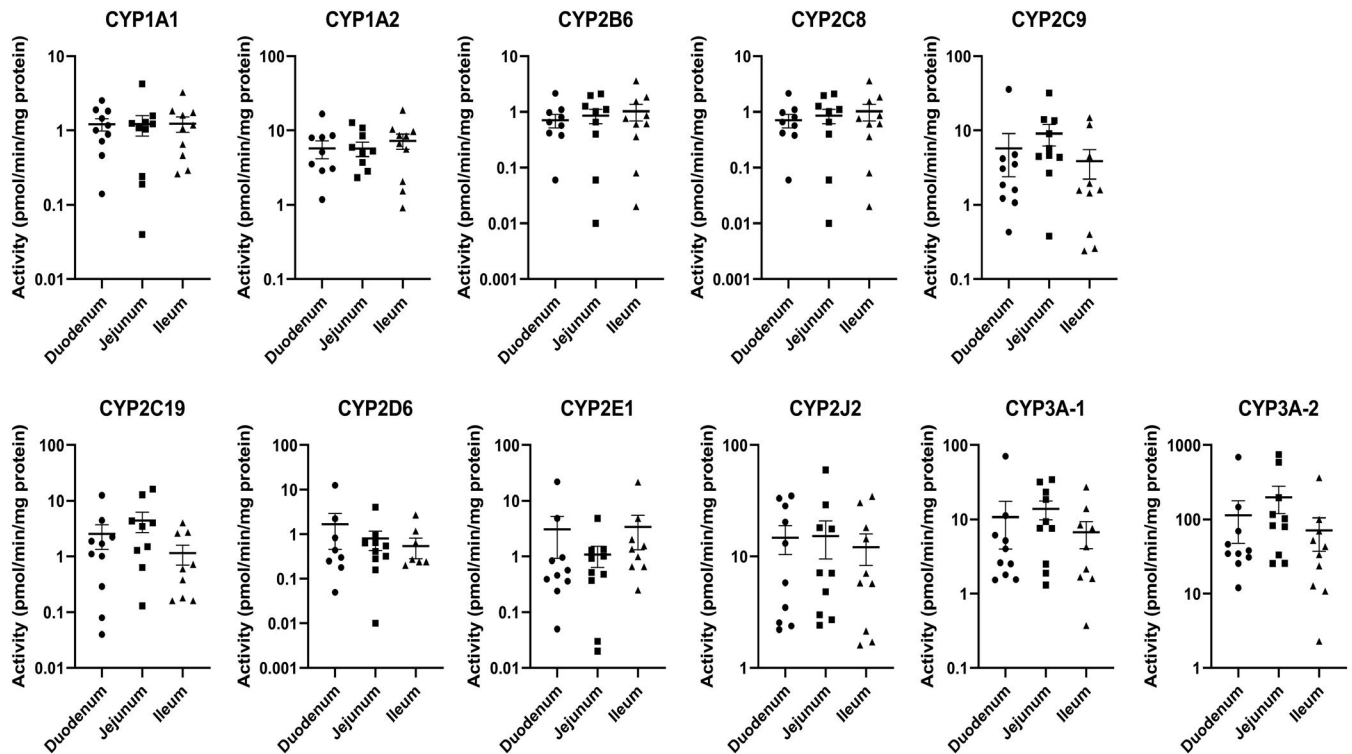


FIGURE 3 P450 isoform-selective drug metabolizing enzyme activities in CHIM from the duodenum, jejunum, and ileum of the small intestines from 10 individual donors quantified using isoform-selective pathways. Each symbol represents activity from CHIM isolated from each of the regions from a single donor. The horizontal bars represent the mean and standard error values of the 10 donors. CYP3A-1 and CYP3A-2 represent midazolam 1'-hydroxylation and testosterone 6- β hydroxylation activities, respectively. No statistically significant differences were observed among activities from duodenum, jejunum, and ileum

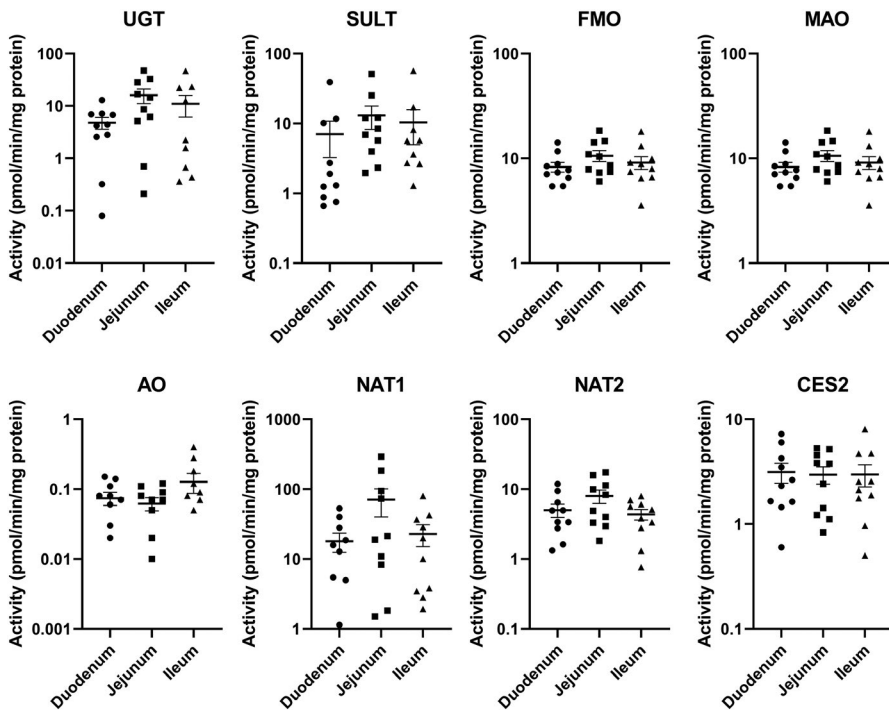


FIGURE 4 Non-P450 drug metabolizing enzyme activities in CHIM from the duodenum, jejunum, and ileum of the small intestines from 10 individual donors. Each symbol represents activity from CHIM isolated from each of the regions from a single donor. The horizontal bars represent the mean and standard error values of the 10 donors. No statistically significant differences were observed among activities from duodenum, jejunum, and ileum

metabolizing enzyme that was quantifiable, with specific activities of 0.06 ± 0.01 (segment J) to 0.71 ± 0.59 (segment B). As in the duodenum, jejunum, and ileum, CYP2A6 activity was not quantifiable (data not shown).

3.2.2 | Regional variations

Drug metabolizing enzyme activities for segments A-J in each of the four donors are shown in Figures 8 to 11. Plots of relative

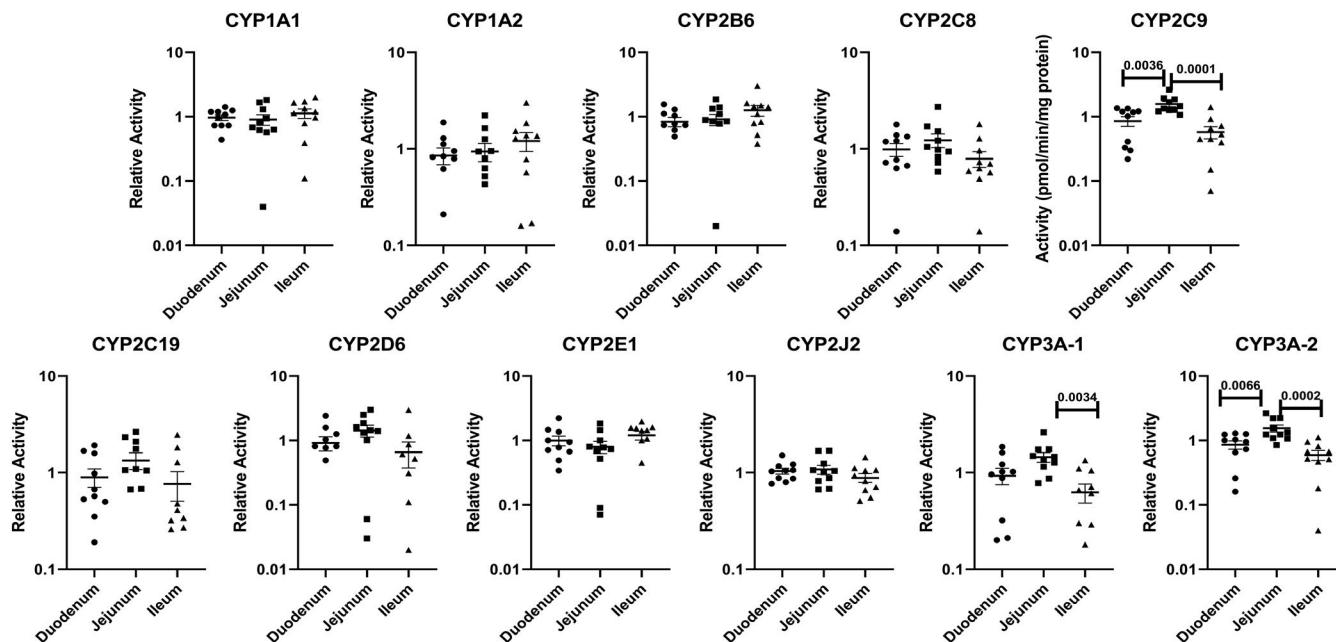
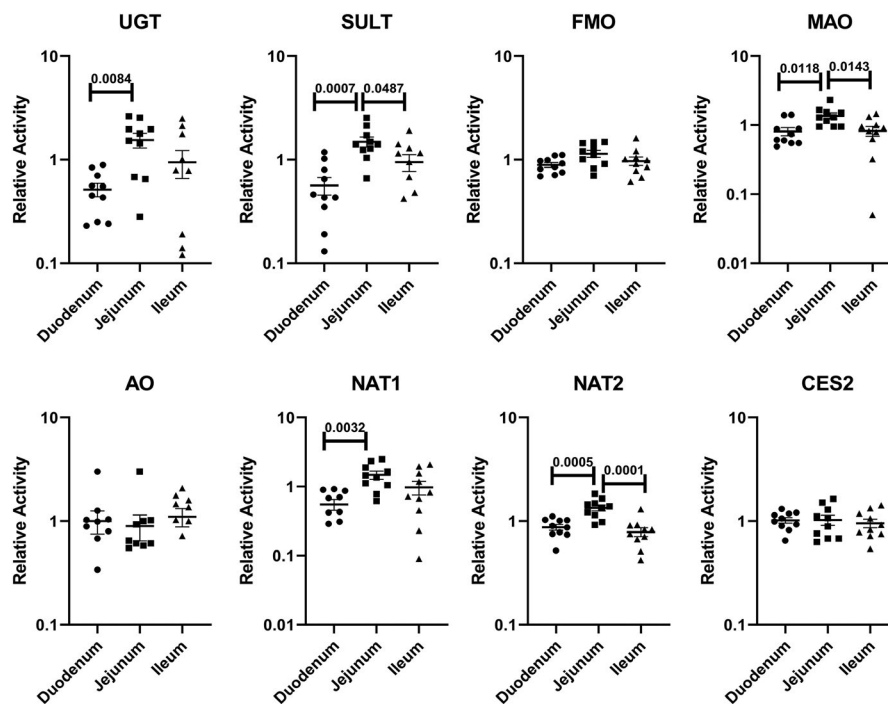


FIGURE 5 Relative activities for P450 isoforms in CHIM from the duodenum, jejunum, and ileum of the small intestines from 10 individual donors. The relative activities were calculated as a ratio of the specific activity of each of the three regions (duodenum, jejunum, and ileum) to the average specific activity of all three regions for each donor. Each symbol represents relative activity of CHIM from each of the regions from a single donor. The horizontal bars represent the mean and standard error values of the 10 donors. The probability values are indicated where statistically significant differences ($P < .05$) were observed

FIGURE 6 Relative activities of non-P450 drug metabolizing enzymes in CHIM from the duodenum, jejunum, and ileum of the small intestines from 10 individual donors. Relative activity was calculated as a ratio of the specific activity of each of the three regions (duodenum, jejunum, and ileum) to the average specific activity of all three regions for each donor. Each symbol represents relative activity of CHIM from each of the regions from a single donor. The horizontal bars represent the mean and standard error values of the 10 donors. The probability values are indicated where statistically significant differences ($P < .05$) were observed



regional activity values (mean of the four donors) for each of the pathways are shown in Figure 12. The results show that the proximal regions of the jejunum in general have the higher activities.

4 | DISCUSSION

We have recently reported successful preparation of CHIM with retention of robust drug metabolizing enzyme activities.²⁵ This

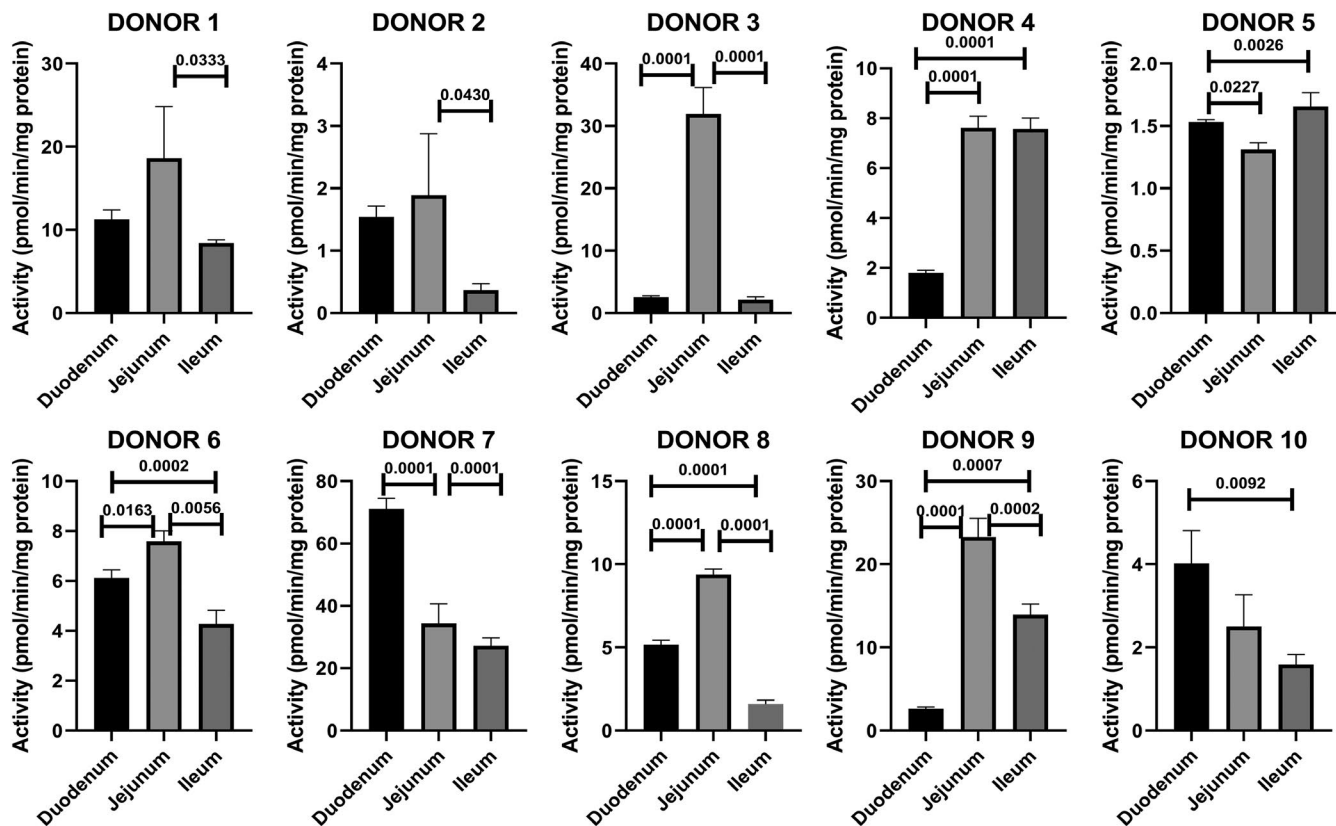


FIGURE 7 CYP3A activity quantified as midazolam 1'-hydroxylation (CYP3A-1) in CHIM derived from the duodenum, jejunum, and ileum of donors 1 to 10. The probability values of regions that are statistically different ($P < .05$) from other regions are shown

novel enteric system is being applied in our laboratory to investigate various aspects of enteric drug metabolism. This report represents our endeavor to evaluate inter-individual and inter-regional variations in enteric drug metabolism. We evaluated P450 (CYPs 1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2J2, and 3A) and non-P450 (UGT, SULT, MAO, FMO, AO, and CES) activities in CHIM isolated from the duodenum, jejunum, and ileum of 10 donors using pathway-selective substrates, with two substrates used for CYP3A, namely midazolam and testosterone, which are known to occupy different regions of the active site.³³ To further define inter-regional variations, we also evaluated CHIM from ten 12-inch segments of the first 10 feet of the small intestine (segments A to J) from four additional donors, with the first segment (A) representing the duodenum, and the second to ninth segments (B to I) representing the proximal to distal regions of the jejunum, and the 10th segment (J) representing the proximal region of the ileum. Our results are intended to be complementary to that previously reported by others on enteric drug metabolizing enzymes based on gene expression and proteomics,³⁴⁻³⁷ and drug metabolizing enzyme activity based on human intestinal microsomes,^{27,28,35,38-43} freshly isolated human small intestinal slices,⁴⁴ as well as a recent report on the activity of various UGT isoforms in CHIM isolated from various regions of the human small intestine.³⁷

A major conclusion from our study is that all regions of the small intestine have similar composition of drug metabolizing enzymes.

CHIM from the various regions of the human small intestines from the 14 donors were found to be active in the drug metabolizing enzyme pathways evaluated except for CYP2A6. Among the active enzyme pathways, MAO was found to have the highest specific activity, and AO the lowest. CYP3A was the most active P450 isoform (especially for testosterone 6β -hydroxylation), a result similar to that previously reported²⁷ based on Western blotting analysis of microsomes prepared from mucosal scrapings from the duodenal/jejunal portion of 31 human donors. The composition of the various drug metabolizing enzyme pathways of the duodenum is shown in Figure 2 which are representative of that for all other segments of the human small intestine. In this study, the MAO activity evaluated based on the metabolism of kynuramine to 4-hydroxyquinoline is likely a function of MAO-A which is known to be extensively expressed in the human intestine.⁴⁵⁻⁴⁷ The results suggest that substrates of MAO and CYP3A are likely to be extensively metabolized by the intestine upon oral administration. While the role of enteric CYP3A in bioavailability of its substrates has been well established,⁴⁸⁻⁵¹ much less information is available for MAO. It is interesting to note that MAO is a drug target to treat various psychological disorders. Oral administration of MAO inhibitor drugs, therefore, is likely to increase Fg of orally administered drugs and food-associated chemicals that are MAO substrates. Patients taking MAO inhibitors, for instance, are advised to avoid foods rich in tyramine, a MAO substrate, to prevent the onset of tyramine-induced hypertension.^{52,53}

TABLE 5 P450 isoform-specific activities in CHIM derived from 10 consecutive 12-inch segments of the small intestines of four donors. Segment A was isolated from the 12-inch segment proximal to the pyloric sphincter, while all other segments were 12-inch segments distal to segment A

DME	Specific activity (pmol/min/mg protein)										
	A	B	C	D	E	F	G	H	I	J	
CYP1A1	Mean	2.08	1.60	1.44	0.99	1.21	1.46	1.59	1.44	1.28	1.37
	SEM	0.23	0.28	0.27	0.27	0.33	0.30	0.16	0.26	0.22	0.17
CYP1A2	Mean	12.32	8.35	17.74	13.60	6.54	6.91	4.14	10.15	9.91	13.84
	SEM	5.19	3.83	12.85	7.73	2.03	1.81	0.68	6.61	4.68	7.37
CYP2B6	Mean	0.95	1.02	1.49	1.54	1.86	1.57	1.67	1.76	1.33	1.94
	SEM	0.37	0.23	0.68	0.75	1.08	0.83	1.05	1.20	0.64	1.08
CYP2C8	Mean	0.23	0.38	0.63	0.79	0.37	0.37	0.54	0.44	0.46	0.41
	SEM	0.09	0.22	0.27	0.39	0.23	0.21	0.18	0.21	0.20	0.20
CYP2C9	Mean	7.24	13.50	12.26	11.30	9.47	8.78	8.13	6.39	6.04	6.00
	SEM	1.69	4.25	4.44	6.37	3.78	2.74	3.01	2.01	1.58	2.47
CYP2C19	Mean	3.26	6.33	4.75	4.86	3.68	3.77	1.82	1.19	1.44	1.48
	SEM	1.79	3.13	2.45	3.60	1.90	1.90	0.67	0.31	0.66	0.47
CYP2D6	Mean	0.42	1.29	1.36	1.15	1.15	1.30	1.28	1.26	0.97	1.07
	SEM	0.21	0.59	0.95	0.87	0.85	1.02	1.01	1.05	0.76	0.93
CYP2E1	Mean	0.73	0.82	1.18	0.89	1.63	1.60	1.07	0.97	1.56	1.49
	SEM	0.27	0.23	0.38	0.22	0.21	0.21	0.44	0.39	0.27	0.21
CYP2J2	Mean	13.79	14.30	33.48	37.89	40.52	42.23	26.02	27.34	71.76	88.12
	SEM	3.96	4.04	7.53	9.55	15.94	13.28	7.82	7.20	39.44	57.24
CYP3A-1	Mean	9.70	27.87	20.25	24.69	18.12	14.71	10.54	6.72	7.14	10.89
	SEM	1.58	13.88	9.44	18.31	8.67	6.94	3.52	1.36	1.83	4.01
CYP3A-2	Mean	235.80	533.65	376.89	303.98	359.48	297.98	182.85	155.63	206.16	301.18
	SEM	106.46	230.85	162.82	150.88	158.01	116.13	87.63	83.52	131.30	199.13

Our results show that all regions of the human small intestine have the capacity to metabolize orally administered substrates of CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A, UGT, SULT, MAO, FMO, AO, CES2, NAT1, and NAT2 but not that for CYP2A6. The lack of quantifiable enteric CYP2A6 activity confirms that reported by others with small intestines from cynomolgus monkeys⁵⁴ and mRNA expression in human small intestines.⁵⁵ The lack of CYP2A6 in the human small intestine is interesting as it is known to be expressed in the respiratory tract⁵⁶ and esophageal mucosa⁵⁷ in addition to the liver. One interesting implication of this confirmation of the lack of CYP2A6 activity in the human small intestine is that the reported correlation between CYP2A6 polymorphism and nicotine addiction⁵⁸⁻⁶⁰ is not likely to be an enteric phenomenon, and that enteric nicotine metabolism is likely due to activities of other P450 isoforms such as CYP2B6.⁶¹

Most investigations on individual differences in drug metabolism, a major contributing factor to inter-individual differences in drug efficacy and toxicity, are focused on liver metabolism.⁶²⁻⁷² Our results with CHIM from the duodenum, jejunum, and ileum from 10 donors show that P450 isoforms, UGT, SULT, and NAT1, demonstrated relatively large inter-individual variations for all

three regions of the small intestine, with specific activities differing by more than three orders of magnitude. FMO, MAO, NAT2, and CES2 had lower inter-individual differences, with the activities from the various donors falling within two orders of magnitude. Of all the pathways, FMO and MAO appear to have the lowest inter-individual variations. The distribution of activities clusters around the mean except for CYP2J2 where the activities from the 10 donors were apparently separated into two distinct groups with activities above and below the mean value. This phenomenon may be related to a chance collection of donors with allelic variants of the human CYP2J2 gene resulting in reduced catalytic function,⁷³⁻⁷⁶ which needs to be further confirmed by genotyping. Our results suggest that inter-individual differences in enteric drug metabolism may contribute to variations in drug bioavailability, toxicity, and efficacy like that attributed to hepatic drug metabolism.

A second objective of our investigation is to evaluate inter-regional differences in enteric drug metabolism. A unique feature of the small intestine is that metabolism of an absorbed drug mainly occurs in the enterocytes that the drug enters and exits, with minimal involvement by enterocytes in the rest of the intestine. This phenomenon is different from that in the liver, where the

TABLE 6 Specific activity of non-P450 drug metabolizing enzymes in CHIM derived from 10 consecutive 12-inch segments of the small intestines of four donors. Segment A was isolated from the 12-inch segment proximal to the pyloric sphincter, while all other segments were 12-inch segments distal to segment A

DME	Specific activity (pmol/min/mg protein)										
	A	B	C	D	E	F	G	H	I	J	
UGT	Mean	11.14	16.08	19.53	24.83	16.69	10.18	17.11	7.09	9.49	16.38
	SEM	5.20	7.28	10.29	16.22	10.73	4.26	10.52	3.85	3.85	9.01
SULT	Mean	2.79	4.09	4.21	3.89	3.71	3.31	4.26	3.77	3.21	3.50
	SEM	0.92	1.24	1.84	2.26	1.71	1.44	2.10	1.70	0.71	1.58
FMO	Mean	31.72	45.13	47.15	56.42	62.63	71.57	91.50	91.83	86.55	137.22
	SEM	20.04	29.36	31.78	39.89	45.18	54.81	79.44	78.53	68.38	117.79
MAO	Mean	1429.44	2400.50	2332.02	1821.18	2991.06	2327.78	2890.56	2882.78	2077.89	1974.44
	SEM	473.95	1420.33	1241.22	1163.93	1564.21	663.93	1306.51	1214.40	823.31	1059.52
AO	Mean	0.33	0.55	0.12	0.11	0.11	0.11	0.20	0.09	0.14	0.09
	SEM	0.21	0.45	0.02	0.03	0.02	0.02	0.11	0.02	0.02	0.03
NAT1	Mean	74.96	118.16	88.24	71.71	36.74	38.83	133.92	89.16	42.92	52.46
	SEM	36.86	57.35	41.08	36.79	13.29	13.69	108.59	65.09	22.03	33.53
NAT2	Mean	15.37	18.56	14.00	14.06	13.64	13.02	16.52	12.71	10.24	10.80
	SEM	4.61	4.13	2.52	3.53	4.26	4.53	8.32	4.70	3.07	4.40
CES2	Mean	3.91	4.24	4.88	3.55	4.63	4.15	5.18	3.27	3.79	3.72
	SEM	1.41	1.58	1.72	1.68	2.21	1.73	1.88	1.05	1.32	1.48

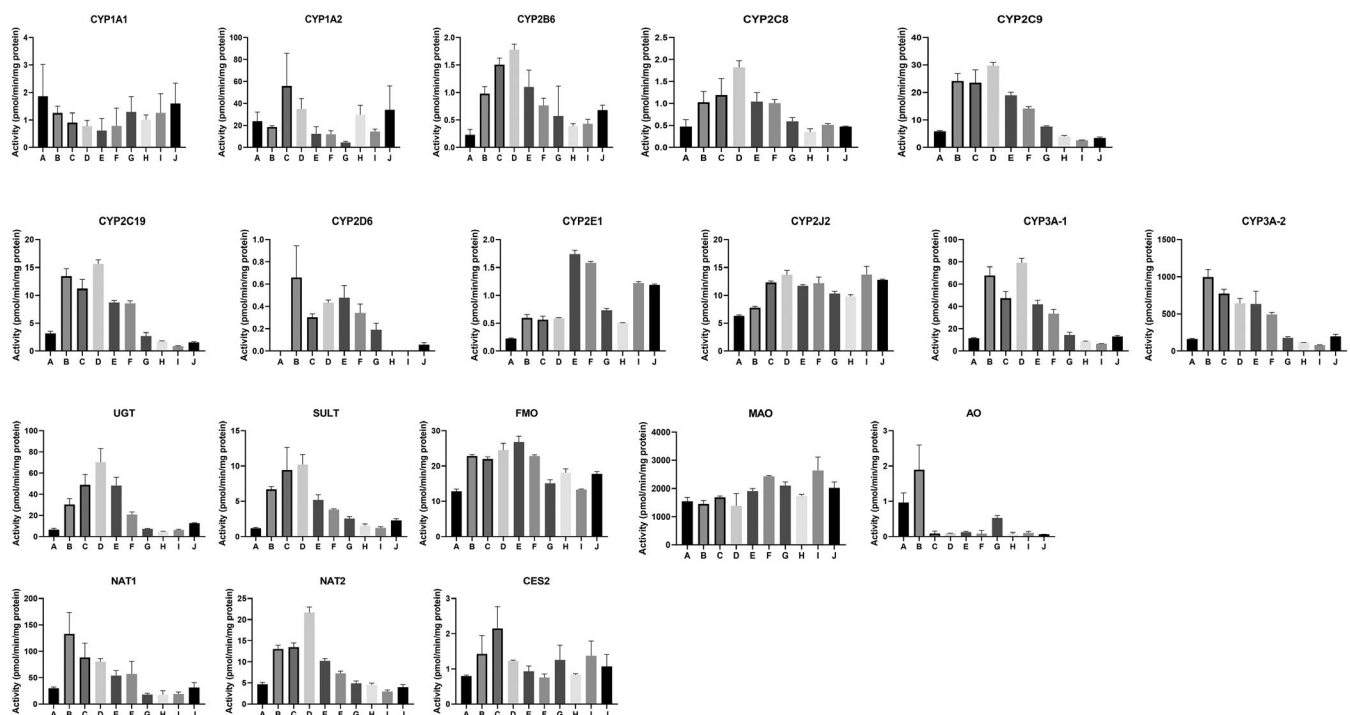


FIGURE 8 Drug metabolizing enzyme activities of CHIM derived from 10 consecutive 12-inch segments of the small intestine of Donor 11 (49 y.o. Caucasian Female)

hepatocytes of the entire liver would participate in the metabolism of a drug that enters the liver. A thorough understanding of the distribution of drug metabolizing enzyme, in combination with other key parameters such as permeability, solubility, and gastric emptying time, will aid the assessment of the extent of enteric

metabolism of an orally administered drug during its transit in the organ.

To minimize the confounding effects of individual differences in specific activities on the evaluation of potential regional differences, the data were expressed as “relative regional activity”,

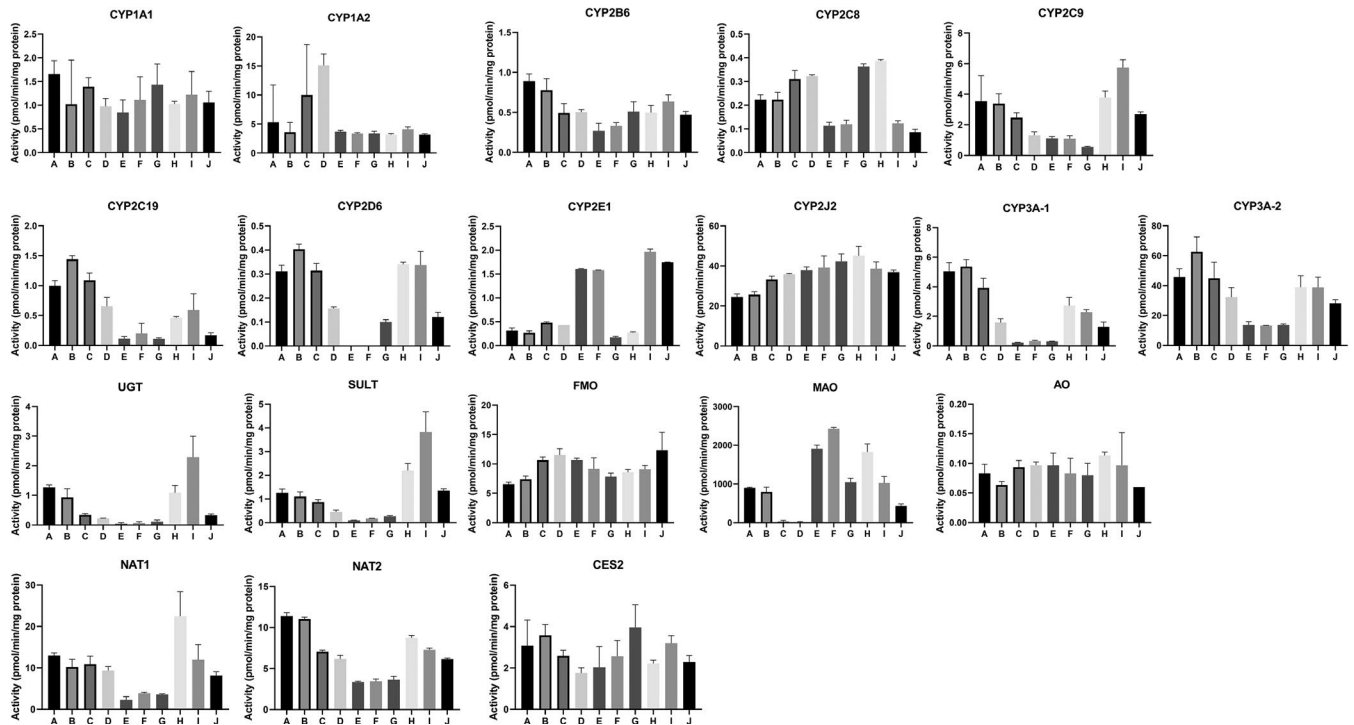


FIGURE 9 Drug metabolizing enzyme activities of CHIM derived from 10 consecutive 12-inch segments of the small intestine of Donor 12 (59 y.o. Samoan Female)

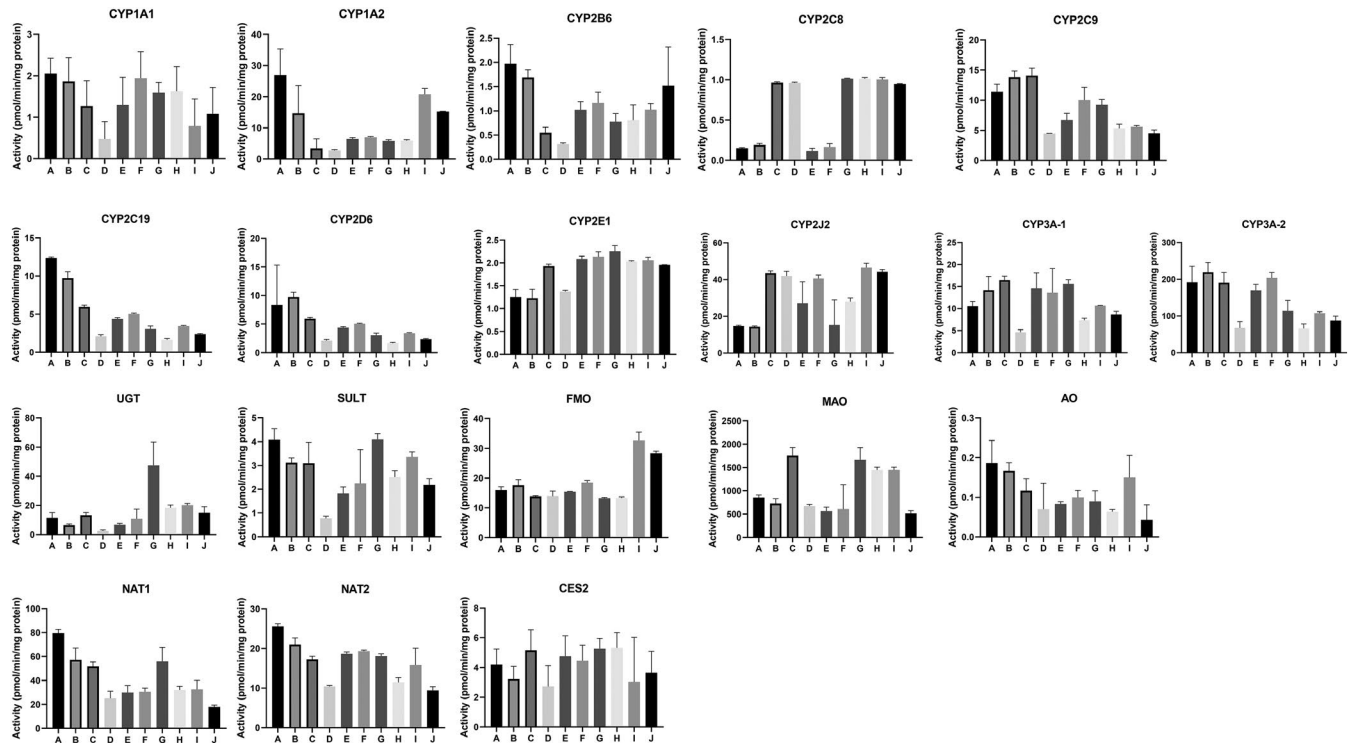


FIGURE 10 Drug metabolizing enzyme activities of CHIM derived from 10 consecutive 12-inch segments of the small intestine of Donor 13 (38 y.o. Caucasian Male)

calculated as a ratio of the activity of each segment to the average of all segments (duodenum, jejunum, and ileum), followed by statistical evaluation of regional differences with data from the 10 donors. Using this approach, the jejunum was found to have the

highest overall relative activity of CYP2C9, CYP3A-1, CYP3A-2, UGT, SULT, NAT1, and NAT2. Results with individual donors substantiate the “relative regional activity” approach, with CYP3A activity found to be significantly higher than duodenum and/or ileum

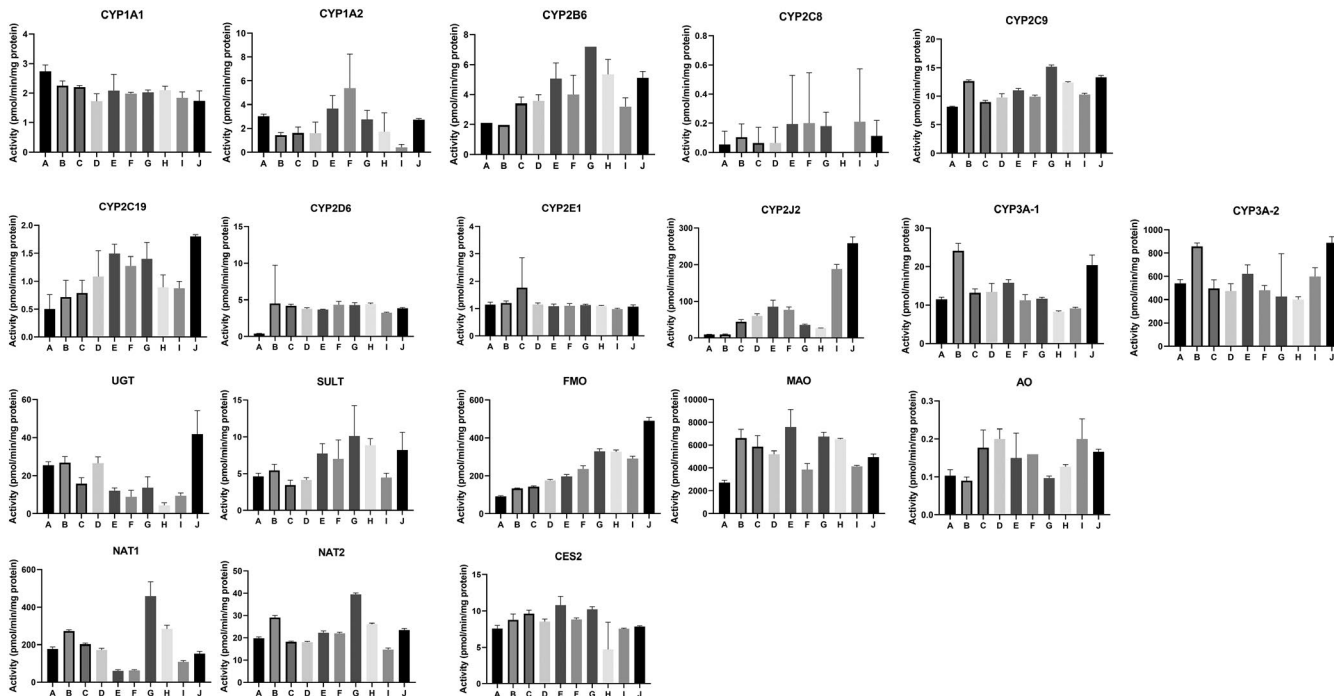


FIGURE 11 Drug metabolizing enzyme activities of CHIM derived from 10 consecutive 12-inch segments of the small intestine of Donor 14 (57 y.o. African American Male)

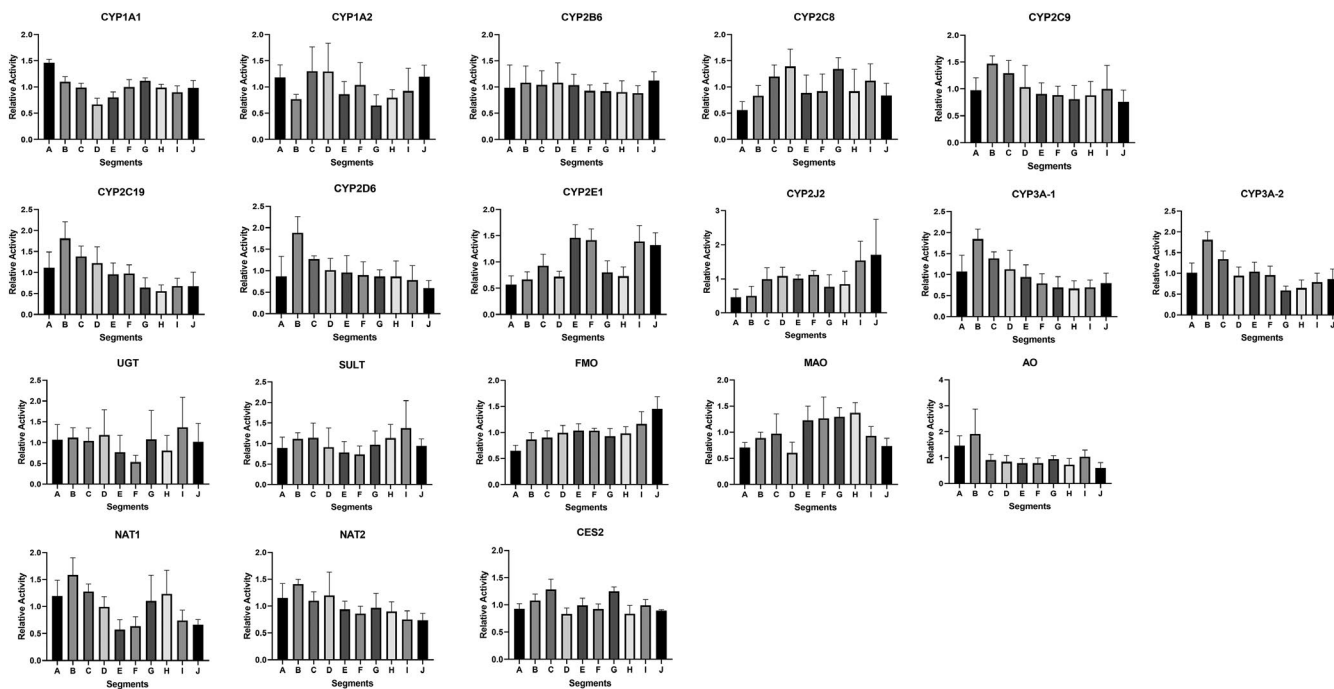


FIGURE 12 Relative activities of P450 and non-P450 drug metabolizing enzymes in CHIM from the 10 consecutive 12-inch segments of the small intestine. Results represent the mean and standard errors of the relative activities for donors 11 - 14

in seven of the 10 donors, a result similar to that reported based on intestinal microsomes.²⁸

Upon demonstrating the jejunum has the highest activities for several drug metabolizing enzymes compared to duodenum and ileum, we proceeded to further define regional variations

within the duodenum and jejunum using CHIM isolated from ten 12-inch segments of the small intestines from four donors. The results show that relative activity peaks in the segments immediately after segment A (representing the duodenum) for CYP2C8, CYP2C9, CYP2C19, CYP3A-1/2, and NAT1, suggesting that the

proximal regions of the jejunum in general have higher drug metabolizing enzyme activities. Our results with UGT, SULT, and CES2 for CHIM from the various regions are similar to that previously reported.^{29,77}

In conclusion, our results provide clear evidence for inter-individual and inter-regional differences in enteric drug metabolism. The variations are likely results of genetic and environmental factors as observed for hepatic drug metabolism. The environmental factors may include foods, drugs, tobacco products, and environmental pollutants which can affect drug metabolizing enzyme activity as inducers and inhibitors, and may also act via the modification of the intestinal microbiome which is known to affect the expression of enteric drug metabolizing enzymes as observed for the hepatic pathways.⁷⁸⁻⁸¹

Our results support our previous assertion that CHIM represents a valuable in vitro experimental system for the evaluation of enteric drug metabolism,²⁵ providing information complementary to the current in vitro human enteric systems including intestinal microsomes,²⁸ intestinal slices (van,⁸² cryopreserved human enterocytes, and MetMax™ cryopreserved human enterocytes.²²⁻²⁴ CHIM, due to the presence of virtually all cell types in the intestinal mucosal epithelium, represent the most complete model for the evaluation of enteric drug properties, while cryopreserved enterocytes can be used to define enterocyte-specific events, and MetMax™ cryopreserved enterocytes for the evaluation of specific pathways. Investigations with CHIM from various regions of the human small intestine can aid the estimation of enteric metabolic fate of drug candidate in drug development, with results that can be applied toward IVIVC approaches in the assessment of the metabolic fate of orally administered drugs.^{83,84} We will continue to further define enteric drug metabolism, for instance, for the various UGT isoforms, and to develop approaches to evaluate uptake and efflux transporters using CHIM for the application of this experimental system to evaluate enteric drug properties.

AUTHOR CONTRIBUTIONS

Participated in research design: Li, AP, Ho, D.; conducted experiments: Ho, D., Alam, N., Mitchell, W.; performed data analysis: Li, A. P., Ho, D., Alam, N., Mitchell, W., Wong, S., Yan, Z., Kenny, J., Hop, M.; manuscript preparation: Li, A. P., Ho, D., Amaral, K., Alam, N., Wong, S., Yan, Z., Kenny, J., Hop, M.

DATA AVAILABILITY STATEMENT

Please contact the corresponding author for additional data requests.

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