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Gastrointestinal diseases and their impact on drug solubility: Celiac disease

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Abstract

The aim of this study was to develop an *in vitro* tool for predicting drug solubility and dissolution in intestinal fluids of patients with Celiac disease (CED). Biorelevant media for patients with CED were developed based on published information and a Design of Experiment (DoE) approach. The CED biorelevant media were characterised according to their surface tension, osmolality, dynamic viscosity and buffer capacity. By performing solubility studies of six drugs with different physicochemical properties in CED media, we aimed to identify drugs at high risk of altered luminal solubility in CED patients. Identified differences in CED patients compared to healthy subjects were related to a higher concentration of bile salts, lecithin and cholesterol and included as factors in the DoE resulting in 8 CED biorelevant media. Differences in media properties were observed for the surface tension between biorelevant media based on CED patients and healthy subjects. In terms of solubility, only a minimal effect of CED on the solubility of the hydrophilic neutral compound azathioprine was observed. For neutral moderately lipophilic compounds (budesonide, celecoxib) a higher surfactant concentration resulted in most cases in a higher drug solubility, while it was specific to each drug whether this was mainly driven by bile salts or lecithin. In comparison, drug solubilisation of ionisable compounds with moderate to high lipophilicity was less impacted by CED differences. The developed biorelevant CED media serve as *in vitro* tool to identify the main media factors impacting on drug solubility.

Keywords

Gastrointestinal diseases; Celiac disease; Biorelevant media; Physicochemical properties; Solubility

1. Introduction

Celiac disease (CED) is a chronic auto-inflammatory disease induced by an intolerance to dietary gluten, a storage protein of wheat, rye, barley and oats. Approximately 1% of the population is affected by CED and its aetiology is a combination of genetic predisposition and environmental factors (e.g., breastfeeding, time of gluten introduction and the microbiota) (Koehler et al., 2014). CED mainly affects the small intestine resulting in gastrointestinal (GI) symptoms such as bloating, diarrhoea, malabsorptive symptoms and weight loss. Additionally, CED patients can present extra-intestinal symptoms such as dermatitis herpetiformis, anaemia or osteoporosis (Leffler et al., 2015). The diagnosis involves serological testing for autoantibodies (anti-tTG, anti-EMA) and an endoscopic biopsy (Turner et al., 2015). Depending on the damage to the small intestine, the disease can be classified in different disease grades based on histological findings such as crypt hyperplasia, the constitution of the villi and the intra-epithelial lymphocytes in the jejunum and duodenum (Oberhuber et al., 1999). For the treatment of CED, patients need to adhere to a gluten-free diet, the only known effective treatment to date, since the reintroduction of dietary gluten results in a relapse of the disease (Gottlieb et al., 2015). More treatment options are expected to emerge in the near future, since several new active pharmaceutical ingredients have reached clinical phases of drug development in recent years (Gottlieb et al., 2015).

Patient convenience dictates that oral administration is the preferred route of drug administration for most drugs. Consequently, patients with CED are likely to be treated with orally administered drug products for concomitant conditions or extra-intestinal manifestations of CED. Since oral drug administration is, apart from drug and formulation properties, dependent on gastrointestinal physiology, pathophysiological changes in CED could affect drug safety and efficacy. GI diseases can affect various processes involved in oral drug delivery e.g., drug release from the formulation, drug dissolution, permeation through the GI membrane

and gut or hepatic metabolism (Effinger et al., 2019). Altered drug absorption in CED patients compared to healthy subjects has previously been attributed to a reduced small intestinal surface area, a different intestinal CYP enzyme abundance, a higher jejunal permeability and differences in gastric emptying (Tran et al., 2013).

So far, there is only a small number of drugs for which drug product performance has been investigated in CED patients and these studies included only a small number of patients (Tran et al., 2013). Due to the high costs of clinical trials, it is expected that in the future investigations in CED patients will remain rare.

For poorly soluble drugs, drug absorption can be limited by the dissolution rate or the solubility of the drug in gastrointestinal fluids (Amidon et al., 1995). If this is the case, *in vitro* release and dissolution testing can be used as surrogate for a drug's *in vivo* performance (Amidon et al., 1995). To simulate closely the conditions present in the GI tract, biorelevant media were developed mimicking the composition of the gastrointestinal fluids of healthy subjects (Galia et al., 1998; Jantratid et al., 2008; Markopoulos et al., 2015; Vertzoni et al., 2010; Vertzoni et al., 2005). The composition of the gastrointestinal fluids can be altered in patients with GI disease and therefore, *in vitro* dissolution and solubility studies with biorelevant media adapted to pathophysiological conditions could result in better predictions of drug product performance in patient populations (Effinger et al., 2019).

This study aims to identify drugs at risk of altered solubility in GI fluids of CED patients. Biorelevant media for patients with CED representative of the small intestinal fluid in the fasted and fed state were developed. Information from literature was collected to identify differences in the composition of luminal contents of patients with CED compared to healthy subjects. Biorelevant media for CED patients were developed based on biorelevant media for healthy subjects and a Design of Experiment (DoE) approach by integrating the identified differences

as factors with two levels. Subsequently, the CED biorelevant media were characterised in terms of surface tension, osmolality, buffer capacity and dynamic viscosity. Additionally, the solubility of six compounds with different physicochemical properties (including azathioprine, budesonide, celecoxib, dipyridamole, loperamide and sulfasalazine), in the developed biorelevant media based on CED patients and healthy subjects was determined.

2. Materials

Acetic acid High Performance Liquid Chromatography (HPLC) grade, chloroform, sodium oleate, budesonide, phosphoric acid and sodium hydroxide were purchased from Sigma-Aldrich Company Ltd., Dorset, England. Sulfasalazine, loperamide hydrochloride, dipyridamole, celecoxib, azathioprine, methanol HPLC grade and acetonitrile HPLC grade were purchased from VWR International Ltd, Lutterworth, UK. Sodium chloride, trifluoroacetic acid (TFA), potassium dihydrogen orthophosphate, dimethyl sulfoxide and maleic acid were used from Fisher Scientific UK Ltd., Loughborough, England. Other chemicals used included sodium taurocholate (Prodotti Chimici Alimentari S.P.A., Basaluzzo, Italy), egg lecithin–Lipoid EPCS (Lipoid GmbH, Ludwigshafen, Germany), glyceryl monooleate–Rylo Mg 19 (Danisco, Brabrand, Denmark) and cholesterol (95%, Acros Organics, Geel, Belgium). Water was ultra-pure (Milli-Q) laboratory grade.

3. Methods

3.1. Media development

3.1.1. GI physiological differences in CED compared to healthy subjects

To identify differences in the composition of GI fluids of untreated CED patients compared to healthy subjects, a literature search was performed. Since to date the GI fluids of CED patients have not been directly characterised, studies investigating parameters that most likely impact on GI fluids were considered.

The bile flow and biliary lipid output has previously been measured in untreated CED patients and healthy subjects during a constant infusion of a liquid formula diet using a duodenal intubation technique (Vuoristo and Miettinen, 1985). Biliary lipid outputs such as cholesterol, bile acids and phospholipids could be estimated in comparison to the dilution of a marker (polyethylene glycol 4000). The bile flow was with 232±29 mL/h (mean ± SD) significantly higher in CED patients compared to 132±24 mL/h in healthy subjects (Student's t-test, $p<0.05$). The biliary cholesterol output normalised to the body weight was significantly increased in CED patients (0.82±0.10 vs 0.43±0.06 mg/kg*h, $p<0.02$). Similarly, the biliary output of phospholipids was also highly increased in CED patients compared to healthy subjects (0.26±0.05 vs 0.08±0.02 mg/kg*h, $p<0.02$). Additionally, a higher bile acid output was observed in CED patients (9.28±1.65 vs 4.64±0.45 mg/kg*h). In accordance, it was observed that the bile salt pool is three times higher in CED patients compared to healthy subjects, which could be related to a very effective ileal reabsorption of bile acids or a sluggish contraction of the gall bladder (Low-Beer et al., 1973). Since our study was based on untreated CED patients (not adhering to a gluten-free diet), dietary differences between CED patients and healthy subjects were not considered.

3.1.2. Development of CED media with Design of Experiment

The development of biorelevant media for CED patients followed a DoE approach and CED biorelevant media representative of the small intestinal fluid in the fasted and fed state were developed. Biorelevant media previously developed based on healthy subjects were used as the basis for CED biorelevant media and included Fasted-State Simulated Intestinal Fluid-Version 2 (FaSSIF-V2) and Fed-State Simulated Intestinal Fluid-Version 2 (FeSSIF-V2) (Jantratid et al., 2008). According to the identified differences described in Section 3.1.1, biorelevant media based on healthy subjects were modified by including the differences as factors in the experimental design. For both prandial states, the integrated factors in the experimental design

were the concentration of bile salts, lecithin and cholesterol. Since the biliary secretion is the main source of bile salts, lecithin and cholesterol present in the intestinal fluids, a direct correlation between biliary output and intestinal concentration was assumed. Since the three parameters were not directly measured in the GI fluids, an indirect percental approach was followed to determine the level of the corresponding factor according to

$$x_{CED-BM} = \frac{y_{CED}}{y_H} * x_{H-BM} \quad (1)$$

where x_{CED-BM} is the high level of the factor in CED media, y_{CED} and y_H are the median of the corresponding biliary output observed in CED patients and healthy subjects, respectively and x_{H-BM} is the level of the factor in biorelevant media based on healthy subjects.

The three factors were integrated with two levels in the experimental design, a low and a high level. The low level was based on the concentration in biorelevant media based on healthy subjects (Table 1) and the high level corresponded to the median percentage of the respective concentration in the healthy medium. For cholesterol, the low level concentration was based on the median concentration of cholesterol observed in human intestinal fluid as observed by Riethorst et al. (2016) [fasted state: 0.08 mM, fed state: 0.57 mM], since cholesterol is not a component of FaSSIF-V2 and FeSSIF-V2.

The DoE was performed with Statgraphics Centurion 18 (Statpoint Technologies Inc., VA, US) with a full factorial design for CED intestinal biorelevant media for the fasted and fed state. An overview of the DoE is given in Figure 1. Biorelevant media were prepared as previously described with an additional step of adding cholesterol (Jantratid et al., 2008). The cholesterol solution (50 mg/mL in chloroform) was mixed with a lecithin solution (100 mg/ml in dichloromethane) using a magnetic stirrer, before being added to the bile salt/buffer mixture and driven off using a rotary evaporator Büchi Rotovapor R-114 (Büchi Labotechnik, Flawil, Switzerland) according to the published protocol. The osmolality of CED media was set to the

value in the corresponding biorelevant medium based on healthy subjects by adjusting the concentration of sodium chloride.

3.1.3. Media characterisation

Surface tension, osmolality, dynamic viscosity and buffer capacity of biorelevant media previously developed based on healthy subjects and newly developed for CED patients were measured in triplicate. The results are reported as mean with standard deviation.

3.1.3.1. Surface tension

Surface tension measurements were performed at room temperature with a ring tensiometer (Sigma 700 Force tensiometer, Attension, UK) using approximately 10 mL of each medium, placed in a glass vessel with a diameter of 46 mm. A platinum Du Noüy ring was lowered below the meniscus of the medium. Subsequently, by pushing and pulling the ring through the surface of the medium, the force exerted by the meniscus was measured and related to the surface tension of the medium (Butt et al., 2004).

3.1.3.2. Osmolality

Osmolality was determined with an Advanced Instruments Inc. micro-osmometer Model 3300 (Norwood, MA, US). Therefore, the freezing-point depression of a 20 µl sample was measured with a high-precision thermistor following the supercooling and induced crystallisation of the sample.

3.1.3.3. Dynamic viscosity

The dynamic viscosity at 37ºC was measured with a Bohlin Rheometer C-VOR (Malvern instruments, UK). Therefore, a cone-plate measuring system, including a rotating upper cone (4°, 40mm) and a fixed lower plate with the medium contained between them, was used. The shear rate was measured while twenty different shear stresses, logarithmically distributed in the range of 0.05 to 0.15 Pa, were exerted on the sample of the medium. The ratio of shear stress to shear rate corresponds to the dynamic viscosity.

3.1.3.4. Buffer capacity

Buffer capacity was determined using a potentiometric titration method. Therefore, small volumes of 0.5 M hydrochloric acid were added to 10 mL of sample until a change of one pH unit was recorded by a Mettler Toledo SevenCompact S220 pH meter (Schwerzenbach, Switzerland). Equation (2) was used to calculate the buffer capacity (β) according to

$$\beta = \left(\frac{0.5M * V_{acid}}{\Delta pH}\right) * \frac{1000}{V_s} \quad (2)$$

where V_{acid} is the volume of the acid added, V_s is the volume of the sample and ΔpH corresponds to the change in pH (Rabbie et al., 2015).

3.2. Compound selection

For the solubility studies, low soluble compounds belonging to Biopharmaceutics Classification System (BCS) class II (low solubility, high permeability) or IV (low solubility, low permeability) were selected as shown in Table 1. Additionally, the selected drugs varied in their ionization properties (pKa) and lipophilicity (logP). Drugs with indication for gastrointestinal diseases were preferred.

Table 1: Properties and indication of selected compounds for solubility studies.

Drug	pKa (acid/base)	logP	BCS class	Intrinsic aqueous solubility [mg/mL]	Indication
Azathioprine	7.9 (acid) (Mitra and Narurkar, 1987)	0.1 (Hansch et al., 1995)	IV (Lindenberg et al., 2004)	0.171 (Llinas et al., 2008)	Immunosuppressive
Budesonide	12.0 (acid) (Corey and Fossel, 2016)	2.6 (Bharate et al., 2016)	II (Bhatt et al., 2014)	0.028 (Ali et al., 2010)	Locally acting corticosteroid in IBD
Celecoxib	11.1 (acid) (G.D. Searle LLC Division of Pfizer Inc, 2019)	3.5 (G.D. Searle LLC Division of Pfizer Inc, 2019)	II (Paulson et al., 2001)	0.003 - 0.007 (Paulson et al., 2001)	Nonsteroidal anti-inflammatory drug
Dipyridamole	6.4 (base) (Pedersen, 1979)	2.2 (Betageri and Dipali, 1993)	II (Zaki et al., 2010)	0.003 (Hopfinger et al., 2009)	Platelet aggregation inhibitor
Loperamide	8.6 (base) (Manallack, 2007)	5.5 (Dickson et al., 2017)	II (Zaki et al., 2010)		Anti-diarrheal agent
Sulfasalazine	2.3, 7.9 (acid) (Shalaeva et al., 2008)	2.9 (Graham and Pile, 2015)	II/IV (Lindenberg et al., 2004)	0.29×10^{-3} (Llinas et al., 2008)	Anti-inflammatory agent in IBD

3.3. Solubility studies

The shake-flask method was used to determine the solubility of the investigated compounds (Baka et al., 2008). Therefore, an excess amount of drug was added to 5 mL of the respective medium in a glass tube, which was then placed in a shaking water bath (Grant instruments, UK) and maintained at 37°C and 200 strokes/min for 24 h. Subsequently, GF/D membrane filters with a pore size of 2.7 µm (Whatman® Puradisc, diameter 13 mm) were used to filter the sample followed by quantitative analysis with HPLC/UV. The solubility studies were

performed in triplicate in CED disease media and healthy media and average solubility differences between CED media and healthy media were expressed as a % Relative effect on solubility [$((S_{CED}-S_{Healthy})/ S_{Healthy}) \times 100$]. A higher drug solubility in CED media compared to healthy media is indicated by a positive value, whereas the opposite is indicated for negative values. HPLC analysis was performed with an Agilent Technologies 1200 series HPLC system (Santa Clara, CA) including a binary pump (G1212A), an autosampler (G1329A), a thermostatted column compartment (G1316A) and a diode array detector (G1315D). The methods used for the HPLC-UV analysis of the six drugs were modifications of previously published methods (presented in Gastrointestinal diseases and their impact on drug solubility: Crohn's disease) (Effinger et al., 2020).

3.4. Statistical analysis

Differences between media properties and drug solubility in biorelevant media based on CED patients compared to healthy subjects were identified with the software XLSTAT (Addinsoft, France) using one-way analysis of variance (ANOVA) with a post-hoc Tukey's test and a significance level of $p \leq 0.05$.

A multifactorial ANOVA performed in Statgraphics Centurion 18 (Statpoint Technologies Inc., VA, US) was used to estimate the effects of the three categorical variables (bile salts, lecithin, cholesterol) and two-factor interactions in the DoE on the solubility of each of the six investigated compounds. Factors were considered statistically significant if the p-value was less than 0.05, indicating an effect on drug solubility at the 95.00% confidence level.

4. Results and discussion

4.1. Media characterisation

The surface tension of intestinal CED biorelevant media is shown in Figure 2 and was in the range of 45.5 to 51.6 mN/m and of 26.6 to 35.7 mN/m for the fasted and fed state, respectively. In the fasted state, the surface tension of all media with low bile salt concentration was higher compared to the healthy medium ($p<0.05$). This finding is consistent with another study, where a higher surface tension was observed for reduced bile salt concentrations in fasted state simulating fluids without cholesterol (Xie et al., 2014). Additionally, media with at the same time high bile salt and lecithin concentrations possessed a significantly higher surface tension compared to the healthy medium but a lower surface tension compared to all CED media with low bile salt concentrations ($p<0.05$). In the fed state, the surface tension of all CED media with low lecithin concentrations, except for the medium with at the same time low bile salt and cholesterol concentrations, was significantly decreased ($p<0.05$).

The osmolality of biorelevant media based on CED patients and healthy subjects was not significantly different.

The measured dynamic viscosities of CED biorelevant media at a shear stress of 0.06 Pa, 0.08 Pa and 0.15 Pa are presented in Figure 3. All healthy and CED media showed shear thinning behaviour. The viscosity of CED biorelevant media at an applied shear stress of 0.15 Pa was in the range of 3.26 to 3.56 mPas, at 0.08 Pa in the range of 3.70 to 4.56 mPas and at 0.06 Pa in the range of 4.28 to 6.42 mPas, respectively. No significant differences between biorelevant media based on CED patients and healthy subjects were observed considering all three different shear stresses ($p<0.05$).

The buffer capacity was not significantly different in fasted and fed state intestinal media based on healthy subjects compared to CED patients, since the same buffer composition was used and no changes of the media pH were applied (data not shown).

4.2. Solubility of drugs in CED biorelevant media

Considering the final pH value of the medium after 24 h, the pH was within 6.5 ± 0.1 and 5.8 ± 0.1 in all cases except for the sulfasalazine studies in fasted (final medium pH: 6.2 ± 0.1) and fed state (final medium pH 5.7 ± 0.1) intestinal media.

4.2.1. Neutral drugs

The results of the solubility studies with neutral compounds in CED fasted and fed state intestinal media are illustrated in Figure 4.

For azathioprine, the solubility in the fasted state was not significantly different in CED media compared to healthy media. In the fed state, the solubility of azathioprine was significantly higher in CED biorelevant media with high concentrations of bile salts but the relative increase was for all media below 15%.

For budesonide, the solubility in all fasted state CED biorelevant media was significantly higher compared to the healthy medium ($p<0.05$), whereby the solubility of budesonide was highest in CED media with high bile salt concentrations. The positive effect of bile salts is in accordance with a previous study showing that an increase of the concentration of bile salts in a fixed 4:1 ratio of bile salts to lecithin resulted in an increase in budesonide solubility (Soderlind et al., 2010). Additionally, the positive effect of cholesterol on budesonide solubilisation indicates a drug-cholesterol interaction or a positive solubilisation effect of more complex vesicles (sodium taurocholate-lecithin-cholesterol) as previously reported for fenofibrate (Khoshakhlagh et al., 2015).

In the fed state, the solubility of budesonide in the CED media with at the same time low concentrations of bile salts and lecithin was significantly decreased compared to the healthy medium ($p<0.05$), indicating a competition for solubilisation between cholesterol and budesonide possibly due to the similarity of their chemical structure. In contrast, a significantly higher solubility was observed in CED media with high concentrations of bile salts and lecithin and CED media with either a high concentration of bile salts or lecithin and a low concentration of cholesterol ($p<0.05$), indicating a positive effect of higher surfactant concentration and a negative effect of cholesterol on budesonide solubility.

For celecoxib, the solubility in fasted state CED media with a high concentration of lecithin and a low concentration of cholesterol was significantly higher compared to the healthy medium. In contrast, in all other CED fasted state media, the solubility of celecoxib was significantly lower ($p<0.05$). The positive effect of lecithin on celecoxib solubility is in accordance with previous results revealing a higher solubility of celecoxib in FaSSIF (higher concentration of lecithin) compared to FaSSIF-V2 (Shono et al., 2009).

In the fed state, the solubility of celecoxib was significantly higher in CED media with at the same time high concentrations of bile salts and lecithin ($p<0.05$), suggesting a positive effect of luminal surfactants on celecoxib solubility.

4.2.2. Weak acid

The results of the solubility studies in CED fasted and fed state intestinal media with compounds possessing different ionisation properties are presented in Figure 5.

For the weak acid sulfasalazine, the solubility in fasted state CED media with at the same time high concentrations of lecithin and low concentrations of cholesterol is significantly lower compared to the healthy medium ($p<0.05$). In fed state intestinal media, the solubility of sulfasalazine was significantly higher in CED media with high bile salt concentrations and in

the medium with a low concentration of bile salts and lecithin and a high concentration of cholesterol.

4.2.3. Weak bases

For the weak base dipyridamole, the solubility was significantly higher in fasted state CED media with high bile salt concentrations and to a lower extent also in the medium with a high concentration of lecithin and low concentrations of bile salts and cholesterol ($p<0.05$). The positive effect of bile salts on the solubility of dipyridamole is most likely the result of electrostatic interactions of the weak base with sodium taurocholate. In the fed state, the solubility of dipyridamole in the CED medium with a high concentration of lecithin and low concentrations of bile salts and cholesterol was significantly lower compared to the corresponding healthy medium ($p<0.05$).

For loperamide hydrochloride, the solubility in the fasted state CED media with high concentrations of lecithin and cholesterol and a low concentration of bile salts was significantly lower compared to the corresponding healthy medium ($p<0.05$). This is possibly due to less bile salts being available for drug solubilisation due to the need for lecithin and cholesterol solubilisation. In the fed state, the solubility of loperamide hydrochloride was not significantly different in CED media compared to the corresponding healthy medium ($p<0.05$).

4.3. Multifactorial statistical analysis of solubility in CED media

For CED fasted state intestinal media, the significant effects and two-factor interactions affecting the drug solubility of the six investigated drugs are presented in Table 2.

For azathioprine and budesonide, only the bile salt concentration had a positive impact on their solubility. For celecoxib, the highest positive effect on solubility was observed for the lecithin concentration, followed by a negative effect of cholesterol. Additionally, all two-factor interactions were significant for the solubility of celecoxib but less influential in comparison

to both main effects. For dipyridamole, the highest positive impact on its solubility was observed for bile salts. Other significant effects for dipyridamole were a positive effect of lecithin, a negative effect of cholesterol and the interaction between bile salts and cholesterol was significant. Considering loperamide, bile salts showed a positive and cholesterol a negative impact on solubility, respectively. For sulfasalazine solubility, a positive effect of cholesterol was observed, followed by a significant interaction of bile salts and cholesterol and a positive effect of the bile salt concentration.

Table 2: Significant effects and two-factor interactions in CED fasted state intestinal media.

Main effects/ interactions	**AZA**	**BUD**	**CEL**	**DIP**	**LOP**	**SSZ**
BS	+	+		+	+	+
Lec			+	+		
Chol			-	-	-	+
BS/Lec			-			
BS/Chol			+	+		+
Lec/Chol			-			

+: positive effect, -: negative effect, BS: bile salts, Lec: lecithin, Chol: cholesterol, AZA: azathioprine, BUD: budesonide, CEL: celecoxib, DIP: dipyridamole, LOP: loperamide, SSZ: sulfasalazine

For CED fed state intestinal media, the significant effects and two-factor interactions with an impact on the drug solubility of all six drugs are shown in Table 3.

For azathioprine, the bile salt concentration had the highest positive impact on solubility, followed by a positive impact of cholesterol. Considering budesonide solubility, all three main effects were significant with the highest positive impact of bile salts, followed by a positive impact of lecithin and a negative impact of cholesterol. The two-factor interactions bile

salts/cholesterol and lecithin/cholesterol were also significant but less influential compared to the main effects. For celecoxib, the lecithin concentration had the highest positive impact on its solubility, followed by a positive effect of the bile salt concentration. For dipyridamole, bile salts and cholesterol had a positive impact on solubility. Additionally, the interaction of bile salts and cholesterol was significant. Considering loperamide solubility, a negative impact of cholesterol was observed and a smaller positive effect of the lecithin concentration. For sulfasalazine, only the bile salt concentration had a positive impact on its solubility.

Table 3: Significant effects and two-factor interactions in CED fed state intestinal media.

Main effects/ interactions	**AZA**	**BUD**	**CEL**	**DIP**	**LOP**	**SSZ**
BS	+	+	+	+		+
Lec		+	+		+	
Chol	+	-		+	-	
BS/Lec						
BS/Chol		-		+		
Lec/Chol		-				

+: positive effect, -: negative effect, BS: bile salts, Lec: lecithin, Chol: cholesterol, AZA: azathioprine, BUD: budesonide, CEL: celecoxib, DIP: dipyridamole, LOP: loperamide, SSZ: sulfasalazine

4.4. Drugs at risk of altered solubility in luminal fluids of CED patients

For hydrophilic compounds, only small differences in drug solubility are expected between intestinal fluids of CED patients and healthy subjects as shown by the low impact of CED alterations on azathioprine solubility.

A higher impact of CED on drug solubility is expected for neutral compounds with moderate to high lipophilicity. For these drugs, a higher luminal surfactant concentration (bile salts, lecithin) is expected to result in a higher solubility. It seems to be specific to each drug whether this increase in solubility is mainly driven by bile salts as in the case of budesonide or lecithin as in the case of celecoxib.

A lower risk of altered intestinal solubility in CED is expected for ionisable compounds with moderate to high lipophilicity since drug solubilisation was less impacted by CED changes integrated in the DoE compared to neutral lipophilic compounds.

The investigation of solubility differences for six compounds in simulated gastrointestinal fluids representing CED patients compared to healthy subjects provided an initial biopharmaceutics risk assessment in CED patients. To reach broader conclusions a bigger database including additional compounds is needed.

The present study considered differences in CED patients in terms of luminal concentrations of bile salts, lecithin and cholesterol. More studies are needed to characterise the luminal fluid composition of CED patients to investigate additional differences (e.g., luminal pH since a higher jejunal surface pH has been reported, luminal protein concentrations that are potentially increased by protein leakage through the intestinal membrane), which could not be adequately explored in this study (Kitis et al., 1982).

5. Conclusion

In the current study, biorelevant media developed to be representative of the small intestinal fluids in fasted and fed state of CED patients showed differences in media properties and drug solubilisation compared to biorelevant media developed based on healthy subjects. In terms of media properties, some CED media showed a higher surface tension in the fasted state compared to biorelevant media based on healthy subjects, whereas a lower surface tension was

observed in some CED media in the fed state. Differences in drug solubility in CED media compared to biorelevant media based on healthy subjects were mainly observed for moderately lipophilic compounds with a higher surfactant concentration (bile salts, lecithin) resulting in most cases in a higher drug solubility. The driving factor behind the increase in drug solubility (higher bile salt or lecithin concentration) seemed to be specific to each drug. Further solubility studies with additional compounds would increase the database for biopharmaceutics risk assessment in CED patients and additional studies investigating the composition of luminal contents in CED patients are needed.

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7. Declaration of interest

None.

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Figure Legends

Figure 1: Design of Experiments for the development of Celiac disease intestinal biorelevant media (*value observed in human intestinal fluids (Riethorst et al., 2016)).

Figure 2: Surface tension (blue, left y-axis) and osmolality (rose, right y-axis) of Celiac disease biorelevant media according to the Design of Experiment (green: high level, red: low level, white: healthy) and healthy media (H).

Figure 3: Dynamic viscosity of biorelevant media based on Celiac disease patients and the corresponding biorelevant media based on healthy subjects (H) at different shear stress (0.06 Pa: blue, 0.08 Pa: red, 0.15 Pa: black) according to the Design of Experiments (green: high level, red: low level, white: healthy).

Figure 4: % Relative effect (RE) on the solubility of neutral (at pH 5.8-6.5) investigated drugs in Celiac disease intestinal biorelevant media compared to the corresponding media based on healthy subjects according to Design of Experiments (red: low concentration of cholesterol, blue: high concentration of cholesterol, grey point: medium based on healthy subjects).

Figure 5: % Relative effect on the solubility of weak acids and bases in Celiac disease intestinal biorelevant media compared to the corresponding media based on healthy subjects according to Design of Experiments (red: low concentration of cholesterol, blue: high concentration of cholesterol, grey point: medium based on healthy subjects).

536

	Celiac disease			
Prandial state	**Fasted state**		**Fed state**	
Compartment	**intestine**		**intestine**	
Level	**low**	**high**	**low**	**high**
Bile salts [mM]	3.0	5.1	10.0	17.0
Lecithin [mM]	0.2	0.6	2.0	6.0
Cholesterol [mM]	0.08*	0.16	0.57*	1.14

	increase
	value in healthy biorelevant media

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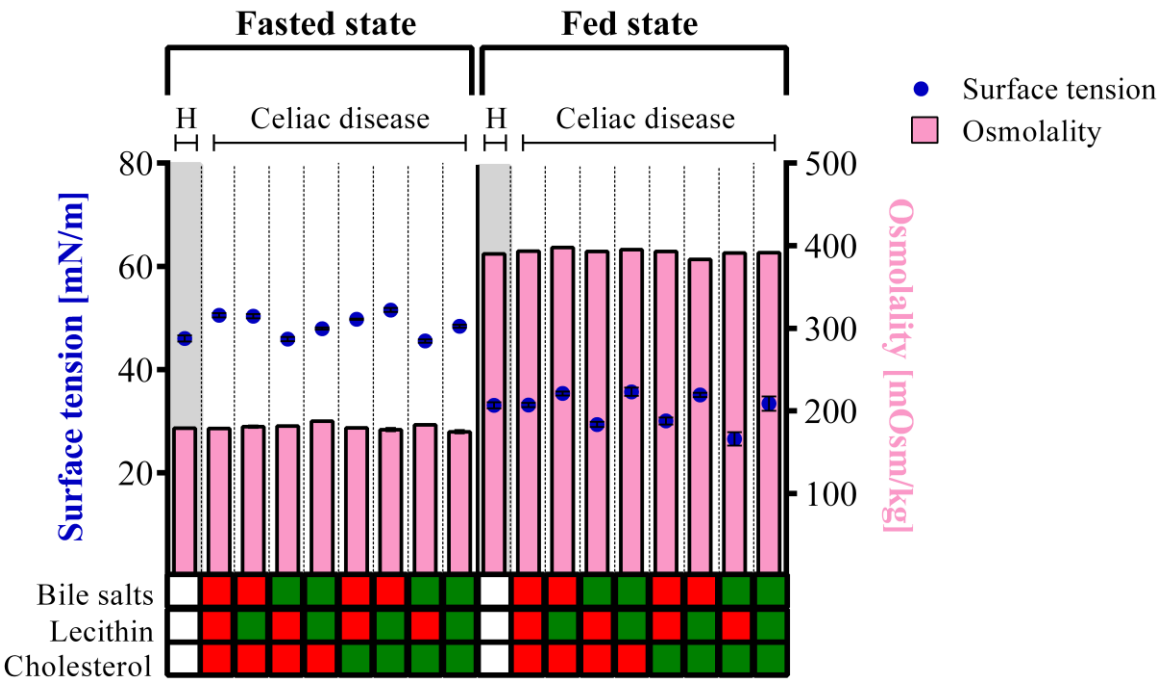
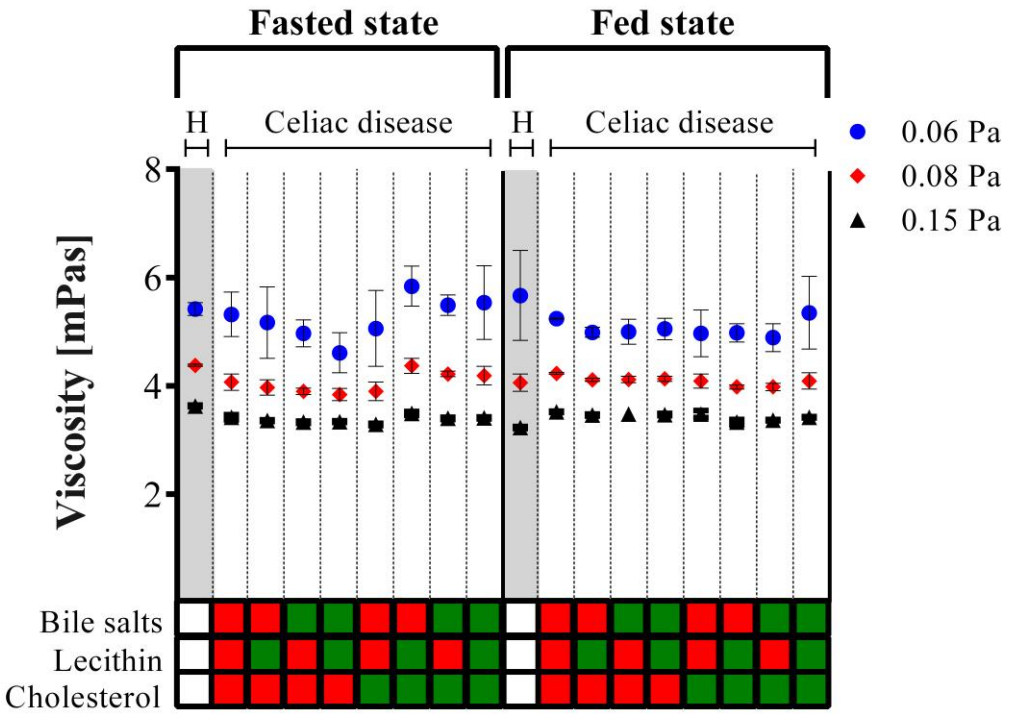
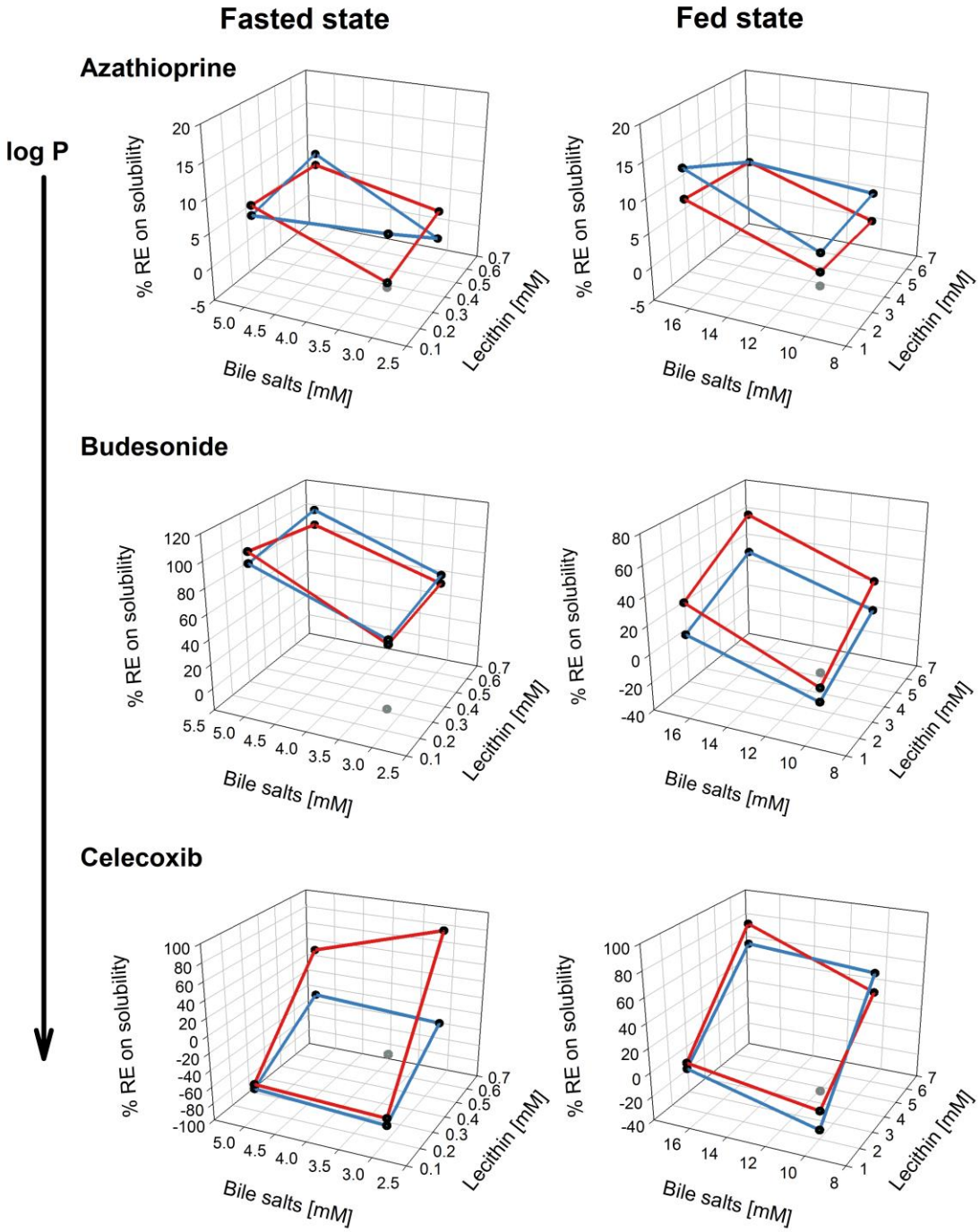

Fasted state
Fed state
H
Celiac disease
H
Celiac disease
Surface tension
Osmolality
Surface tension [mN/m]
Osmolality [mOsm/kg]
80
60
40
20
500
400
300
200
100
Bile salts
Lecithin
Cholesterol


Fasted state
Fed state
H
Celiac disease
H
Celiac disease
0.06 Pa
0.08 Pa
0.15 Pa
Viscosity [mPas]
8
6
4
2
Bile salts
Lecithin
Cholesterol

548



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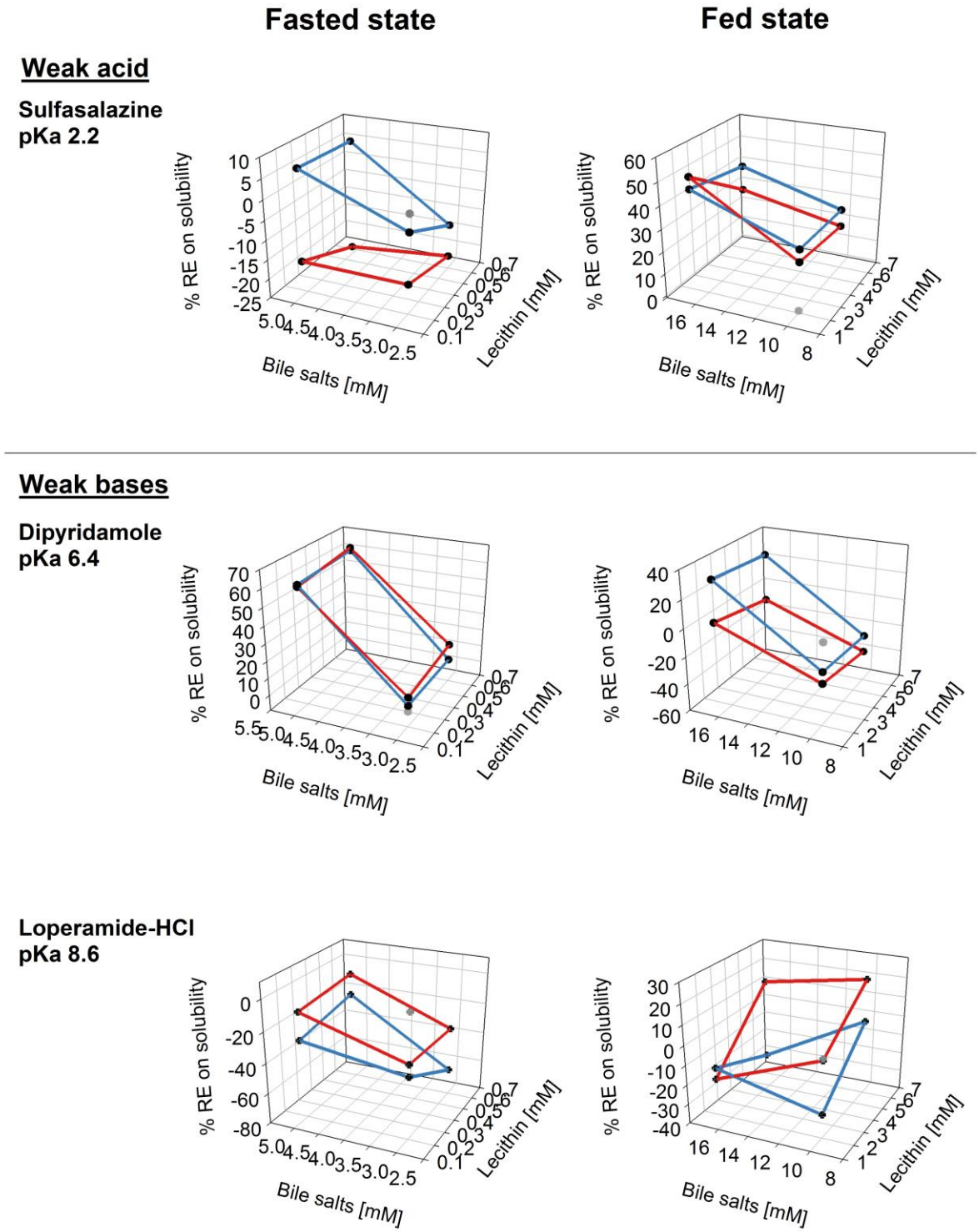
Fasted state
Fed state
Weak acid
Sulfasalazine
pKa 2.2
% RE on solubility
Bile salts [mM]
Lecithin [mM]
Weak bases
Dipyridamole
pKa 6.4
Loperamide-HCl
pKa 8.6

553