




# Altered Bioavailability and Pharmacokinetics in Crohn's Disease: Capturing Systems Parameters for PBPK to Assist with Predicting the Fate of Orally Administered Drugs

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## Abstract

**Background and Objective** Crohn's disease (CD) is a chronic inflammatory bowel disease that affects a wide age range. Hence, CD patients receive a variety of drugs over their life beyond those used for CD itself. The changes to the integrity of the intestine and its drug metabolising enzymes and transporters (DMETs) can alter the oral bioavailability of drugs. However, there are other changes in systems parameters determining the fate of drugs in CD, and understanding these is essential for dose adjustment in patients with CD.

**Methods** The current analysis gathered all the available clinical data on the kinetics of drugs in CD (by March 2021), focusing on orally administered small molecule drugs. A meta-analysis of the systems parameters affecting oral drug pharmacokinetics was conducted. The systems information gathered on intestine, liver and blood proteins and other physiological parameters was incorporated into a physiologically based pharmacokinetic (PBPK) platform to create a virtual population of CD patients, with a view for guiding dose adjustment in the absence of clinical data in CD.

**Results** There were no uniform trends in the reported changes in reported oral bioavailability. The nature of the drug as well as the formulation affected the direction and magnitude of variation in kinetics in CD patients relative to healthy volunteers. Even for the same drug, the reported changes in exposure varied, possibly due to a lack of distinction between the activity states of CD. The highest alteration was seen with *S*-verapamil and midazolam, 8.7- and 5.3-fold greater exposure, respectively, in active CD patients relative to healthy volunteers. Only one report was available on liver DMETs in CD, and indicated reduced CYP3A4 activity. In a number of reports, mRNA expression of DMETs in the ileum and colon of CD patients was measured, focussing on P-glycoprotein (p-gp) transporter and CYP3A4 enzyme, and showed contradictory results. No data were available on protein expression in duodenum and jejunum despite their dominant role in oral drug absorption.

**Conclusion** There are currently inadequate dedicated clinical or quantitative proteomic studies in CD to enable predictive PBPK models with high confidence and adequate verification. The PBPK models for CD with the available systems parameters were able to capture the major physiological influencers and the gaps to be filled by future research. Quantification of DMETs in the intestine and the liver in CD is warranted, alongside well-defined clinical drug disposition studies with a number of index drugs as biomarkers of changes in DMETs in these patients, to avoid large-scale dedicated studies for every drug to determine the effects of disease on the drug's metabolism and disposition and the consequential safety and therapeutic concerns.

## 1 Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) that can affect any part of the gastrointestinal (GI) system. The mucosal layers of the intestine, ileum and colon segments are predominantly affected [1, 2]. Although

duodenum and jejunum involvement is not as common, these segments play a major role in drug pharmacokinetics (PK), as they represent the largest surface area and have an abundance of metabolising enzymes and transporters [3, 4]. Most CD patients are diagnosed before the age of 30, and patients are susceptible to cancer and arthritis, as well as cardiovascular, respiratory, kidney and liver diseases [5, 6]. Hence, over a lifetime, these patients receive many different drugs beyond those aimed to control CD itself. It is estimated that the prevalence of CD is up to 322 per 100,000

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## Key Points

There was no uniform and simple trend linking the changes in physiology/biology in Crohn's disease patients to changes in drug bioavailability and disposition.

Inadequate clinical and proteomics data were identified as hurdles to incorporate relevant information into physiologically based pharmacokinetic models for creation of reliable predictive virtual populations of Crohn's disease patients. Initial assessment of the models identified the major physiological influencers and the knowledge gaps for prudent prediction of altered pharmacokinetics in Crohn's disease.

persons in Europe, with corresponding numbers in Asia and North America being 67.9 and 319, respectively [7]. Despite the high prevalence of CD, unlike other organ impairments such as those related to renal or hepatic function [8, 9], there are no mandatory requirements by regulatory agencies for clinical studies to be conducted on drugs entering market, to assess variable exposure to drugs in these patients. It might be assumed that changes to the fate of drugs in CD, based on the current knowledge on drug studies in CD, indicates minimal variations from healthy volunteers, which has led to a lack of legislation. However, this is not the case, and the more likely reason is associated with practical issues in adding further dedicated studies to those already in place for renal and hepatic impairment. Indeed, many other comorbidities may be excluded from clinical trials during drug development, for example, a typical, similar case would be patients undergoing bariatric surgery [10]. The issue of a lack of diversity in patient populations during drug development has been addressed in recent guidance by the US Food and Drug Administration (FDA), which encouraged the sponsors to broaden the inclusion criteria for clinical studies [11].

Following several position papers on special populations [12, 13], the possibility of using physiologically based pharmacokinetic (PBPK) models to fill in the gaps in guiding informed dosage adjustment (when necessary) in a sub-group of patients, as an alternative to leaving the drug labels silent, seems a prudent approach until dedicated studies become available. Indeed, the recent FDA guidance for the conduct of renal impairment studies during drug development [9] has mentioned PBPK as a potential approach for informing prescribers regarding any actions until adequate data are gathered from dedicated clinical studies or real-world data analysis.

The major aims of the current report are twofold:

1. Gather evidence regarding changes to the fate of drugs in CD, with a view to indicating any trend that can be discerned.
2. Extract relevant systems information for PBPK in CD and create a repository that helps with the creation of CD population files in PBPK modelling.

We also conducted a preliminary application of PBPK for CD for two case examples (the benzodiazepine midazolam and the corticosteroid budesonide) for verification and testing the adequacy of the model versus existing major gaps. The two drugs are both substrates of cytochrome P450 3A4 (CYP3A4). However, as would be expected from their different uses, there are subtle differences between midazolam and budesonide that may be used to identify the influence of different systems parameters. Midazolam is a well-known CYP3A4 probe, which allows investigation of the influence of CD on the liver and intestine separately by comparing relative PK in CD patients versus healthy volunteers after an intravenous (IV) and oral dose. The investigated dosage form is given as a solution, allowing for assessment of the effects on the upper intestine. On the other hand, budesonide is a substrate for several enzymes (CYP3A4, CYP2C9 and CYP1A2) and P-glycoprotein (p-gp), which allows us to investigate the influence of other factors beyond CYP3A4. Budesonide is a very high extraction ratio drug ( $E_h = 0.9$ ) [14]; hence, it allows the assessment of the effects from changes to mesenteric blood flow (cardiac output) in CD. Budesonide is given in a controlled-release formulation, where its release is triggered by entering a basic environment ( $\text{pH} > 5.5$ ); it is expected to be sensitive to changes that occur in the lower intestine. The two drugs are highly bound to albumin (80–90%). Both drugs have shown significant alteration in oral bioavailability in CD patients, based on clinical studies. Moreover, they are clinically relevant to CD patients, as IV midazolam is used before endoscopy procedures as an anxiolytic, while oral budesonide is used for treatment of CD itself.

## 2 Methods

### 2.1 Data Gathering and Analysis for Kinetics of Orally Administered Drugs in the Crohn's Disease (CD) Population

A systematic review of the reported PK in the CD population was conducted with the specific aim of assessing any exposure (bioavailability/clearance) changes compared to what was known for the same drug in healthy volunteers. A literature search concluded by March 2021 was run in PubMed

(<http://www.ncbi.nlm.nih.gov/pubmed/>) using the terms (“Crohn’s disease” and “inflammatory bowel diseases”) in combination with (“pharmacokinetic”, “bioavailability”) for oral and IV drug formulations (Fig. 1). The search was not limited to particular time periods. Only original research articles concerning small molecule agents were included. An initial screening of titles and abstracts was carried out to identify the inclusion of a PK analysis of the drug in CD adult patients. This was followed by extended screening of the results to identify the presence of the required PK data to allow the drug exposure/clearance analysis. Restriction of the search to reports where PK were assessed in both CD and healthy volunteers within the same study could severely reduce the number of cases for analysing the trends. The same was true in narrowing the studies only to those conducted in the fasting state or excluding the studies due to small sample size. Whenever a drug lacked the information in healthy volunteers in the same study, a literature search was done by the name of the drug and the terms “pharmacokinetic” and “bioavailability”. Hence, the only exclusion was dis-similarity of the trial methodology (dosage regimens, fed or fasted state, formulation and route of administration) between the studies conducted in CD patients versus those in healthy volunteers, particularly when a large dose normalisation was required.

The area under the curve (AUC) of the concentration–time profile for each of the drugs both in CD patients

and healthy volunteers was the basis for the calculation of the relative drug exposure following dose normalisation (when needed) and the relative clearance (CL) when accounting for IV drug in each group, respectively. Since the values of the two populations could arise from different studies, we had to ensure the nature of the AUC for comparison was the same (e.g. extrapolated  $AUC_{0-\infty}$ , steady-state  $AUC_{ss}$  or truncated  $AUC_{0-t}$ ). All drugs without information on AUC (for oral) and CL (for IV) from healthy volunteers in studies reasonably similar in design to those performed in the CD population were excluded, since no reliable relative bioavailability or clearance could be inferred.

The calculation of relative exposure and clearance between the CD and healthy population (CD/healthy),  $\frac{a}{b}$ , and its 95% confidence interval (CI), applied Fieller’s Theorem [15–17], as per Eq. 1:

$$CI_{\frac{a}{b}(1-\alpha)} = \frac{a}{b \times (1-g)} \pm t_{\alpha,df} SE_{\frac{a}{b}} \quad (1)$$

where  $a$  is the mean AUC for the CD population and  $b$  is the mean AUC for the healthy volunteer population,  $t_{\alpha,df}$  is the inverse of the cumulative  $t$  distribution (two tailed) for a significance level of  $\alpha$  (0.05), and degrees of freedom ( $df$ ) is equal to  $n_a + n_b - 2$ , where  $n_a$  and  $n_b$  are the number of participants in the study for the estimation of  $a$  and  $b$ , respectively, and  $SE_{\frac{a}{b}}$  is the combined standard error for the ratio  $\frac{a}{b}$ , and it is calculated from Eq. 2:

$$SE_{\frac{a}{b}} = \frac{a}{b \times (1-g)} \sqrt{(1-g) \times \frac{SE_a^2}{a^2} + \frac{SE_b^2}{b^2}} \quad (2)$$

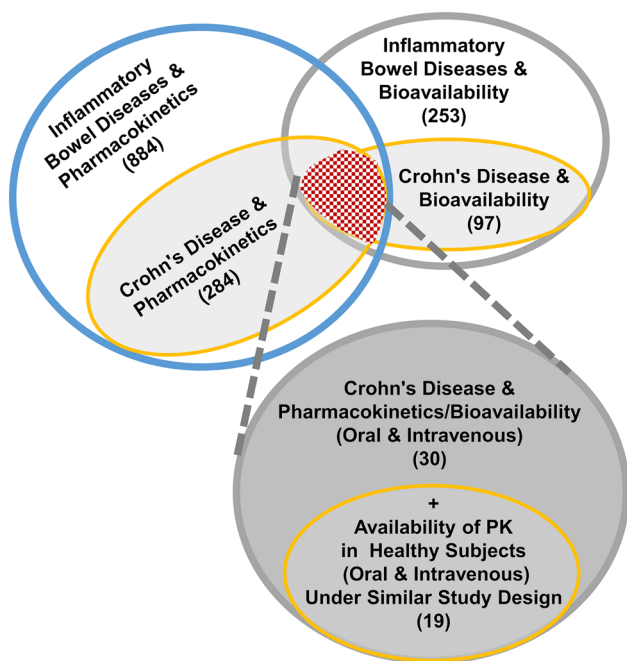
where the quantity  $g$  is given by Eq. 3, and  $SE_a$  and  $SE_b$  are the standard error of the means  $a$  and  $b$ , respectively:

$$g = \frac{t_{\alpha,df}^2 \times SE_b^2}{b^2}. \quad (3)$$

## 2.2 Demography of the CD Population

In general, CD is more common in the Caucasian population than in people of African and Hispanic ethnicities in North America. Various prevalence values are reported for Europe, North America, Oceania, Africa, South America and Asia in the literature [7, 18, 19].

For adults, the peak incidence of CD is between the ages of 20 and 40, with a less common second peak around the age of 60–70, making the demography of CD bimodal [20–22]. The incidence of CD is marginally higher in females compared with males, as suggested by several studies [23–28].



**Fig. 1** An illustration of the process of literature search in relation reports on the systemic exposure of drugs in adults Crohn’s disease relative to healthy volunteers (carried out using PubMed; March 2021). PK pharmacokinetics

In our study, the demography used mimics the demography of the corresponding clinical studies that we were simulating for the model verification.

### 2.3 Systems Parameters Defining Physiologically Based Pharmacokinetics (PBPK) in CD Patients

A meta-analysis of the literature data was conducted to identify the relevant sources of changes in the bioavailability of oral drugs, and to extract data related to the physiological and anatomical changes of the intestine and whole body in CD patients. The literature search was run in PubMed (October 2021), using the following terms (“small intestine pH”, “large intestine pH”, “gastric emptying time”, “intestine transit time”, “serum albumin”, “alpha acid glycoprotein”, “liver blood flow”, “portal vein flow”, “superior mesenteric artery blood flow”, “cardiac output”, “ABC transporter”, “solute carrier transporter”, “metabolising enzymes”, “CYP” and “proteomics”) in combination with “Crohn’s disease”. Screening of titles and abstracts was carried out to identify the studies with a clear indication of the required information in CD patients. No time frame of the research was applied.

Inclusion of data was restricted to original articles, studies that provided information where determination of the mean, SD and CI is possible, studies that reported the level of the parameter at the baseline (before the treatment intervention), and studies including both CD patients and healthy volunteers. Only the adult population and English publications were considered. Studies that did not report the values of the CD group separately from other diseases were excluded. Some studies included non-IBD patients as the control group instead of healthy volunteers. When such studies are included in the analysis (owing to the low number of the available studies), it is indicated in the results section. For all parameters, differentiation of the results based on active or inactive CD phase is considered. If it was not clearly indicated in the study whether the patients were active or inactive, they were considered mixed (active and inactive).

The activity of CD disease is defined by a subjective scoring index based on the clinical manifestation of the patient in most of the included studies. The most common scoring systems used are the Harvey-Bradshaw Index (HBI) [29] and Crohn’s Disease Activity Index (CDAI) [30]. In the case of reporting the activity based on an objective index where biomarkers, endoscopy or other markers are assessed, this is reported in the results section if applicable. Clinical remission does not always reflect histological/endoscopic remission, hence the reported activity according to the symptom-based scores and the objective markers [31, 32].

For drug metabolising enzymes and transporters (DMET) expression, all studies are included if a fold change of CD

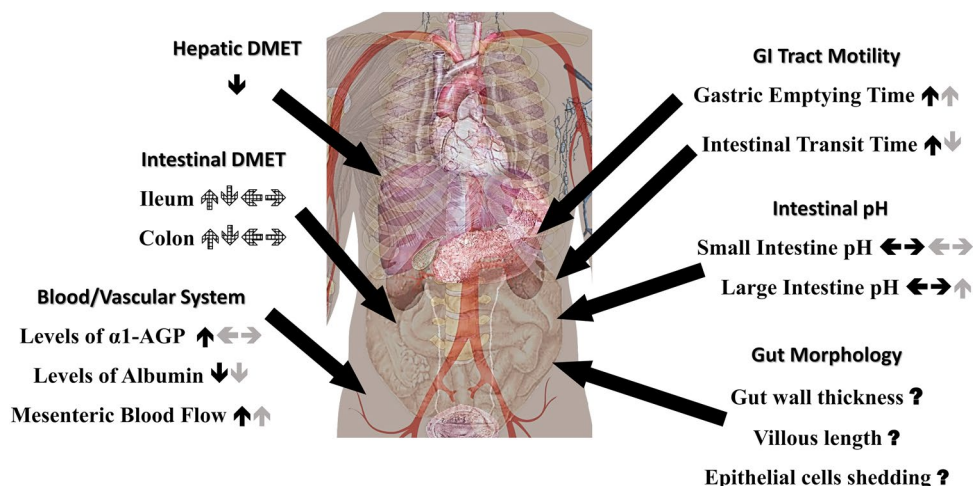
value to control is reported or can be determined without distinguishing between inflamed or non-inflamed tissue. If both values from inflamed and non-inflamed tissues are separately available, only the values of inflamed tissues are included. Only studies or values that reported the expression level in CD patients and not in a mixed set of IBD (ulcerative colitis [UC] and CD) patients are included. Several reports did not provide the raw values; thus, the values used here are based on a digitally extracted estimate from the figures. As only a few reports are available, the weighted ratio was calculated from all the included studies regardless of the nature of the assay used to determine the expression.

The identified physiological aspects that can alter oral drug bioavailability depending on the nature of the drug and formulation are summarised in Fig. 2. Previous review [33] and analysis [34] covered some of the aspects considered in our analysis in CD patients, which was checked to assure inclusion of the collected available data. The collected information was stratified based on different segments of the intestine, differentiation between active and inactive CD, sex and fasting state whenever possible. Figure 3 shows a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram of the flow for inclusion and exclusion of studies in the meta-analysis. Inadequate or incomplete information is highlighted, as such information can compromise the accuracy of the PBPK model projections.

The collected physiological parameters were analysed based on the weighted mean and overall standard deviation using the reported mean and SD as previously reported [35]. Similarly, we followed the previously reported procedure for converting median values and ranges to mean and SD when not provided in the publications [36].

### 2.4 PBPK Application for Obtaining Systems Parameters and Its Applications for Verification of the CD Model

*Reverse translation* Two CYP3A substrates, midazolam and budesonide, were investigated by means of PBPK modelling, utilising the Simcyp<sup>®</sup> Simulator M-ADAM absorption model. The details of model assumptions and infrastructure for a multilayer gut wall [37] and the general attributes of the advanced dissolution, absorption and metabolism (ADAM) model are described elsewhere [38]. However, to build a CD population with all relevant known physiological differences in intestine and other organs, we had to incorporate relative changes in each parameter into the population library files within the simulator, based on our analysis of the systems parameters described in the previous section. Additionally, a reverse translation approach was used based on a midazolam IV clinical study in CD [39] to determine the value of the liver CYP3A4. Hence, the value of hepatic clearance ( $CL_H$ )



**Fig. 2** Illustration of physiological parameters that were the subject of the systematic literature review and subsequent meta-analysis in relation to CD. The arrows represent the general trend seen in active (black arrow), inactive (grey arrow) and mixed (undistinguished active and inactive) [stripe arrow] CD patients. The question marks represent the parameters that are not incorporated in the model due to unavailability of data in the targeted population, inconsistencies in the

methods or unclear utility in Simcyp<sup>®</sup> simulator. (Figure is adapted using a public domain image by Mikael Häggström, CC0, via Wikimedia Commons: [https://commons.wikimedia.org/wiki/File:Adult\\_male\\_with\\_organs.png](https://commons.wikimedia.org/wiki/File:Adult_male_with_organs.png)). *AGP* acid glycoprotein, *CD* Crohn's disease, *DMET* drug metabolising enzymes and transporters, *GI* gastrointestinal

was used to estimate the intrinsic unbound metabolic clearance ( $CL_{u-int}$ ), knowing the hepatic blood flow ( $Q_H$ ) and fraction unbound in blood ( $f_{u_B}$ ) according to Eq. 4:

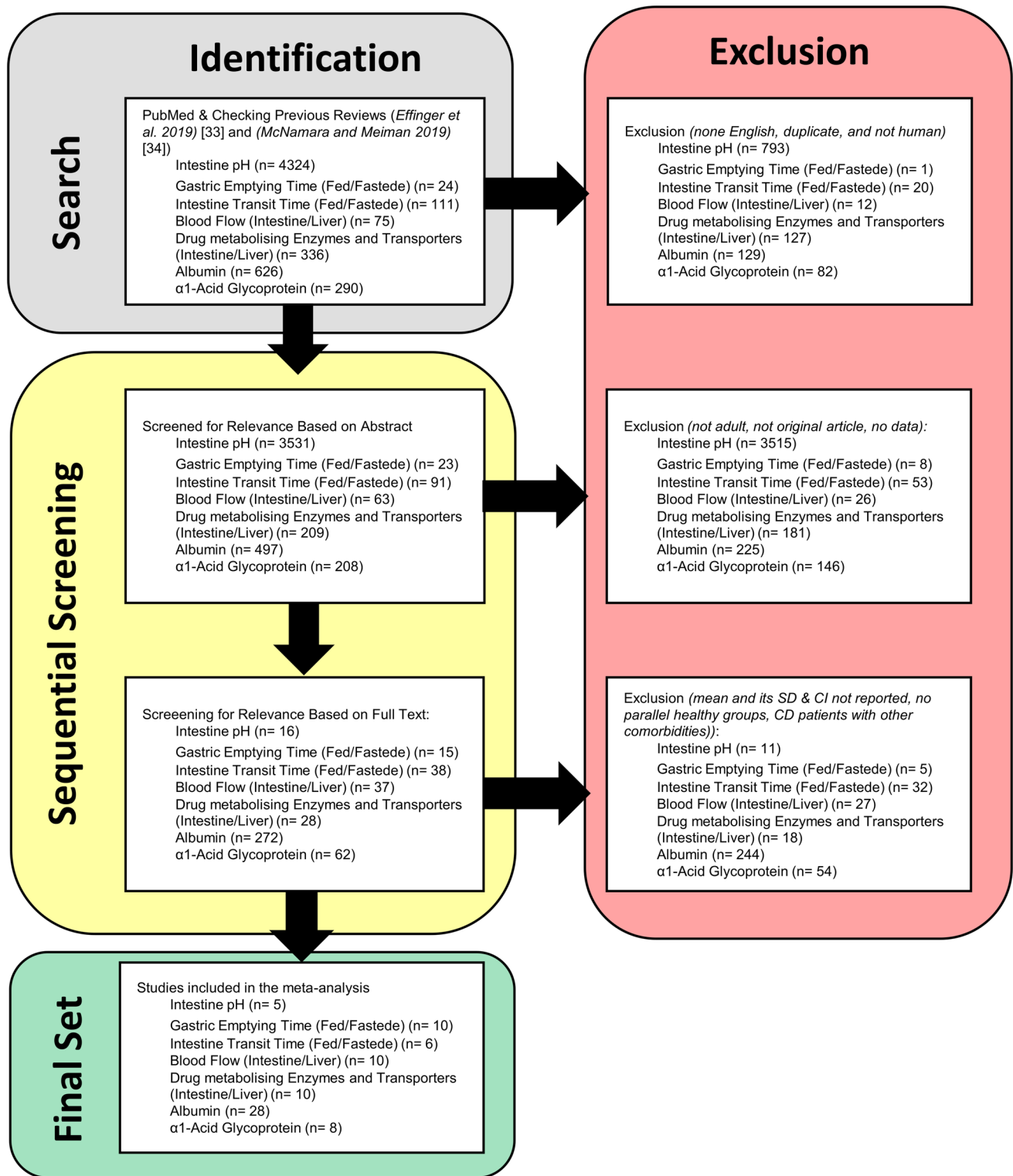
$$CL_{u-int} = \frac{Q_H \times CL_H}{f_{u_B} \times (Q_H - CL_H)}. \quad (4)$$

The relative reduction in intrinsic clearance ( $CL_{int}$ ) in CD patients compared to healthy volunteers [39, 40] was considered as a reflection of reduced hepatic CYP3A4 expression. These changes were incorporated in the CD population file that was used in modelling other cases. The input parameters used to create the inactive and active CD population framework are summarised in Table 1.

The study design taken from each clinical case was used for building and verifying the drug PBPK models in healthy volunteers (drug PBPK models in healthy volunteers: budesonide IV [41], budesonide oral as Entocort<sup>®</sup> [42], midazolam oral [40]) in the Simcyp<sup>®</sup> Simulator V19 and using the default healthy volunteer population. The study design included the information on age range, female-to-male ratio, fasting status and dosage regimen. The clinical studies for budesonide in the CD population were conducted under different conditions [39, 42–44]. Our focus was on the study that showed a significant difference [42] in budesonide bioavailability in CD patients compared to healthy volunteers. Midazolam was studied in only one study for assessing bioavailability difference in CD [39]. The drug-specific parameters of budesonide were mainly taken from the report by Effinger et al. [45], since there is no budesonide default

library in the library set of compounds in the Simcyp<sup>®</sup> simulator. For midazolam, the available Simcyp<sup>®</sup> drug model from the compound library was used as a starting point; this is verified and published in several reports and frequently used in regulatory submissions [46, 47]. The key modifications that we had to make, for the purpose of later applications in CD, were the selection of the M-ADAM absorption model option (in preference to the default ADAM) and the selection of full-PBPK (as opposed to the default of minimal-PBPK). Input parameters used in the Simcyp<sup>®</sup> simulator for building budesonide and midazolam drug profiles are summarised in Tables S1 and S2, respectively (see the Electronic Supplementary Material).

**Application** The observed concentration–time profiles of budesonide and midazolam were derived using GetData Graph Digitizer 2.26. We considered females and males as having different CYP3A4 abundance in all simulations (one of the options in the Simcyp<sup>®</sup> simulator), as this is more consistent with the gender difference reported for CYP3A4 substrate clearance values [48]. All trial sizes were based on 100 participants (ten participants in ten trials). Details of the trial design for clinical studies of budesonide IV [43], budesonide controlled-release formulation [42] and midazolam oral solution [39] are in Table S3 (see the electronic supplementary material). The simulated concentration–time profiles of budesonide and midazolam were visually inspected by comparing the mean and 95% prediction interval against the observed clinical data. The similarity of the predicted relative mean values of maximum drug concentration ( $C_{max}$ ),



**Fig. 3** PRISMA flowchart of studies systemically reviewed to be included in the meta-analysis of the physiological parameters related to CD. CD Crohn's disease, CI confidence interval, PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses

**Table 1** Summary of the Input system parameters in Simcyp® simulator of the active and inactive CD population model verification, based on their relative alteration from healthy population as per the data analysis carried out in this study

Physiological parameter	Inactive CD (fed)	Active CD (fasted)	Source of alteration in CD
<b>GIT alterations</b>			
pH proximal SI	<i>Fed</i> Unaltered from normal values <sup>a</sup>	<i>Fed</i> Unaltered from normal values <sup>a</sup>	<i>Reference</i> Relative change based on our meta-analysis
pH distal SI	<i>Fed</i> Unaltered from normal values <sup>a</sup>	<i>Fasted</i> Duodenum = 6.3 Jejunum I and II = 6.4 and 6.5 <i>Fasted</i> Ileum (I–IV) = 7, 7.2, 7.3 and 7.5 <i>Fasted</i> Ileum (I–IV) = 7.1, 7.2, 7.3 and 7.4	<i>Fasted</i> Duodenum = 6.7 Jejunum I and II = 6.8 and 6.9 <i>Fasted</i> Ileum (I–IV) = 7.1, 7.2, 7.3 and 7.4
pH large intestine	<i>Fed</i> Unaltered from normal values <sup>a</sup>	<i>Fasted</i> Colon = 7.6 <i>Fed</i> Unaltered from normal values <sup>a</sup>	<i>Fasted</i> Colon = 6.5
<b>GIT alterations, segregated transit time model</b>			
Gastric MRT (h)	<i>Fed</i> 1.46	<i>Fasted</i> 0.3	<i>Reference</i> Relative change based on our analysis
SITT (h)	<i>Fed</i> 3.8	<i>Fasted</i> 3.2	<i>Fasted</i> 4.2
Ascending colon transit time (h)	<i>Fed</i> Male = 9.9 Female = 12.1	<i>Fasted</i> Unaltered from normal values <sup>a</sup>	<i>Fasted</i> Unaltered from normal values <sup>a</sup>
Intestine CYP3A4	<i>SI</i> (nmol/SI) 52.3	<i>Colon</i> (nmol/colon) 2.4	<i>Colon</i> (nmol/colon) 2.4
Intestine CYP2C9 <sup>b</sup>	<i>SI</i> (nmol/SI) 13.75	<i>Colon</i> (nmol/colon) 0.1	<i>Colon</i> (nmol/colon) 0.1
Intestine p-gp <sup>b</sup>	<i>SI</i> (pmol/mg) Ileum I–IV = 1.2	<i>Colon</i> (pmol/mg) 0.17	<i>Colon</i> (pmol/mg) 0.55
<b>Systemic circulation alterations</b>			
SI blood flow (% of CO)	<i>Male</i> 11.1	<i>Female</i> 12.2	<i>Female</i> 16.8
$Q_H$ (% of CO)	<i>Male</i> 21	<i>Female</i> 23.8	<i>Female</i> 27.39
			<i>Reference</i> Relative change based on our meta-analysis <i>Reference</i> Proportional to the increase in the intestine flow rate <sup>c</sup>

Table 1 (continued)

Physiological parameter	Inactive CD (fed)	Active CD (fasted)	Source of alteration in CD
HSA (g/L)	<i>Scenario 1</i>		
	<i>Male</i> 44.8	<i>Female</i> 43.9	<i>Male</i> 30.13 <i>Female</i> 25.2
<i>Reference</i>	Inactive based on the relative change based on our analysis (no differentiation between active and inactive CD)		
<i>Scenario 2</i>			
Unaltered from normal values in one simulation. (Two <i>active</i> CD simulations were carried out once with reduced HSA and once with normal HSA levels)			
<i>Reference</i>	The reduction of HSA was not assumed to be the definite state of CD patients, as only around 20% of CD population suffer from HSA drop based on the collected literature data		
Liver alterations			
Liver CYP3A4 (pmol/mg protein)	Unaltered from normal values <sup>a</sup>	<i>Male</i> 55.4	<i>Female</i> 80.5 <i>Reference</i> Reverse translation based on $F_H$ value [31]

CD Crohn's disease, CO cardiac output, GIT gastrointestinal track, HSA human serum albumin, MRT mean residence time,  $Q_H$  hepatic blood flow, p-gp P-glycoprotein, SI small intestine, SITT small intestine transit time,  $F_H$  the fraction of drug escaping first-pass hepatic metabolism and biliary secretion

<sup>a</sup>No available information that supports alteration from normal values

<sup>b</sup>Used with budesonide only

<sup>c</sup>No available literature data on the liver blood flow



$AUC_{0-\infty}$  and time to reach  $C_{max}$  ( $T_{max}$ ) to the observed oral budesonide and midazolam values were assessed.

## 2.5 Global Sensitivity Analysis (GSA)

The impact of the identified systems parameters in Table 1 on the PK properties of the oral drugs of interest is investigated utilising the Global Sensitivity Analysis (GSA) function within Simcyp®. GSA is important as the physiological alterations encountered by CD patients vary in their effects on different oral drugs based on several disease and drug factors. The determination of the impact spectrum and identification of the key parameters were performed based on physicochemical and PK attributes of budesonide and midazolam and the integrated systems data in the CD population framework. The frequency distributions of the investigated systems parameter values, within the selected range for each parameter, were considered to be uniform. The applied range of the lower bound and upper bound was based on the collected literature systems parameters in healthy volunteers and the active CD population. The Morris method for determining GSA was selected, where it defines the parameters' impact based on their influence on the output variable. It also accounts partly for the interaction between the investigated parameters [49].

The influence of the different systems parameters on altered bioavailability is reflected in the simulated PK properties  $C_{max}$ , AUC and  $T_{max}$  of budesonide and midazolam oral formulations based on their trial specifications. The influential parameters determined by the GSA were compared with the extracted available literature data, to identify the gaps in the key parameters and to guide the decision for future research needed to be carried out to fill these gaps, to allow for the reliable prediction of active and inactive CD PBPK population models.

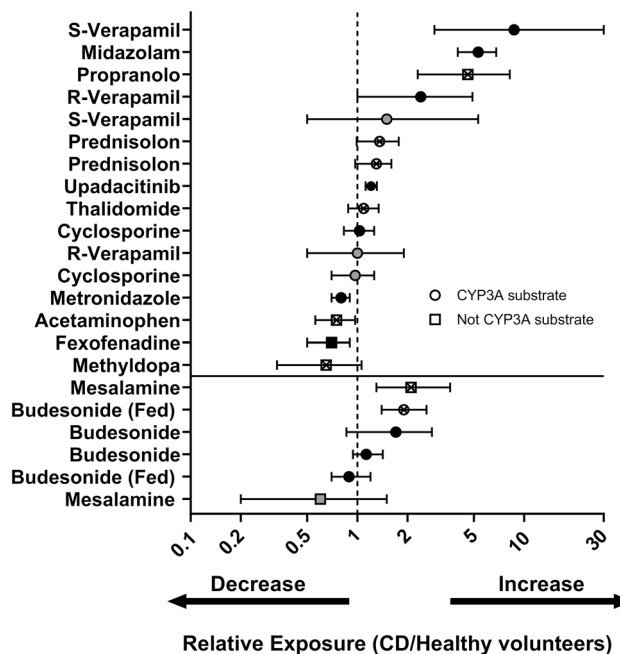
## 3 Results

### 3.1 Altered Kinetics of Orally Administered Drugs in the CD Population

The systematic data analysis on the exposure of orally administered drugs in the CD population relative to healthy volunteers, based on the criteria set in Sect. 2, is summarised in Fig. 4. Knowing there are several modified physiological parameters in the GI tract of CD patients, the number of cases that passed the rigor of the inclusion criteria was not sufficiently high to indicate a consistent trend. For instance, the exposure of CYP3A substrates did not show the same magnitude of change [39, 42, 43, 50–56] in the same phase of the disease (active/inactive). Also, when *S*- and *R*-verapamil [50] and midazolam and budesonide [39] were given

to the same CD patients, the exposure behaviour was not similar. The results from different studies on the same drug were also inconsistent, as seen with budesonide and mesalamine [39, 42, 43, 57, 58]. It should be noted that the type of formulation and other attributes of the drugs could play a role in these observations. Although *S*-verapamil showed the highest level of increased relative exposure (8.7-fold, with 9.6-fold higher  $C_{max}$ ), since there was no comparative IV data in the CD population, these could not be assigned solely to changes in bioavailability. The relative fold changes in exposure for midazolam and budesonide were 5.3 and 1.9 higher, respectively. There were comparative IV data for CL in CD and healthy populations to discern the source of variation [39, 43].

A number of studies showed comparable exposure in CD patients and healthy volunteers [43, 51, 53, 54]. p-gp substrates (propranolol, fexofenadine and budesonide) did not show the same direction of change [39, 42, 59, 60]. Of the three drugs, fexofenadine is a p-gp probe, while propranolol and budesonide are metabolised by CYP enzymes. The activity of the CD patients in the propranolol study was determined based on the erythrocyte sedimentation rate (ESR). The details of the relative exposure of orally



**Fig. 4** The exposure to given oral doses of drugs given to Crohn's disease (CD) patients relative to healthy volunteers. The lower panel represents controlled-release formulations, and the upper panel shows the values in the case of immediate-release/solution formulations. The markers represent the mean of relative exposure and the bars represent the 95% confidence intervals. The black, grey and patterned fill of markers indicates active, inactive and a mixture of CD patients, respectively. All studies were done under fasted conditions unless indicated as 'fed'

administered drugs in CD patients are in Table S4 (see the Electronic Supplementary Material).

Some of the included drugs were studied under specific conditions that might have contributed to the outcome. One of the two prednisolone studies included had inactive CD patients as the control group, and the diseased group was a mix of active UC and CD patients, where the activity was determined by clinical, biochemical, endoscopic and biopsy criteria [53]. Yet, the exposure result was similar to the other study, where the control group was healthy volunteers and only CD patients were in the test group [52]. For mesalamine, one of the two included studies had CD patients in the remission state or with low activity only [57]. In the other mesalamine study, six out of the nine CD patients had undergone resection of the terminal ileum, where they have reported lower AUC values [58]. A similar observation was reported with cyclosporine [54] and metronidazole [51] in patients with a history of bowel resection, indicating a lower absorption profile compared with other CD patients with an intact intestine.

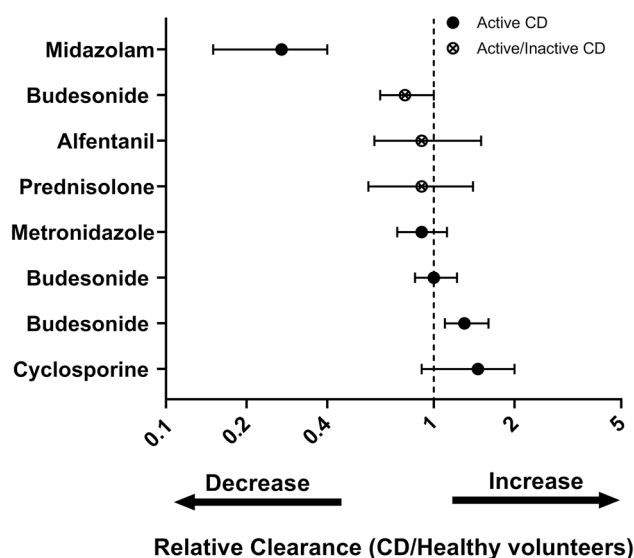
The reports on CL values for various drugs following IV administration to CD patients showed no substantial difference compared to healthy volunteers except in the case of midazolam, which was 3.7-fold lower (Fig. 5). Since all the other drugs included in the comparison were also CYP3A4 substrates, midazolam being distinct from the other drugs cannot be related to the route of metabolism alone. The details of the relative CL for drugs administered via the IV route in CD patients are in Table S5 (see the Electronic Supplementary Material).

### 3.2 Demography of the CD Population

Although the simulation of a large group of CD patients requires implementation of the general demography for the CD population, the objective in our study was not to simulate such a large CD cohort, but was to focus on replicating the observed clinical studies on the kinetics of midazolam and budesonide. Hence, we only applied the demography of the patients reported in each study, rather than the overall demography of CD patients in the general population.

### 3.3 Major Systems Parameters Defining PBPK in CD Patients

**Intestinal pH** The pH values of the small and large intestines of CD patients were reported in five studies (Fig. 6a) based on the criteria previously described in Sect. 2 [61–65]. The CD activity in one study was based on the level of serum proteins [65], but it did not show difference in the alteration of the pH compared to the other studies where the CDAI was used to indicate the activity of CD. All the studies were relatively small ( $\leq 15$  CD patients). From our meta-analysis

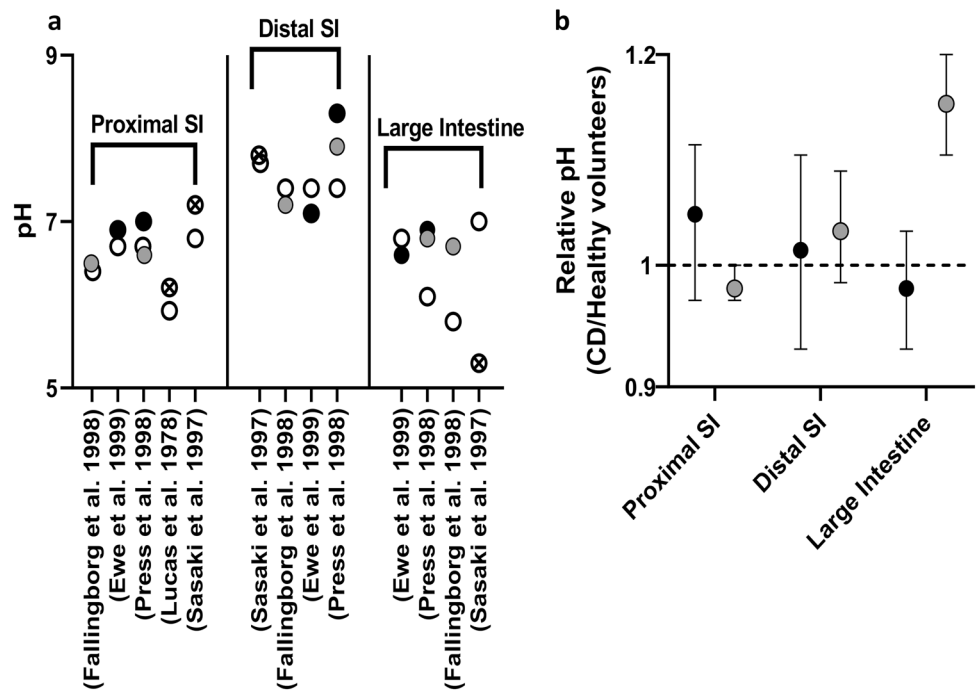


**Fig. 5** The clearance of drugs in Crohn's disease (CD) patients relative to corresponding values in healthy volunteers following intravenous (IV) dose. The markers represent the mean of relative clearance and the bars represent the 95% confidence intervals. The black and patterned fill of markers indicates active or a mixture of active/inactive CD patients, respectively. All drugs are substrates of CYP3A to varying degrees

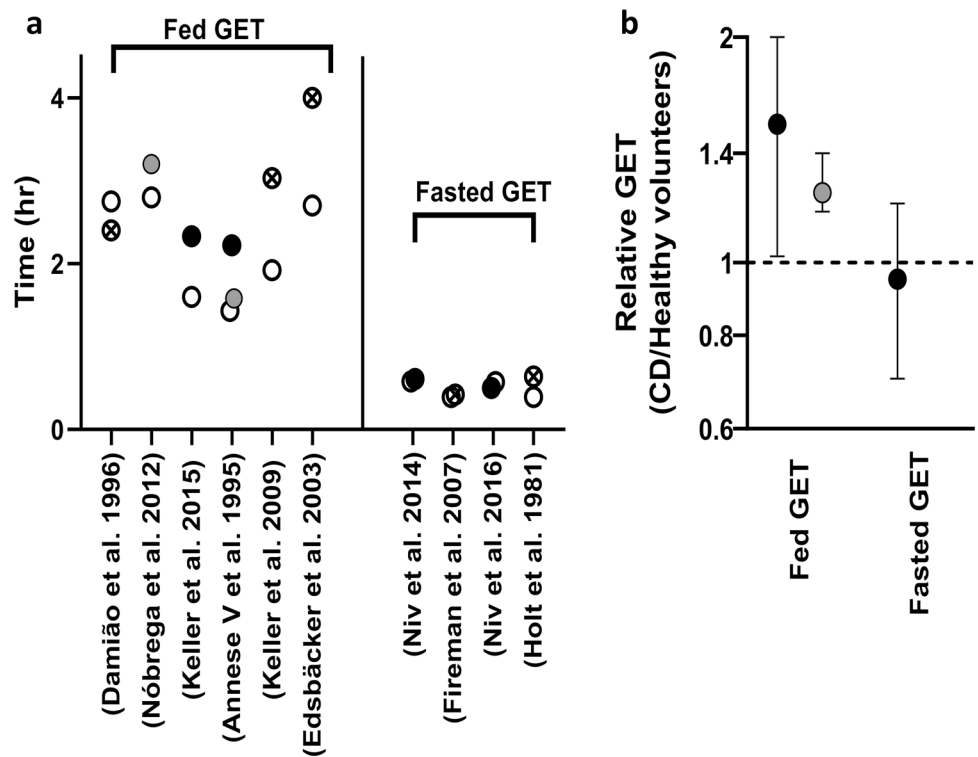
(Fig. 6b), only the pH of the large intestine in inactive CD patients showed a statistically significant increase relative to healthy volunteers. The reports were not consistent, but the reported pH of the proximal small intestine showed a general trend towards higher values, particularly in the active stage. The trends for the distal small intestine and large intestine were slightly more divergent, showing pH values to be higher, similar or lower than those for healthy volunteers (Fig. 6a). Only two studies reported the pH values for the active and inactive groups separately. No pH data in CD patients were reported in the fed state, although pH values are reported in a mix of IBD (UC and CD) patients in the fed state. Details of the reported values in the literature are given in Table S6 (see the Electronic Supplementary Material).

**Gastric emptying time** Ten studies reported gastric emptying time in CD patients alongside the control groups, under fed [42, 66–70] and fasted conditions [71–74]. Under fed conditions, a general trend of a higher gastric emptying time in CD patients was observed, while a similar value was seen under fasted conditions (Fig. 7a). Two studies had non-IBD patients as the control group [73, 74] and one had a mix of healthy and non-IBD patients [72], where gastric emptying time was measured under fasted conditions. Our analysis showed statistical significance of the gastric emptying time in both active and inactive CD patients compared to healthy volunteers only under fed conditions (Fig. 7b). Five of the included studies reported mixed active and inactive

**Fig. 6** The pH of the proximal and distal small intestine (SI) and the large intestine in active and inactive Crohn's disease (CD) populations compared to healthy volunteers in the fasted state. Part **a** shows the reported pH values. Part **b** shows the weighted mean pH from all the studies and the associated 95% confidence intervals. The white, black, grey and patterned fill of markers indicates healthy, active, inactive and a mixture of CD, respectively



**Fig. 7** The gastric emptying time (GET) in Crohn's disease (CD) patients compared to healthy volunteers in fasted and fed states. Part **a** shows the values of GET in each study. Part **b** shows the weighted value of GET from all studies associated 95% confidence intervals. The white, black, grey and patterned fill of markers indicates healthy, active, inactive and mixture of CD, respectively



CD patients without distinguishing between the two states of the disease [42, 67, 70–72]. The sizes of the studies were between six and 96 CD patients. Details of reports on gastric emptying time in CD patients are provided in Table S7 (see the Electronic Supplementary Material).

*Intestinal motility and transit time* Three studies reported the small intestine transit time in CD patients alongside the control healthy groups [42, 62, 71], and three studies had non-IBD patients as the control group [73–75]. The observations from these studies are summarised in Fig. 8a. One

study reported the transit time in the fed state and in the colon [42], which does not allow for comparative analysis of the changes between the diseased and healthy states. The highest reduction in small intestine transit time was seen in a study in which patients with inactive CD who had undergone ileocecal resection [62] were compared with controls. A statically significant prolonged small intestine transit time in active CD and shortened time in inactive CD compared to healthy volunteers are indicated from our analysis (Fig. 8b). The sizes of the studies were between nine and 96 CD patients. Details of all studies on intestinal transit time in CD patients are included in Table S8 (see the Electronic Supplementary Material).

**Vascularity and haemodynamics of blood flow** The intestine morphology and vascularity differ in the active stage of the disease and in the stage where the inflammation is controlled. The key identified differences were linked to gut blood flow and, hence, oral drug PK; our focus here is on the change of the superior mesenteric artery (SMA) blood flow rate, as it represents an important aspect for oral drugs with high extraction. Ten studies reported the SMA blood flow in CD patients alongside that of healthy volunteers (Fig. 9a) [76–85] based on our inclusion criteria. The meta-analysis showed a statistically significant increase in the SMA flow in both active and inactive CD patients relative to healthy volunteers (Fig. 9b), with a higher increase recorded in the active CD phase. One study used X-ray alongside clinical signs to indicate the disease activity [77]; the alteration in

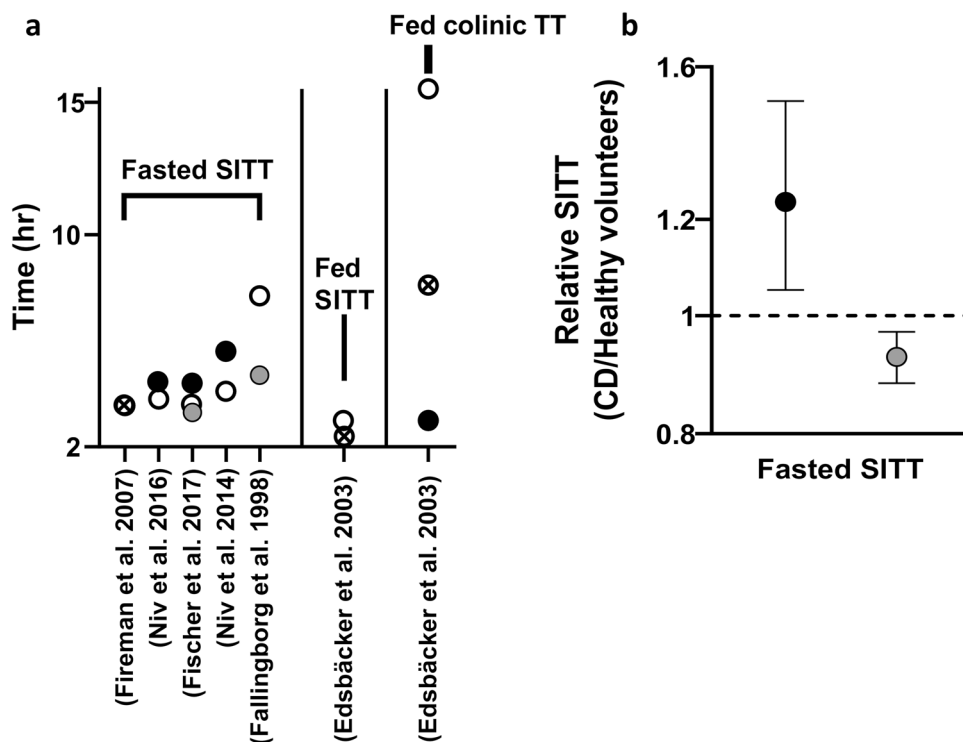
the SMA of both active and inactive CD patients was very similar to the observed alteration in other studies where the CDAI was used. The sizes of the studies were between nine and 74 CD patients. Details of all studies on intestinal transit time in CD patients are included in Table S9 (see the Electronic Supplementary Material).

There are few studies measuring the blood flow of the portal vein. An increase is seen in the portal vein blood flow [83, 86], which is ~ 50% higher in active and inactive CD patients than in healthy volunteers [87].

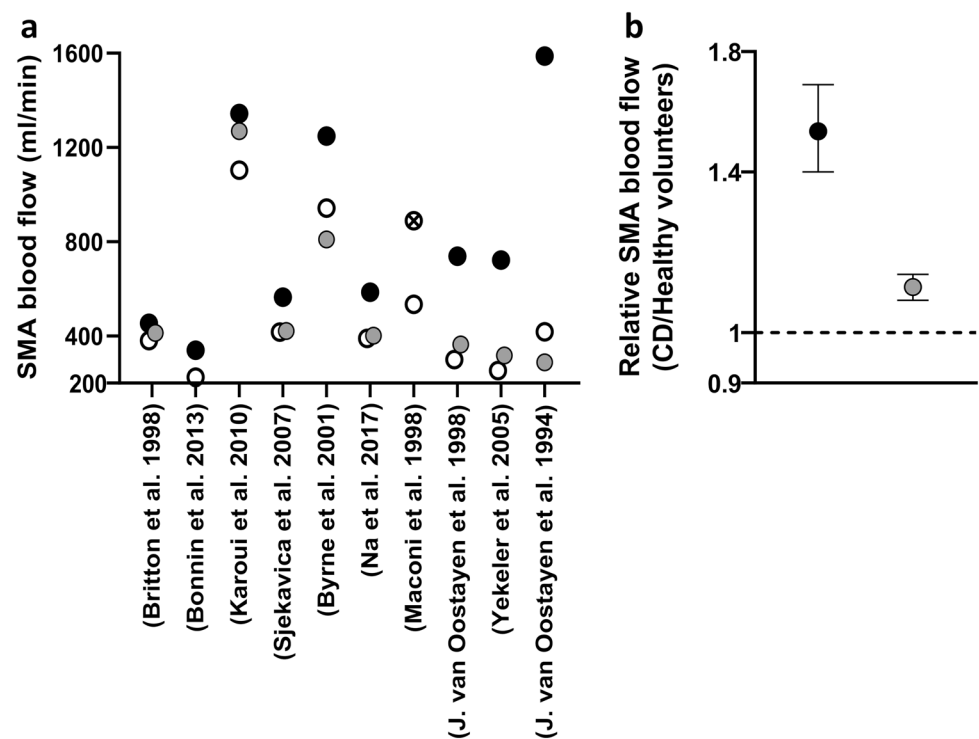
Another aspect is the intestine regional blood flow, which was shown to differ between intestinal regions based on the activity of the disease, where the colonic blood flow was significantly increased in the active stage, while the ileum blood flow was significantly decreased in the inactive stage compared with healthy levels [88]. Other changes encountered by the intestine due to inflammation in CD that might affect the PK of oral drugs are reported. These include dilation of the blood vessels [78, 89], change of the intestine vascularity [90–92], change of intestine blood perfusion [90–94], increase in the thickness of the intestine wall (active CD > 4 mm) [78, 80, 81, 83, 89, 93], submucosal fibrosis and muscularisation [91] and wrapping of the intestinal wall in fat wrap [95], but they are beyond the focus of this analysis.

**Expression of DMETs in the intestine** The effect of CD inflammation on the expression of DMETs has not been extensively studied. In total, we found 13 publications that measured DMETs from CD patients in inflamed and

**Fig. 8** The small intestine transit time (SITT) and the colonic transit time (TT) in Crohn's disease (CD) patients compared to healthy volunteers. Part **a** shows the values of TT in each individual study. Part **b** shows the weighted values for SITT from all studies and the associated 95% confidence intervals. The white, black, grey and patterned fill of markers indicates healthy, active, inactive and mixture of CD, respectively



**Fig. 9** The superior mesenteric artery (SMA) blood flow (mL/min) in Crohn's disease (CD) patients compared to healthy volunteers. Part **a** shows the SMA blood flow from each study. Part **b** shows the weighted value of SMA blood flow from all the studies and the associated 95% confidence intervals. The white, black, grey and patterned fill of markers indicates healthy, active, inactive and mixture of CD, respectively



non-inflamed intestinal tissues. Seven reported drug metabolising enzymes (DMEs) (mainly CYPs) [96–102], seven reported ABC transporters [96–101, 103, 104] and six reported solute carrier (SLC) and/or solute carrier organic anion (SLOC) expression [98, 101, 105–108]. In addition, a case report study on a duodenum biopsy reported an increase in p-gp expression of greater than twofold in a CD patient, as compared with healthy specimens [109].

Four studies were excluded from our analysis. Two had combined UC and CD patients, considered gene expression values from rectum inflamed tissue [96] and non-inflamed tissue from upper and lower segments of the intestine, and had no control group to extract fold change values for use in our analysis [101]. One study reported the expression value of DMEs from the ileum and colon combined, which did not allow for differential analysis of the expression based on the segment [102]. The last study had the values reported in a previous study compared to healthy instead to non-inflamed tissue [104].

The available abundance data are of two kinds: relative abundances of proteins measured at the protein level by immunoblotting techniques, and reports of mRNA levels being used as proxies for the protein abundances. The gold standard for measuring absolute abundances of proteins in complex mixtures is now generally considered to be tandem mass spectrometry (LC/MS-MS) of protein digests, and in no case has such an analysis been reported. Six of the included publications had healthy volunteers as the control group [97, 98, 100, 103, 106, 107], while the rest had

non-IBD patients as the control group [99, 105, 108]. DMET abundances in the ileum, which is known to have a more dominant role in oral drugs than the colon, were reported in six studies. There are no reports of expression of DMETs in the upper intestine segments (duodenum, jejunum) in CD, but these are rarely affected by inflammation and are, therefore, less relevant.

The lack of a clear distinction between active and inactive CD in the available studies makes it hard to determine the extent of inflammation-caused changes in DMET expression in the different phases. Only one study compared expression levels in active and inactive CD patients [98]. In both disease phases, the investigated DMETs were altered compared with control, but, unsurprisingly, alterations were more pronounced in active CD patients. The expression of absorption, distribution, metabolism and excretion (ADME) proteins was reported in samples that were not differentiated for being in an active or inactive state of the disease [100, 105], with an indication that the active state does not affect the expression of CYP3A4 and p-gp [100]. The degree of change of expression from normal was shown to be proportional to the tissue inflammation [103, 105, 106].

The reported expression data show high variability in the changes (CD/control) of the ileum (Fig. 10a) and colon (Fig. 10b) DMETs of CD patients. From our analysis, OATP2B1 showed the highest increase in its expression in the ileum and colon, seven- and eightfold, respectively. Another protein, ASBT, showed the highest reduction in its expression in CD (~3.5- and 35-fold in the ileum and

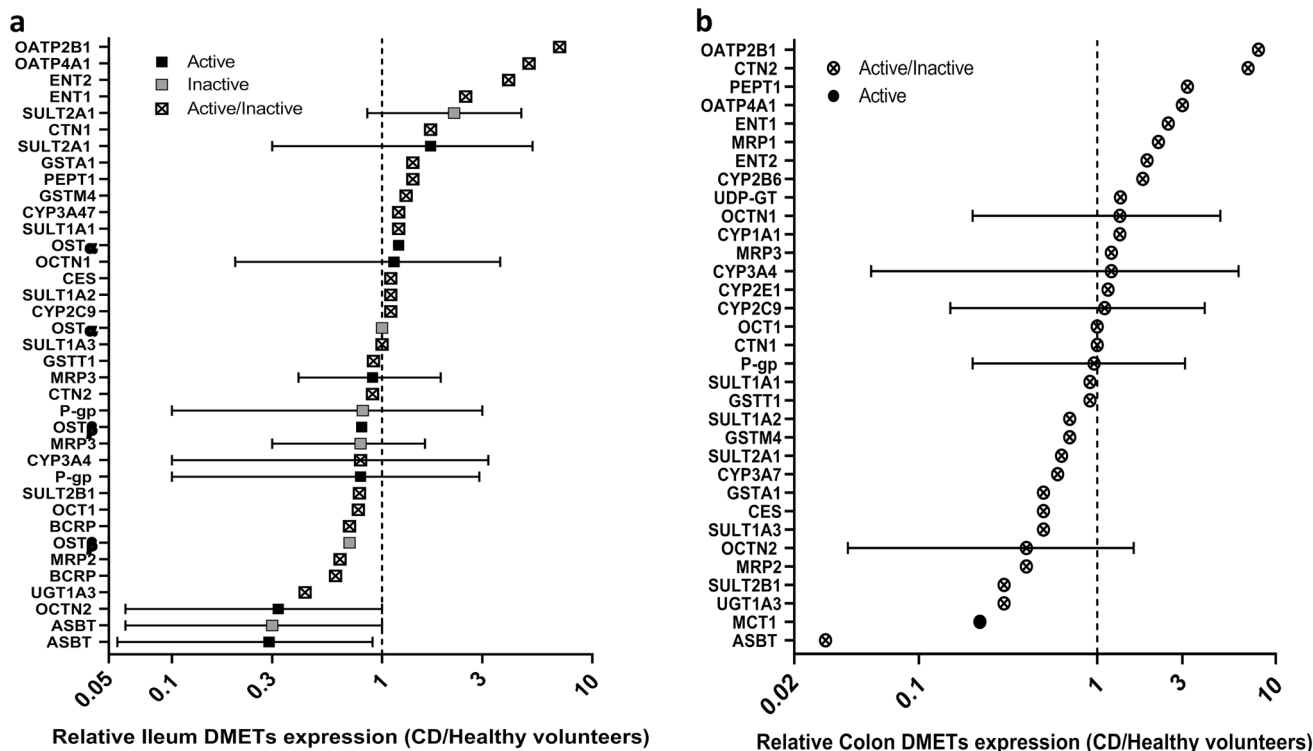
colon, respectively). In general, limited numbers of DMET isoforms were studied and very few were reported in more than one publication. UGTs are strikingly absent from these reports, with only UGT1A3 reported. In addition, there is no in vitro activity data on the human CD population. Details of the studied DMET abundances are summarised in Table S10 and S11 (see the electronic supplementary material).

**Expression of drug metabolising enzymes and transporters in the liver** There is no available direct information on the abundance or activity of DMETs in the livers of CD patients. One study reported a reduction of the hepatic extraction ratio ( $E_H$ ) in eight active CD patients using midazolam as a CYP3A4 probe [39]. From this study, we calculated the fraction of drug entering the liver and escaping first pass hepatic metabolism and biliary secretion ( $F_H$ ) in CD patients as 0.89 and in healthy volunteers as 0.57 [40]. This shows that the liver clearance (CYP3A4 activity) is decreased in CD and midazolam absorption has increased by ~ 56% compared with samples from healthy volunteers. We used an  $F_H$  relative change (CD patients/healthy volunteers) of 1.5 as a reflection of the fold reduction in CYP3A4 abundance. Although, the  $F_H$  value is not linearly related to the change in abundance or activity of CYP3A4, and a back-calculation of intrinsic clearance through Eq. 4 would be needed.

We assessed the suppression of CYP3A4 by back-calculation from the observed midazolam clearance after IV administration and accounted for the changes to blood flow and protein binding. An alternative approach would be to estimate the level of suppression based on the inflammatory proteins circulating in blood, particularly interleukin-6 (IL-6) [110–115]. The suppressive level of hepatic IL-6 is not known in CD patients. However, considering relative higher levels of IL-6 in the blood stream of CD patients [116, 117], some inferences on the lower level of CYP3A4 could be made. A recent simulation study has used such an approach [118]. Broadening the cases related to 3A4 activity in gut and liver as well as proteomics analysis of tissue samples from CD patients will give more confidence on the quantitative level of the changes that occur in these patients.

**Blood proteins (albumin and  $\alpha$ 1-AGP)** Human serum albumin (HSA) and  $\alpha$ 1-acid glycoprotein ( $\alpha$ 1-AGP) levels show dysregulation during CD. Dysregulation of drug–blood protein binding levels would be expected to affect the bioavailability of drugs, depending on the extent of drug–protein binding.

A literature search for HSA in CD patients and healthy adults identified a total of 27 studies (Fig. 11a). In 26 studies, participants with active and inactive CD had a lower



**Fig. 10** The drug metabolising enzymes and transporters (DMETs) expression in Crohn's disease (CD) patients relative to healthy volunteers. Part **a** shows the expression in ileum based on individual

studies, and part **b** shows corresponding values in the colon. The bars represent the 95% confidence intervals (no bar means there was only a single measure)

albumin level compared with controls [52, 55, 57, 119–142], where one study used serum sialic acid as the activity indicator [134], which did not show a difference in the albumin changes compared to other studies that used the CDAI and HBI, and one study showed a slightly higher albumin level in inactive CD patients, where all participants had undergone an ileocecal resection [62]. Our analysis showed a statistically significant decrease in HSA in both CD phases, with active CD exhibiting a higher drop relative to healthy volunteers (Fig. 11b). The sizes of the studies were between nine and 247 CD patients. Details of HSA level studies in CD patients are summarised in Table S12 (see the Electronic Supplementary Material).

Among active CD patients, females showed a greater depletion in HSA than males, with both being significantly lower than healthy values [143]. The HSA level was significantly lower when the same set of CD patients was examined in active state compared with after remission [144]. The results of this study are in agreement with other publications where active CD patients showed a significant drop in albumin compared with inactive CD patients [57, 62, 145].

Importantly, the albumin level is not always low in CD patients, as illustrated by two large-scale publications of 117 [146] and 6082 [147] CD patients. The percentage of the patients who encountered a severe drop in their albumin (< 30 g/L) during active inflammation did not exceed 22%.

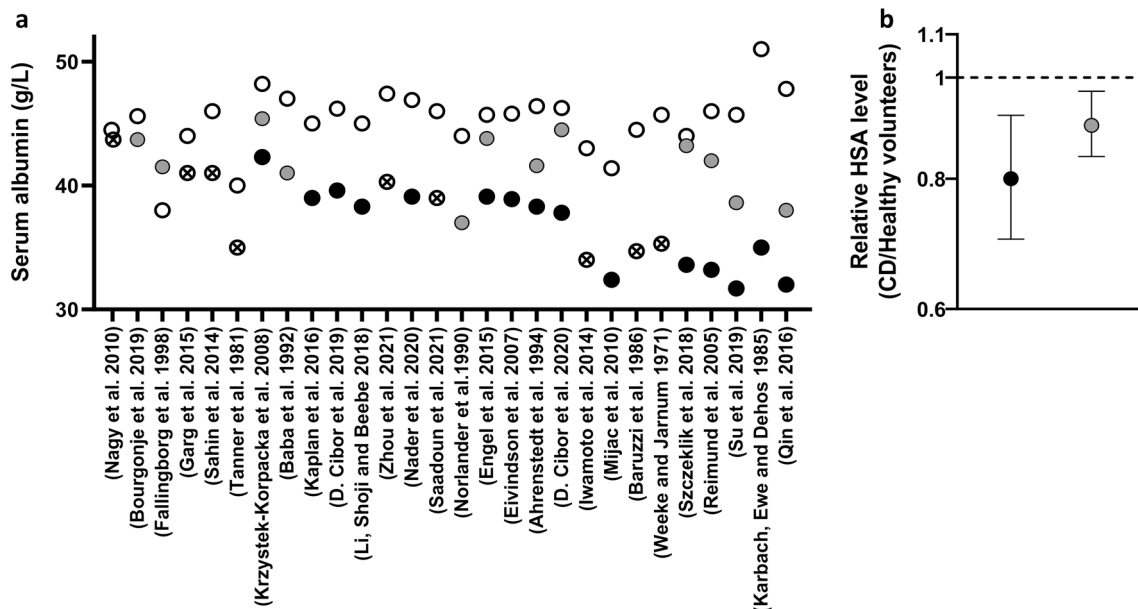
A total of eight studies were identified reporting  $\alpha$ 1-AGP levels in CD patients based on our research criteria [119,

120, 135, 142, 148–151]. The number of CD patients was between two and 51. The reported values of  $\alpha$ 1-AGP in active and inactive CD patients compared to healthy volunteers (Table S13; see the Electronic Supplementary Material) showed that only active CD patients had a significantly higher level compared to healthy volunteers, with the weighted mean determined to be 1.59 g/L (95% CI 0.75–2.97) compared with 0.85 g/L (95% CI 0.37–1.7) in healthy volunteers [135, 142, 148–150]. The  $\alpha$ 1-AGP of the inactive CD patients was in close proximity to that of healthy volunteers, with weighted means (95% CIs) of 1.15 g/L (0.7–1.8) and 0.86 g/L (0.34–1.8), respectively.

### 3.4 Verification Status PBPK Model with Current Systems Data

We created two separate files for the CD patients within the Simcyp<sup>®</sup> simulator for the active and non-active stage, respectively, based on all available data gathered in the report. The created CD populations were alternately applied to budesonide and midazolam (IV and oral administration). The simulations of budesonide and midazolam in Fig. 12 show the overlay of plasma concentration–time profiles of Simcyp<sup>®</sup> healthy volunteers and active and inactive CD population models on the observed clinical data in CD.

As seen from the overlay of the created populations and the PK parameters predicted/observed ratio (Table 2), the inactive population did not capture the observed clinical



**Fig. 11** The human serum albumin (HSA) level (g/L) in Crohn's disease (CD) patients compared to healthy volunteers. Part **a** shows the HSA level reported in individual studies. Part **b** shows the weighted HSA level relative to healthy volunteers from all the studies and the

associated 95% confidence intervals. The white, black, grey and patterned fill of markers indicates healthy, active, inactive and mixture of CD, respectively

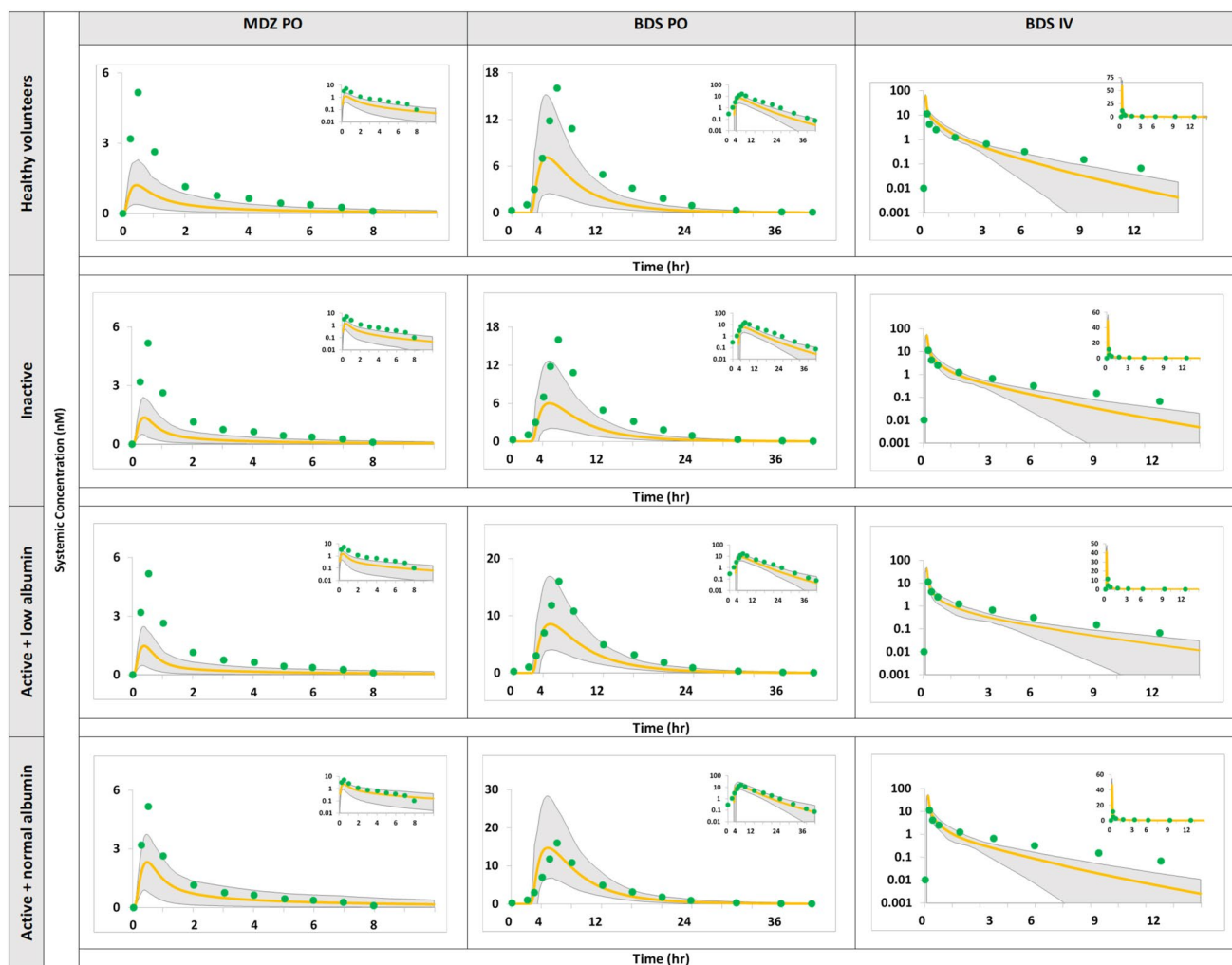
outcome. Both the budesonide and midazolam simulations were greater than twofold relative to the observed.

The simulated PK of oral budesonide was compared with the clinical observations using two different models, one assuming normal levels of albumin and another assuming lower albumin than healthy individuals. Although both predictions were within twofold of the observed data, the predictions assuming normal levels of albumin were closer to the observed values. PK parameters were all within twofold of the observed data as well (Tables 3 and 4). The reason the active CD model (with normal albumin levels) was considered superior to the alternative model with low albumin levels was the fact that the latter was crossing the observed plasma concentration on the borders of the 95% prediction interval. The 95% prediction interval of the simulated

midazolam shows that none of the active CD models were able to capture its in vivo plasma concentration (Tables 3 and 4). Thus, these models cannot be relied on as reproducible PBPK models for the CD population. A global sensitivity analysis was, therefore, carried out to identify the literature gaps that primarily determine the observed alteration of the bioavailability in CD patients.

### 3.5 GSA

All the identified systems parameters that are altered during the course of CD were included in the GSA, but only the parameters that showed the highest influence on AUC,  $C_{\max}$  and  $T_{\max}$  are shown in Fig. 13a–c. The other parameters with low impact are excluded from the presented output data.



**Fig. 12** Simulation of concentration–time profile of midazolam (MDZ) following oral (PO) administration of 0.1 mg solution in the fasted state [39] and budesonide (BDS) after administration of an 18-mg controlled-release PO dose in the fed state and a 0.5-mg intravenous (IV) dose [42], 43. Observed data (green circles) are

compared with the 95% prediction interval (grey region) and the central tendency of profile (yellow line) generated from physiologically based pharmacokinetic models of active and inactive Crohn's disease patients and healthy volunteers



**Table 2** Comparison of the predicted and observed PK parameters of oral midazolam and budesonide in inactive CD populations

Parameter	Midazolam oral solution, fasted			Parameter	Budesonide controlled release, fed		
	Predicted	Observed Mean $\pm$ SD	Predicted/ Observed		Predicted	Observed Mean (95% CI)	Predicted/ Observed
AUC <sub>0-∞</sub> (nM*h)	2.1	14 $\pm$ 6.38	0.15	AUC <sub>0-∞</sub> (nM*h)	46.7	114 (81.4–159.5)	0.41
C <sub>max</sub> (nM)	1.3	8.4 $\pm$ 5.13	0.16	C <sub>max</sub> (nM)	6.22	14.3 (6–13.7)	0.43
T <sub>max</sub> (h)	0.39	0.53 $\pm$ 1.3	0.5	T <sub>max</sub> (h)	4.8	6 (3–8)	0.8
F %	22	31 $\pm$ 22	0.8	F %	10	20.5 (8.8–15)	0.5

AUC area under the curve, CD Crohn's disease, CI confidence interval, C<sub>max</sub> maximum drug concentration, PK pharmacokinetics, T<sub>max</sub> time to reach C<sub>max</sub>, F oral bioavailability

**Table 3** Comparison of the predicted and observed PK parameters of oral midazolam and budesonide based on in active CD population with reduced HSA

Parameter	Midazolam oral solution, fasted			Parameter	Budesonide controlled-release, fed		
	Predicted	Observed Mean $\pm$ SD	Predicted/ observed		Predicted	Observed Mean (95% CI)	Predicted/ observed
AUC <sub>0-∞</sub> (nM*h)	2.4	14 $\pm$ 6.38	0.2	AUC <sub>0-∞</sub> (nmol*h/L)	61.6	114 (81.4–159.5)	0.54
C <sub>max</sub> (nM)	1.45	8.4 $\pm$ 5.13	0.2	C <sub>max</sub> (nM)	7.8	14.3 (6–13.7)	0.55
T <sub>max</sub> (h)	0.37	0.53 $\pm$ 1.3	0.7	T <sub>max</sub> (h)	4.84	6 (3–8)	0.81
F %	27	31 $\pm$ 22	0.9	F %	14	20.5 (8.8–15)	0.7

AUC area under the curve, CD Crohn's disease, CI confidence interval, C<sub>max</sub> maximum drug concentration, HSA human serum albumin, PK pharmacokinetics, T<sub>max</sub> time to reach C<sub>max</sub>, F oral bioavailability

**Table 4** Comparison of the predicted and observed PK parameters of oral midazolam and budesonide in active CD populations with normal HSA

Parameter	Midazolam oral solution, fasted			Parameter	Budesonide controlled release, fed		
	Predicted	Observed Mean $\pm$ SD	Predicted/ Observed		Predicted	Observed Mean (95% CI)	Predicted/ Observed
AUC <sub>0-∞</sub> (nM*h)	5	14 $\pm$ 6.38	0.36	AUC <sub>0-∞</sub> (nM*h)	104.6	114 (81.4–159.5)	0.92
C <sub>max</sub> (nM)	2.31	8.4 $\pm$ 5.13	0.3	C <sub>max</sub> (nM)	14.1	14.3 (6–13.7)	0.99
T <sub>max</sub> (h)	0.45	0.53 $\pm$ 1.3	0.85	T <sub>max</sub> (h)	4.75	6 (3–8)	0.8
F %	34	31 $\pm$ 22	1.1	F %	21	20.5 (8.8–15)	1.02

AUC area under the curve, CD Crohn's disease, CI confidence interval, C<sub>max</sub> maximum drug concentration, HSA human serum albumin, PK pharmacokinetics, T<sub>max</sub> time to reach C<sub>max</sub>, F oral bioavailability

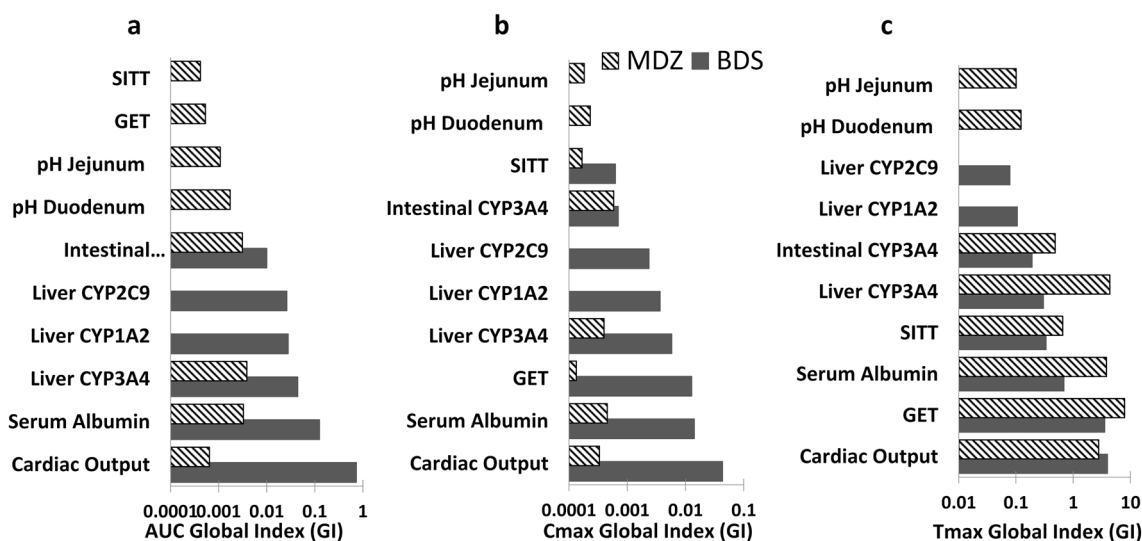
Cardiac output is found to have the highest influence on AUC, C<sub>max</sub> and T<sub>max</sub> of the budesonide PK parameters investigated. HSA comes second for AUC and C<sub>max</sub> and third for T<sub>max</sub>. Gastric emptying time is one of the highest contributors in C<sub>max</sub> and T<sub>max</sub> observations. When it comes to CYP influence, liver CYP3A4 is the most impactful on all three investigated parameters, while small intestine CYP3A4 abundance has its highest impact on T<sub>max</sub>.

Liver CYP3A4 abundance was found to be the major influencer of midazolam AUC, while small intestine CYP3A4 abundance is the major influencer of C<sub>max</sub>. Gastric emptying time has an important influence on T<sub>max</sub>. HSA

comes second for AUC and C<sub>max</sub> and third for T<sub>max</sub>, and liver CYP3A4 abundance comes second for T<sub>max</sub> and third for C<sub>max</sub>. Cardiac output, upper intestine pH and small intestine transit time have varying influences on the three investigated PK parameters.

## 4 Discussion

The systematic data analysis for the kinetics of drugs in CD patients demonstrated clearly that the internal exposure to given doses of drugs can be significantly different from that



**Fig. 13** Relative sensitivity of budesonide (BDS) and midazolam (MDZ) kinetics to the systems parameters from global sensitivity analysis based on physiologically based pharmacokinetics in Crohn's

disease: **a** area under the curve (AUC), **b** maximum drug concentration ( $C_{\max}$ ) and **c** time to reach  $C_{\max}$  ( $T_{\max}$ ). *GET* gastric emptying time, *SITT* small intestine transit time

of healthy volunteers. However, there is no consistent and uniform effect for different drugs or formulations to enable a “one size fits all” recommendation for alteration of a dosage regimen or, indeed, of keeping it the same as for other patients. Unlike in the case of hepatic and renal impairment, where there are regulatory requirements to assess the potential changes in the kinetics, there are no such requirements. Moreover, whilst the changes, if there are any, in the cases of renal and hepatic impairment are in one direction (reduced clearance), the changes in the case of CD patients seem to happen in both directions; exposure may be increased or decreased compared with healthy volunteers.

The classification of CD can be addressed differently when considering the clinical representation of the patient or the objective disease biomarkers. The most commonly used classification of CD in the literature is the symptoms-based system, classifying CD into active and inactive. This is simple, but not very accurate, due to patient variations and the apparent lack of use of more reliable objective markers to define disease activity. For example, magnetic resonance enterography (MRE) can reliably evaluate small bowel CD activity [152], which was not reported in any of the studies assessing the CD activity of this segment. A new classification of CD was introduced based on the localisation (ileal and colonic) of the disease and its relevant clinical presentation and biomarkers profile [153]. If this classification were to be considered, several PBPK population models of CD would be distinct according to the different clinical phenotypes.

Fistula and stricture are common complications of CD that can present alongside the initial diagnosis of CD or

occur later [154]. Epithelial to mesenchymal transition (EMT) and inflammatory biomarkers are associated with their formation. Such complications do not heal with the control of inflammation, and no preventive treatment is in place. Developing an appropriate PBPK model for such CD patients should be done independently from the current models, as their presence alters CD from an inflammatory disease phenotype to a strictured or fistulated phenotype. Currently, this is hard to apply, as various drawbacks prevent accurate assessment of CD activity. The literature does not usually distinguish between CD types based on the location of the disease; rather, it distinguishes the disease, based on the clinical profile of the patient, as active and inactive CD.

When examining the systemic exposure of CYP3A oral substrates, which represent the majority of drugs with available clinical data, a trend of higher exposure is more common, but cannot be generalised, as the available data on active and inactive CD are clouded by uncertainties, which hinders obtaining a definite conclusion. The same applies for the clearance of IV drugs. For the non-CYP3A substrates, no clear trend could be concluded. A higher variability is seen, but is inferred from a small number of studies. The changes are multi-factorial, and the interplay of the drug/formulation with the changes in CD can help in postulating the likely effects. This necessitates further clinical data to establish the disease impact and the magnitude of its activity.

Hence, application of PBPK might be the best chance for rationalising the dosage changes (when needed) in CD patients, as suggested in the case of bariatric surgery patients, who also do not get dedicated studies for various drugs coming to the market [10].

To develop the CD PBPK population, the current systems data defining active and inactive CD patients are captured in this article, whether obtained from independent studies or from a reverse translation approach. The data are not abundant, and some parameters lack definition. More dramatic alteration is seen with active disease patients relative to patients in remission. The predominant alterations are seen with the increase of SMA flow, small intestine transit time and reduction of albumin, where the dysregulation severity in HSA level, SMA and the intestine flow were reported to correlate with the active disease severity [88, 94, 136, 155–157]. The increase of gastric emptying time and small intestine transit time will affect the absorption rate and solubility of oral drugs.

Although an increase of gut wall thickness can affect gut fluid dynamics, a meta-analysis was not carried out for this parameter even though it showed an approximately twofold increase in active CD patients compared to healthy volunteers in the few studies encountered [78, 80, 81, 93, 158]. This is because the utility of the intestine loop morphology is not clear in the Simcyp® M-ADAM model; instead, the villous morphology is established in Simcyp®. For CD patients, reduction of the duodenum and ileum villous length was reported in paediatric patients [159]. From the carried GSA in this study, the villous morphological changes had little impact on midazolam and budesonide AUC and  $C_{\max}$ , and only a very limited impact was seen on midazolam  $T_{\max}$ ; thus, this was not incorporated in our model.

Enterocyte turnover rate is another aspect not included in our model. Turnover of the epithelial cells in CD patients was reported to be faster than in healthy volunteers, but the measurement varies from one study to another. Moreover, some of these reports are on preclinical models of CD, rather than real patients [160]. There are also reports on faster production of cytokines, cadherin or other proteins [161–164] and the excretion of bile acids [165] as an indicator of the different cell turnover. Therefore, currently, it is difficult to reach a consensus regarding a specific value for enterocyte turnover in CD patients, despite general agreement that it would be faster than in healthy individuals.

The intestine microbiota is an important aspect to include when creating a CD virtual population. Unfortunately, the topic suffers from the same problem, as it is not clearly defined for the different clinical phenotypes of the disease, and the diversity of the addressed bacteria and their role hinders its accurate representation in the current, developed models. CD patients suffer from change in the intestine microbiota, which might alter the intestine environment and homeostasis [166–168], affecting oral drug bioavailability, although, the evidence for such effects is lacking and most oral drug absorption takes place before the drug comes into contact with the microbiota. In future work, focus on this topic should be prioritised, especially if the studied drugs are

activated/metabolised by the bacterial enzymes of the intestine [169, 170]. Dysregulation of DMETs in the liver has not been addressed except for the reduction of CYP3A4 activity in active CD patients [39]. This reduction might be a major contributor in the significant difference of the systemic exposure seen with CYP3A substrates midazolam, verapamil and budesonide in the CD population. In patients with primary sclerosing cholangitis (PSC), a comorbidity associated with CD [171], investigators reported an insignificant reduction of liver CYP3A4 expression and a notable increase of OST $\beta$  in participants with diseases other than CD [172, 173]. This supports the possible deterioration of liver metabolism in CD and promotes investigation into the abundance and activity of liver DMETs in the CD population, with and without relation to comorbidities. Liver DMETs alteration will cause a considerable change in drug clearance, as the liver is the major xenobiotic-metabolising organ [174].

CYP3A4 expression has shown different results in the ileum and colon [97, 99, 100], but overall, a reduction of its expression is seen. Similar observations are seen with p-gp expression [97–100, 103]. The intestine CYP3A4 reduction influence might seem less pronounced, but the simulation of midazolam and budesonide based on the liver CYP3A4 alteration did not capture the clinical data and the intestine involvement is greater than anticipated based on our GSA results. Other non-CYP DMEs abundance/activity should be addressed in the liver and the intestine, as they metabolise about 30% of approved drugs [175]. Undesired drug–disease interactions can occur, which might result in several complications and delays in disease control.

Intestinal permeability is another major player in the observed changes. It is altered in response to the elevation of inflammatory biomarkers changing the drug transporter expression during the course of CD [176–178]. The available data on the intestine transporters in CD are mainly based on gene expression levels. The gene expression analysis does not have a high correlation with direct protein expression measurements for all proteins [179–190]. Establishing a relationship that would reflect the correlation between protein mRNA expression and direct concentration is challenging, as it was found that in around 60% of proteins, mRNA levels do not highly correlate with abundance [191].

Propranolol, a CYP (2C9, 1A2, 2D6) and p-gp substrate, showed a significant difference between its exposure in CD patients and that in healthy volunteers [59, 192]. More severe inflammation indicated by higher ESR was associated with higher propranolol concentration. When ESR was low, the concentration of propranolol in rheumatoid arthritis patients was similar to that in the healthy group, but this was not the case in the CD group, as the concentration remained significantly higher [59]. This might be attributed to localisation of the inflammation to the intestine in CD causing alteration of the DMET protein abundances. Therefore,

systemic exposure of oral drugs in CD cannot be explained by the liver factors alone or intestine factors alone.

In vitro data for DMETs are lacking in the CD population; these data would allow identification of the intestinal intrinsic clearance and the microsomal fraction unbound to enable reproducible and more accurate prediction of oral drug PK, and differentiation between the involvement of the main metabolising organs.

In inflammatory conditions, albumin synthesis by the liver can be shifted [193, 194]. Albumin reduction will affect the drug fraction unbound in the plasma ( $f_{up}$ ). This will affect drug disposition and lead to an increase in free drug plasma concentration. The volume of distribution ( $V_{ss}$ ) and clearance are sensitive to  $f_{up}$  values [195, 196]. Underprediction of  $f_{up}$  can lead to underprediction of clearance and hinder the prediction accuracy for drugs with a high protein binding affinity [197, 198]. Correlation between albumin elevation and IL-6 reduction was reported as an indicator of medication effectiveness and progression to a remission state in CD patients [144] and also to the severity of the disease of IBD patients (CD and UC) [199]. The displacement of the high extraction ratio drugs like budesonide from plasma proteins will be of similar magnitude to the displacement of low extraction ratio drugs.

Patients who underwent resection surgery would suffer from consequences that might alter the physiological aspects that affect oral drugs absorption. Short bowel syndrome (SBS) is a disorder where patients suffer from malabsorption due to bowel resection [200]. SBS complications, alteration in the gut microbiota, reduction of gastric emptying time and small intestine transit time, higher intestine pH, reduction of the absorption area and altered transporters profile [62, 201–207], can affect the absorption of a wide range of drugs [208]. Further to these complications, the bile acid composition is altered as a result of its malabsorption [209, 210], especially when the surgical procedure included removal of the upper intestine parts (jejunum and ileum), where the bile acids get absorbed [211, 212]. Surgeries targeting the ileum could lead to loss of ASBT transporter, a major contributor in the absorption of bile acid [213]. Loss of the bile acid would affect the absorption and solubilisation of lipophilic drugs such as cyclosporine [214], lovastatin [215] and ampicillin [216], impairing their bioavailability. Along with the size of the resection, the location of the surgery influences the extent of the impact on absorption following resection. Colostomy's influence is expected to be more restrained, affecting special formulation drugs directed to be released in the lower segments of the intestine. Ileostomy, on the other hand, could lead to more drastic absorption alteration, since it represents a larger surface area and higher abundance of DMETs, where most conventional oral formulations are absorbed [54, 217]. Therefore, caution should be taken when dealing with patients with prior bowel surgery, as their

physiological profile can be significantly different from that of other CD patients. Thus, rectification of the created PBPK model is necessary to deliver a more accurate insight into the appropriate dose adjustment for these patients.

The case of oral budesonide requires further investigation as the different studies reporting its bioavailability in CD did not show significant variation from healthy volunteers [39, 43, 44] except for one [42]. The different studies followed different dose regimens under fed and fasted conditions, but this was reported to carry no significant effect on the drug PK. The male-to-female proportion and the inclusion of active and inactive study participants varies among the studies and could contribute to the disparities noticed between studies. Budesonide is a high extraction ratio drug that is highly affected by the blood flow, which is subject to individual variability. The fed state results in increasing the splanchnic blood flow, which leads to increased absorption [218]. This indicates that when an oral high extraction drug is under investigation, many different factors contribute to its PK behaviour, and these factors are changeable, specifically in a disease situation, where the change is caused by a combination of organ dysfunction and alterations in physiological factors all together at the same time.

The budesonide immediate-release and controlled-release formulations encountered varying changes in systemic exposure, which might be linked to the additive relationship of CYP3A4 and p-gp, as it is a substrate of both. The function of CYP3A4 and p-gp as a barrier for drug absorption [219–221] has been supported by in silico modelling [222–225] and in vitro experiments [226, 227]. The difference between immediate-release and controlled-release formulations for combined CYP3A4 and p-gp substrates was examined, and no significant differences in the AUC between the two formulations were reported [17].

The active CD model only captured the budesonide [42] plasma concentration–time profile in CD patients, but not the midazolam profile [39]. A previously reported CD population PBPK model was capable of capturing budesonide based on modifying several parameters in CD [45]. The patients included in the budesonide study were both active and inactive CD patients, while in the corresponding midazolam study, there were only active CD patients. Therefore, the models applied to the selected drugs fully characterised in CD patients following IV and oral dose (budesonide and midazolam) are not sufficient to produce robust PBPK models that can predict the likely outcome of PK studies.

To provide a map for future studies, we conducted a global sensitivity analysis showing variations in the intensities of the effects of the most influential physiological parameters on midazolam and budesonide PK. The observed variations are attributed to different factors related to the drug's nature and formulation, in addition to the patient activity state, where the budesonide study included a

mixture of active and inactive patients and the midazolam study included only active state participants.

New oral molecules have been investigated and introduced for the treatment of CD. Filgotinib and upadacitinib are Janus kinase (JAK) inhibitors that have successfully passed phase II of the clinical trials, with promising efficacy and safety profiles. Other JAK agents are still under development [228]. Sphingosine 1-phosphate (S1P) modulators such as ozanimod, etrasimod and amiselimod are another class of oral drugs that has shown promising clinical results for CD [229]. Other oral anti-trafficking therapies and PDE4 inhibitors did not pass clinical trials for CD, but showed efficacy for UC [230]. The use of a reliable PBPK model would aid in the drug development process and give insight into what to expect from the drug in such a special population.

Therefore, going forward, differentiation between active and inactive CD is essential. This is identified from the available literature data collected for the systems parameters as well as limited clinical observations and PBPK simulations. However, the definition of the activity state of the disease in all of the included studies was hard to unify. The activity state was mainly based on subjective clinical patient data, with or without objective biomarkers participating in this variability. Due to the multifactorial physiological alterations of active and inactive CD, attempts to explain observed changes to oral bioavailability in the CD population using single attributes of drugs or simplified modelling is not successful.

## 5 Conclusion

The most influential parameters determining the changes to bioavailability of the two investigated drugs in the CD population were the expression/activity of liver and intestine enzymes. Both of these parameters currently lack a reliable value in CD patients. This gap stands between the aspiration to apply PBPK for predicting the alterations of drug fate in CD populations to guide dosing, and the practical ability to do such predictions. Therefore, we urge all researchers in this field to boost efforts to generate such information, which should be provided separately for the active and inactive status of CD patients when possible.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s40262-022-01169-4>.

## Declarations

**Conflict of interest** The PBPK models were provided by Certara. Amin Rostami-Hodjegan is an employee of Certara UK Limited and holds shares in Certara. The rest of the authors have no conflicts of interest to declare.

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**Author contributions** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by SA and ARH. The first draft of the manuscript was written by SA, and all authors have commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

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**Code availability** Not applicable.

**Availability of data and material** The datasets generated or analysed during the current study are included in this article and in its supplementary material files, which can be found in the online version of this article.

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