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Characterization of luminal contents from the fasted human proximal colon

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ABSTRACT

To treat colonic diseases more effectively, improved therapies are urgently needed. In this respect, delivering drugs locally to the colon is a key strategy to achieve higher local drug concentrations while minimizing systemic side effects. Understanding the luminal environment is crucial to efficiently develop such targeted therapies and to predict drug disposition in the colon. In this clinical study, we collected colonic contents from an undisturbed fasted proximal colon via colonoscopy and characterized their composition with regard to drug disposition. Colonic pH, osmolality, protein content, bile salts, lipids, phospholipids and short-chain fatty acids were investigated in 10 healthy volunteers (8 male and 2 female, age 19–25). The unique environment of the proximal colon was reflected in the composition of the sampled luminal fluids and the effect of the microbiota could be observed on the pH (median 6.55), the composition of bile salts (majority deconjugated and secondary), and the abundance of short-chain fatty acids. At the same time, an increase in phospholipid concentration, osmolality and total protein content compared to reported ileal values was seen, likely resulting from desiccation. Lipids could only be found in low quantities and mainly in the form of cholesterol and free fatty acids, showing almost complete digestion and absorption by the time luminal contents reach the colon. All characteristics also displayed the considerable intersubject variability found in different regions of the gastrointestinal tract. This study contributes to an improved understanding of the luminal conditions in the proximal colon and facilitates the development of new predictive tools to study colonic drug absorption.

1. Introduction

To comprehend and potentially predict the absorption of new drugs after oral administration, understanding the luminal environment of the gastrointestinal tract is key, as it affects absorption-related processes such as drug release, dissolution, precipitation, degradation, and permeation. In this respect, insight into the composition of luminal fluids is crucial to establish new predictive in-vitro and in-silico drug disposition tools. So far, a lot of focus has been placed on the upper regions of the gastrointestinal tract (Vertzoni et al., 2019). However, with diseases like colonic cancer and inflammatory bowel disease (IBD) being of major medical and societal relevance (Alatab et al., 2020; Wang et al., 2024), there is a growing need to better understand processes influencing colonic drug disposition to improve and enhance the development of new colon targeting formulations for local drug delivery in the colonic environment (Lemmens et al., 2021b). The local delivery of drugs is advantageous, as it allows reaching higher drug concentrations in diseased tissue, while avoiding systemic side effects. Furthermore, colonic drug absorption is essential for the performance of extended release formulations, due to the relatively short time window for absorption in the small intestine.

The interplay between a drug or a drug formulation and the local colonic environment plays a crucial role in drug disposition. For instance, knowledge about the composition of colonic fluids is needed to predict the apparent solubility of drugs in the colonic lumen, a key factor for drug uptake. Other aspects that are affected by the colonic fluids are, for example, the stability and mucosal permeation of (pro-)drugs, as well as drug release from modified release dosage forms. For colonic drug delivery, the proximal colon holds particular relevance, as it is the target site for drug release in colon-targeting formulations and provides the most favorable conditions for drug absorption in the large intestine in terms of luminal fluid volumes (Schiller et al., 2005).

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Colonic fluids can be sampled via a colonoscopy procedure, but with characterization purposes in mind, a major challenge concerns the correct subject preparation technique (Augustijns et al., 2020). Ideally, the procedure has only a minimal effect on the colonic fluid composition. However, the limited data that are currently available on the luminal contents in the cecum (Reppas et al., 2015) and ascending colon (Diakidou et al., 2009) have been obtained after the administration of low doses of laxatives (until 44 h prior to sampling) and consumption of a semi-liquid food diet leading up to the time of sample collection.

This clinical study aimed to contribute to a better understanding of the environment a drug or dosage form faces upon arriving in the colon and add to the limited existing literature on this topic. To this end, we collected contents from the cecum and ascending colon of healthy volunteers with only minimal disturbance of the normal physiology, using a sampling technique previously published by our group (Lemmens et al., 2021a, 2020). After an overnight fast, study subjects underwent a colonoscopy without bowel preparation using laxatives. Rather, following a three-day low-fiber diet, the left hemicolon was cleansed using an enema with limited water volume to enable sample collection from an unaltered proximal colon. The contents were subsequently characterized with respect to variables relevant for colonic drug disposition, namely pH, osmolality, protein content, bile salts, lipids, phospholipids and short-chain fatty acids.

2. Materials

Cholic acid (C), glycocholic acid (GC), chenodeoxycholic acid (CDC), glycochenodeoxycholic acid (GCDC), taurochenodeoxycholic acid (TCDC), deoxycholic acid (DC), glycodeoxycholic acid (GDC), taurodeoxycholic acid (TDC), lithocholic acid (LC), glycoursodeoxycholic acid (GUDC), L-tryptophan, tris(hydroxymethyl)aminomethane (TRIS), urea, sodium hydroxide (NaOH), cholesterol (Chol), cholesteryl palmitate (Cholp), tripalmitin (TP), dilinolein (DL), mono-oleate (MO), oleic acid (OA), 1-octadecanol and diethyl ether were bought from Sigma-Aldrich (St. Louis, MO, USA). Taurocholic acid (TC), ursodeoxycholic acid (UDC) and tauroursodeoxycholic acid (TUDC) were obtained from Calbiochem (Darmstadt, Germany). Deuterated cholic acid (C-d4) and deuterated chenodeoxycholic acid (CDC-d4) were acquired from Cayman Chemical (Ann Arbor, MI, USA) and Alsachim (Illkirch Graffenstaden, France), respectively. Acetone (LC-MS grade) and ethyl acetate (LC-MS grade) were purchased from Carl Roth (Karlsruhe, Germany), methanol (HPLC grade) and Na_2SO_4 from Acros Organics (Waltham, MA, USA). Isooctane (for gas chromatography ECD and FID), H_2SO_4 96 %, 2-ethylbutyric acid, propionic acid, isobutyric acid, isovaleric acid, pentanoic acid and hexanoic acid were acquired from Merck (Darmstadt, Germany). Fisher scientific (Waltham, MA, USA) supplied acetonitrile (HPLC gradient grade), hydrochloric acid (HCl) and butyric acid. GC grade helium (5.6) was obtained from Nippon Gases (Schoten, Belgium). Sodium chloride (NaCl) and acetic acid were bought from VWR International (Radnor, PA, USA). Either an ELGA (Woodridge, IL, USA) or Milli-Q (Merck, Darmstadt, Germany) purifying systems were used to obtain ultrapure water.

3. Methods

3.1. Study design

This study was approved by the Ethics Committee Research UZ/KU Leuven in Belgium (S66181). Before inclusion in the study, written informed consent was obtained from all participants. Healthy adults between the ages of 18 and 40 were eligible for participation in the study. Exclusion criteria were a history of an acute or chronic gastrointestinal disease, other disorders which might jeopardize the participants' safety or compliance with the protocol, pregnancy, participation in an interventional trial with an investigational medicinal product (IMP) or device, smoking, use of drugs, HIV or hepatitis infection, medication use (except contraceptives), lactose intolerance, food allergies and constipation.

Colonic contents from the proximal colon were collected via colonoscopy after an overnight fast. To allow sampling from a mostly undisturbed proximal colon, a protocol previously developed in our research group was used (Lemmens et al., 2020). Prior to the procedure, participants followed a three-day low fiber diet. They did not receive any laxatives for the preparation of the colonoscopy. Instead, an enema of 250 mL water was applied on the study day to rinse the left hemicolon and facilitate the safe placement of the colonoscope without disturbing the luminal environment at the sampling site in the proximal colon (cecum and ascending colon). No sedation or anesthesia was given to the subjects to avoid altered gut motility. One sample per volunteer (0.17–1.50 mL liquid fraction) was aspirated from the proximal colon via the suction channel of the colonoscope. After sample collection, the pH was measured immediately, followed by a centrifugation step (5 min, 20,238 g, room temperature) to remove the solid fraction. The supernatant was snap frozen in liquid nitrogen and stored at –26 °C until further analysis.

3.2. Sample analysis

Various characteristics of the collected contents were determined, including pH, osmolality, protein content, bile salts, lipids, phospholipids and short-chain fatty acids. Sample pH was measured in the total colonic contents, before removal of the solid fraction by centrifugation. All other characteristics were determined from the supernatant (liquid fraction).

3.2.1. pH

Sample pH was measured immediately after collection of the colonic contents using a BioTrode glass electrode (Hamilton, Reno, NV, USA). The electrode was calibrated on each study day.

3.2.2. Bile salts

For bile salt quantification, a previously published LC-MS/MS method was used (de Waal et al., 2023a; Riethorst et al., 2016). In short, samples were diluted 100- and 1000-fold with MeOH/water 50:50 (v/v) containing deuterated internal standards C-d4 and CDC-d4 at 200 nM. Following dilution, 5 µL was injected and bile salts were separated on a Kinetex XB-C18 column (2.6 µm, 100 A, 50 × 2.1 mm, Phenomenex) at 35 °C by gradient elution. A Xevo TSQ micro triple quadrupole mass spectrometer (Waters) was used for detection with electrospray ionization operated in positive and negative ion mode. The bile salts analyzed were C, GC, TC, CDC, GCDC, TCDC, DC, GDC, TDC, LC, UDC, GUDC and TUDC.

3.2.3. Osmolality

For osmolality measurement, 50 µL of sample was analyzed in a freezing point osmometer (Gonotec Osmomat 3000). Before use, the osmometer was calibrated using distilled water and a standard solution of 300 mOsmol/kg.

3.2.4. Total protein content

To determine the total protein content, 2 µL of sample was analyzed using the tryptophan fluorescence assay published by Wiśniewski et al. (Wiśniewski and Gaugaz, 2015). This method was used previously to quantify the protein content in various gastrointestinal fluids of different populations (de Waal et al., 2023a, 2023b). Samples were diluted with 200 µL of 8 M urea in 0.1 M Tris-buffer at pH 8.5. Fluorescence emission of diluted samples and tryptophan standards was measured at 350 nm (excitation wavelength 295 nm) using a Tecan infinite M200 plate reader. For the calculation of the total protein content, the tryptophan content of proteins present was assumed to be 1.17 % (Wiśniewski and Gaugaz, 2015).

3.2.5. Phospholipids

Phospholipid content was assessed in 2 μL of sample using the Labassay phospholipid kit (Fujifilm, Tokyo, Japan). Phospholipids that contain choline (i.e., lecithin, sphingomyelin, lysolecithin) were hydrolyzed in the presence of phospholipase D. The produced choline was enzymatically oxidized using choline oxidase resulting in the formation of hydrogen peroxide, which, catalyzed by peroxidase, reacted with 4-aminoantipyrine and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline to form a blue pigment. The phospholipid amount was determined by measuring the absorbance at 600 nm (Tecan infinite M200 plate reader). The lower limit of quantification (LLOQ) was 0.12 mM.

3.2.6. Lipids, lipid digestion products and cholesterol

The concentrations of triacyglycerides (TAG), diacylglycerides (DAG), monoacylglycerides (MAG), free fatty acids (FFA), cholesterol (C) and cholesteryl esters (CE) were determined using an adapted method previously published by de Waal et al. (de Waal et al., 2023a). Short-chain fatty acid and total phospholipid concentrations were determined separately. In short, 100 μL of a 1 M HCl solution and 5 μL of internal standard solution (1-octadecanol at 4 mg/mL) were added to 50 μL of the colonic fluid sample. Subsequently, 200 μL of a chloroform/isooctane mixture (50:50) was added and samples were shaken extensively. Following centrifugation (20,000 g, 15 min, 4 °C), 80 μL of the organic bottom layer were diluted with 120 μL isooctane. After injection of 1 μL of the dilution, lipids, lipid digestion products and cholesterol were separated via high-performance liquid chromatography (HPLC) and detected using a charged aerosol detector (CAD). The lower limit of quantification (LLOQ) was 0.02 mg/mL for TAG, MAG, C and CE, 0.04 mg/mL for DAG, and 0.08 mg/mL for FFA.

3.2.7. Short-chain fatty acids

For quantification of short-chain fatty acid (SCFA) concentrations following a previously developed method (Dalile et al., 2020), 1 mL of saturated NaCl (36 %) solution was added to 100 mg of the colonic fluid samples. Following the addition of an internal standard comprising 50 μL of 10.7 μM 2-ethylbutyric acid in Milli-Q water, sample homogenization was achieved using glass beads. Subsequently, 150 μL of H_2SO_4 96 % was added and SCFAs were extracted with 3 mL diethyl ether. After collecting and drying the organic layer by adding 150 mg of Na_2SO_4, 0.5 μL of supernatant was separated on a DB-FFAP analytical column (30m x 0.53 mm ID, 1.0 μm; Agilent) by gas chromatography. The flow of the carrier gas, GC grade helium (5.6), was set to 4.2 mL/min. A temperature gradient was employed starting from 100 °C for 3 min, followed by an increase of 4 °C/min to 140 °C, kept isothermal for 5 min and ramped further by 40 °C/min to 235 °C (isothermal for 15 min). Acetic acid (AA), propionic acid (PA), butyric acid (BA), valeric acid (VA), caproic acid (CA), isobutyric acid (IBA) and isovaleric acid (IVA) were quantified using a flame ionization detector (Agilent, Santa Clara, California, USA). The lower limits of quantification (LLOQ) were 4.50 mM (AA), 0.94 mM (PA), 1.01 mM (BA), 0.14 mM (VA), 0.64 mM (CA), 0.28 mM (IBA) and 0.10 mM (IVA). ChemStation software (Agilent Technologies) was used to process the resulting chromatograms.

4. Results and discussion

Contents of the fasted proximal colon were collected from 10 healthy volunteers (8 male and 2 female, 19–25 years old). Samples of all volunteers were analyzed for pH, bile salts, phospholipids, total protein content, osmolality, lipids, lipid digestion products, cholesterol and short-chain fatty acids. The composition of the fluids aspirated in the present study reflects the unique conditions in the colon compared to the more proximal regions of the gastrointestinal tract, with the colonic environment being distinctly shaped by the physiological function of the colon, namely desiccation and electrolyte absorption, as well as by the abundance of microbiota. All characteristics exhibited considerable variability, consistent with findings from numerous studies across different gastrointestinal regions (Diakidou et al., 2009; Fuchs and Dressman, 2014; Reppas et al., 2015; Riethorst et al., 2016; Vinarov et al., 2021). Since only one sample was collected from each volunteer, the current study provides a snapshot of the dynamic luminal environment in the proximal colon and its intersubject variability. The extent of intrasubject variability cannot be determined from this study, but should not be overlooked when considering the variability in the colonic lumen.

4.1. pH

One key characteristic affecting solubility and permeation of ionizable compounds, is pH. The median pH measured in the current study was 6.55 (range: 5.58–7.05) (Fig. 1A), which is lower than values reported for aspirates from the distal ileum (median 8.1) (Reppas et al., 2015). This drop can be explained by ongoing bacterial metabolism, resulting in products which lower the pH, e.g., short-chain fatty acids (Den Besten et al., 2013). While literature pH data from aspiration studies in the cecum (median: 7.4, range: 6.2–8.5) (Reppas et al., 2015) and ascending colon (median 7.8, range 6.4–8.4) (Diakidou et al., 2009; Vertzoni et al., 2021) are higher than in the current study, the observed values are in line with various studies where the pH along the gastrointestinal tract was determined using a telemetric capsule (Fig. 1B). Contrary to aspiration studies such as the present one, these do not require subject preparation for a colonoscopy. The fact that the values measured in our study closely resemble those found in the telemetric capsule trials, indicates that the sampling technique used does not significantly alter the environment in the proximal colon.

4.2. Bile salts

Bile salts can influence drug solubility and permeation, as their amphiphilic nature allows them to form micelles at sufficiently high concentrations and to reduce surface tension (Bakatselou et al., 1991; Luner, 2000). The median bile salt concentration measured in the present study was 174 μM, which is a decrease from low mM levels reported for the fasted duodenum (Riethorst et al., 2016). Like most characteristics, the concentration highly varied among subjects (range: 92–806 μM).

The composition of bile salts found in the colon differed noticeably from the small intestine (Riethorst et al., 2016), with mostly deconjugated (95 %) and secondary (68 %) bile salts present as a consequence of bacterial deconjugation and 7α-dehydroxylation (Fig. 2). In comparison, duodenal bile salts are reported to be fully conjugated with the majority being primary (77.6 %) (Riethorst et al., 2016). Those changes in bile salt composition have been shown to affect solubilization capacity and surface tension. Especially after bacterial 7α-dehydroxylation, a significant increase in micellar solubilization capacity was reported for several poorly water-soluble drugs, as well as a reduction in surface tension (Enright et al., 2017). On average (SD), the most abundant bile salt in the proximal colon was deoxycholate (45.5 (27.6) %), followed by cholate (20.2 (18.6) %), lithocholate (18.2 (18.7) %), chenodeoxycholate (6.9 (5.4) %), ursodeoxycholate (4.2 (3.5) %) and taurocholate (1.9 (4.5) %) (Fig. 2). Previous studies observed comparable patterns in bile salt composition, but lower mean (SD) bile salt concentrations of 183 (221) μM in cecum (Reppas et al., 2015) and 115 (119) μM in ascending colon (Diakidou et al., 2009) compared to 275 (228) μM in the current study. Similar trends were also observed in a study by Shalon et al. (2023), wherein bile salt concentrations decreased from the proximal to distal gastrointestinal tract, while the composition shifted towards more deconjugated and secondary bile salts.

As described for the total concentration of bile salts, sizeable differences in their composition could also be observed between individuals (Fig. 3). All samples have in common that each of the measured deconjugated secondary bile salts (deoxycholate, lithocholate, ursodeoxycholate) could be found. Deconjugated primary bile salts

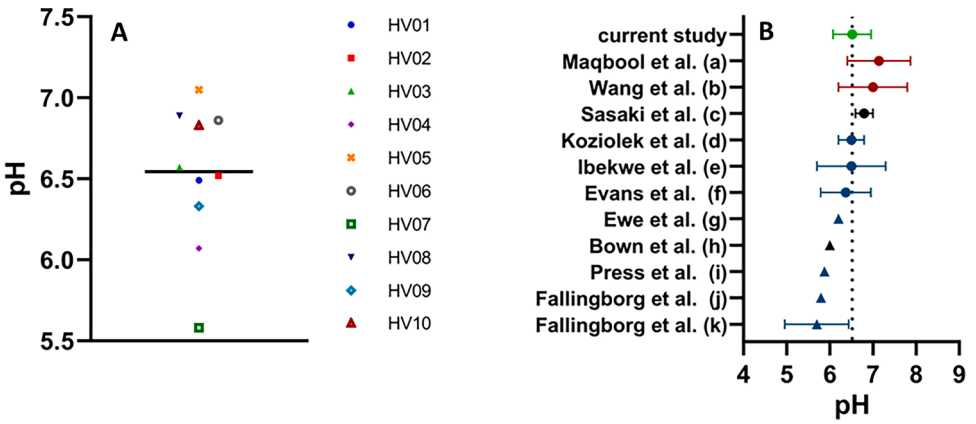

Fig. 1. A: Individual pH values of colonic fluids from 10 healthy volunteers (HV); the solid line represents the median. B: Comparison of the pH values observed in the present study (green) to literature values reported in telemetric capsule studies. Circles represent the mean, triangles the median and error bars the standard deviation. Depending on the prandial state, studies are marked in blue (fasted state), red (fed state) or black (no information on the prandial state was given). (a (Maqbool et al., 2009), b (Wang et al., 2015), c (Sasaki et al., 1997), d (Koziolek et al., 2015), e (Ibekwe et al., 2008), f (Evans et al., 1988), g (Ewe et al., 1999), h (Bown et al., 1974), i (Press et al., 1998), j (Fallingborg et al., 1998), k (Fallingborg et al., 1989)).

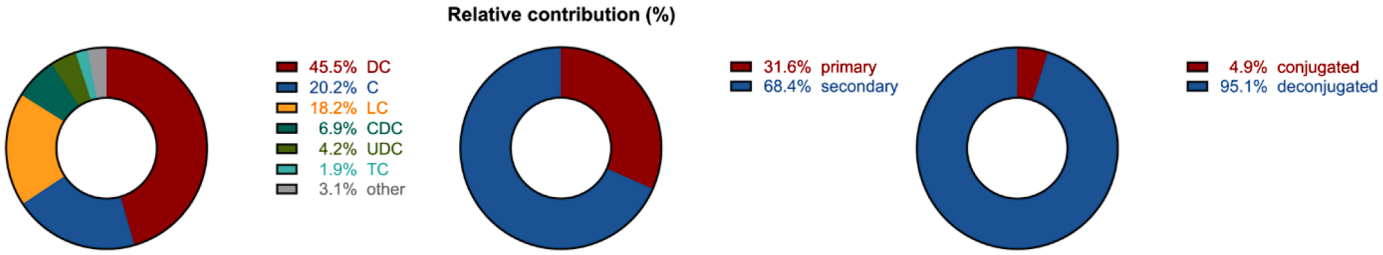

Fig. 2. Average relative bile salt composition of colonic fluids from 10 healthy volunteers (DC: deoxycholate; C: cholate; LC: lithocholate; CDC: chenodeoxycholate; UDC: ursodeoxycholate; TC: taurocholate).

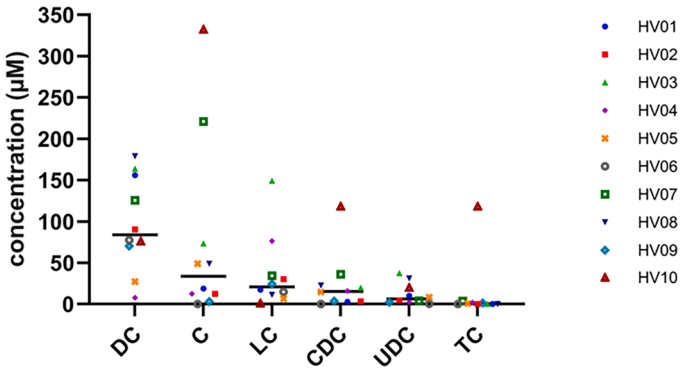

Fig. 3. Individual concentrations of the six most abundant bile salts in colonic fluids from 10 healthy volunteers; lines represent the median of the observed values (DC: deoxycholate; C: cholate; LC: lithocholate; CDC: chenodeoxycholate; UDC: ursodeoxycholate; TC: taurocholate).

cholate and chenodexycholate were quantified in every subject except for HV06. Notably, HV10 did not only have the highest total bile salt concentration (806 µM), but also the highest relative abundance of conjugated (32.1 % vs. < 6 % in all other volunteers) and primary bile salts (84.1 %). The significant concentrations of several conjugated bile salts, especially taurocholate, taurochenodeoxycholate and glycocholate, set HV10 apart from all other volunteers, where conjugated bile salts were either absent or only found in very low quantities. The difference in concentration and composition may be the consequence of a recent expulsion of luminal contents from the ileum to the cecum, resulting in lower microbial metabolism of bile salts at the time of sample collection.

Since the critical micellar concentration (CMC) of most bile salts is in the low mM range (Monte et al., 2009; Natalini et al., 2014), the presence of bile salt micelles in the proximal colon over an extended period of time is unlikely. However, due to the complex composition of colonic fluids, the formation of mixed micelles with other amphiphilic molecules like phospholipids and fatty acids is conceivable. Drug solubilization in aspirates from the ascending colon has been reported for three model compounds (Vertzoni et al., 2010).

4.3. Phospholipids

Like bile salts, phospholipids can affect the apparent solubility of drugs in the gastrointestinal lumen due to their amphiphilic properties, which not only facilitate the formation of (mixed-)micelles, but also lead to a reduction in surface tension (Fuchs and Dressman, 2014). The median phospholipid concentration of 0.26 mM (range: 0.13–0.4 mM, Fig. 4A) detected in the colonic fluids was lower than published values for the fasted duodenum (0.58 mM, range: 0.01–6.33 mM) (Riethorst et al., 2016). While the mean (SD) phospholipid concentration of 266 (110) µM was higher than the 73 (41) µM in the fasted ileum and the 166 (110) µM in the cecum reported by Reppas et al. (Reppas et al., 2015), higher concentrations were reported for the ascending colon by Diakidou et al. (362 (210) µM) (Diakidou et al., 2009).

4.4. Total protein content

The median protein content in colonic fluids amounted to 8.4 mg/mL (Fig. 4B), with a mean (SD) concentration of 12.0 (9.4) mg/mL, which is higher than the 5.1 (3.3) mg/ml reported for the fasted state ileum (Reppas et al., 2015). This higher protein concentration likely stems

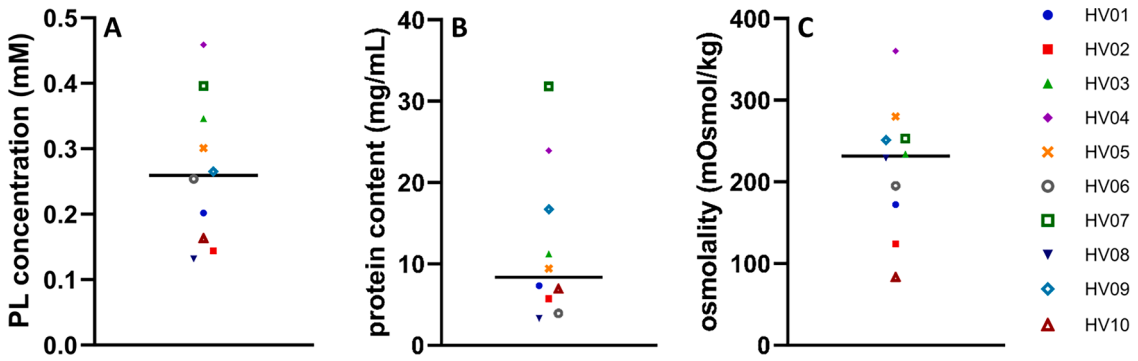

Fig. 4. Individual phospholipid (PL) concentration (A), total protein content (B) and osmolality (C) in colonic fluids from 10 healthy volunteers (HV); lines represent the median.

from a lower total fluid volume in the colon due to desiccation. Published protein contents in the fasted cecum (10.2 (2.2) mg/ml) (Reppas et al., 2015) and ascending colon (9.7 (4.6) mg/mL) (Diakidou et al., 2009) are in the same range as what we observed in the current study. Potential effects of proteins in gastrointestinal fluids on drug absorption still need to be elucidated. Possibly, their presence could enhance drug solubility, while binding to luminal proteins may restrict mucosal permeation.

4.5. Osmolality

It has been shown that changes in osmolality can affect both the release of drugs and the performance of excipients (Jantratid et al., 2008). Furthermore, the difference in osmotic pressure between the intestinal lumen and the mucosa facilitates water absorption in the gastrointestinal tract (Billich and Levitan, 1969). The colonic fluids were mostly hypotonic, having a median osmolality of 232 mOsmol/kg (Fig. 4C). With a mean (SD) of 218 (79) mOsmol/kg, the observed values are considerably higher than those found in literature for the fasted cecum (144 (65) mOsmol/kg) (Reppas et al., 2015) and ascending colon (81 (102) mOsmol/kg) (Diakidou et al., 2009). The differences possibly result from distinct subject preparation and dietary protocols prior to sample collection (see Supplementary Table 1). The osmolality of colonic fluids in this study is also higher than published measurements in fasted ileal fluids (60 (50) mOsmol/kg) (Reppas et al., 2015). This increase may be explained by water absorption from the luminal contents upon reaching the colon, resulting in elevated solute concentrations. As seen for the bile salt concentration and composition, HV10 also stands out in terms of osmolality, exhibiting the lowest measured level at 83 mOsmol/kg. This finding is consistent with the earlier interpretation of a recent transfer of luminal contents from ileum to cecum, as the observed osmolality resembles the one reported for the ileum.

4.6. Lipids, lipid digestion products and cholesterol

Upon ingestion, lipids are extensively digested in the upper gastrointestinal tract. The resulting digestion products like diacylglycerides, monoacylglycerides, free fatty acids and cholesterol can be incorporated in micellar structures and solubilize lipophilic drugs (Fuchs and Dressman, 2014). In the current study, the median total lipid concentration (including cholesterol, cholesteryl esters, triacyglycerides, diacylglycerides, monoacylglycerides and free fatty acids) was 0.58 mg/mL (range: 0.05–3.66 mg/mL), with cholesterol being the most abundant component (median 0.46 mg/mL) (Fig. 5). Cholesterol was found at varying concentrations in samples from all ten volunteers (range 0.03–2.08 mg/mL). Cholesteryl esters could be detected in two out of ten samples (0.28 and 0.02 mg/mL). Those were also the two samples with the highest cholesterol and free fatty acid concentrations. For free fatty acids, relatively high concentrations could be quantified in three

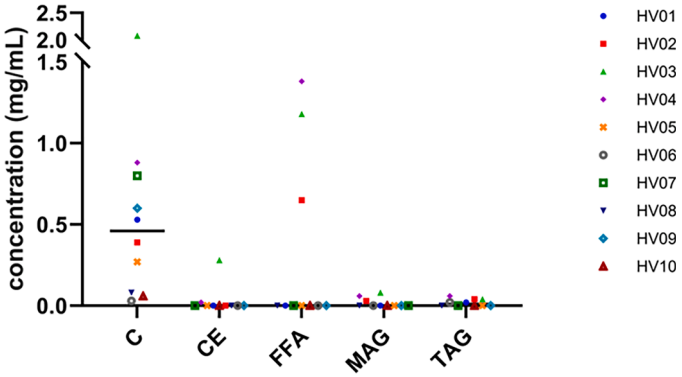

Fig. 5. Individual concentrations of cholesterol (C), cholesteryl esters (CE), free fatty acids (FFA), monoacylglycerides (MAG) and triacylglycerides (TAG) in colonic fluids from 10 healthy volunteers (HV); lines represent the median.

volunteers, ranging between 0.65–1.18 mg/mL. Monoacylglycerides and triacyglycerides were only found at concentrations below 0.1 mg/mL in three and five samples, respectively. Diacylglycerides could not be quantified in any volunteers.

These findings indicate that the digestion and absorption of dietary lipids are almost completed by the time the luminal contents reach the colon. Especially because sampling was done in the fasted state, a low abundance of dietary lipids is expected. Apart from the diet, possible sources of cholesterol, lipids and lipid digestion products include microbiota and dead enterocytes, with the reported mean turnover time of epithelial cells in the colorectal region being around 4 days (Darwich et al., 2014). Our findings are somewhat similar to those reported for the cecum and ascending colon (Diakidou et al., 2009; Reppas et al., 2015), with significant concentrations of cholesterol present, while triacyglycerides, diacylglycerides and monoacylglycerides are only found in low concentrations or below the limit of quantification. The mean abundance of free fatty acids was higher in the current study, which can be attributed to three volunteers displaying exceptionally high free fatty acid levels. Compared to literature ileal (Reppas et al., 2015) values, lipid concentrations in the colon display a similar rise to that observed for other characteristics like protein content and osmolality, reflecting colonic water absorption.

4.7. Short-chain fatty acids

The high abundance of short-chain fatty acids (SCFA) in the ascending colon, compared to the more proximal regions of the gastrointestinal tract, contributes to the unique luminal environment. These SCFA are mainly the result of bacterial fermentation of polysaccharides by the colonic microbiota (Wong et al., 2006) and their

production is widely considered the cause of the pH drop after ileo-cecal transit (Den Besten et al., 2013). In this study, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, isobutyric acid and isovaleric acid were analyzed. In all samples, acetic acid, propionic acid, butyric acid, valeric acid and isovaleric acid could be determined, while caproic acid (LLOQ 0.64 mM) and isobutyric acid (LLOQ 0.28 mM) could only be quantified in seven and two out of ten volunteers, respectively. The median total SCFA concentration was 74.29 mM, with acetic acid being the most abundant (median 48.59 mM), alongside notably high median abundances of propionic acid (13.97 mM) and butyric acid (8.05 mM) (Fig. 6A). Valeric acid (median 1.15 mM) and isovaleric acid (median 0.58 mM) were found in lower concentrations in all samples, as well as caproic acid and isobutyric acid in some of the samples (Fig. 6B).

While concentrations varied between volunteers, the relative SCFA composition remained similar (Fig. 7). The mean relative abundance was 63.0 % acetic acid, 17.4 % propionic acid and 15.5 % butyric acid across all volunteers, with valeric acid, caproic acid, isobutyric acid and isovaleric acid making up the remaining 4.1 %. One notable outlier was HV04, who had butyric acid concentrations more than twice as high as all other volunteers, as well as the highest total SCFA concentration. Similar ratios in composition have been found for cecum (Reppas et al., 2015) and ascending colon (Diakidou et al., 2009), although the reported mean (SD) concentrations of 32.2 (17.6) mM and 30.9 (15.4) mM were considerably lower than those in the current study (78.5 (42.2) mM). These apparent differences in SCFA concentration are likely the cause for the lower pH values observed in the current study and may result from different dietary and subject preparation protocols prior to sampling.

Generally, the concentrations of SCFA (mM range) are high compared to other components of the colonic fluids like bile salts and phospholipids (μM range). These significant concentrations highlight the impact of the microbiome on the luminal environment, especially considering that participants needed to follow a three-day low fiber diet prior to sample collection, which potentially reduces the substrates available for SCFA production

4.8. Implications for in vitro testing

In order to predict the apparent solubility and in vivo dissolution of drugs and dosage forms in the gastrointestinal tract, biorelevant media are commonly used for in vitro testing. These media exist at varying levels of complexity, generally containing multiple components found in the intestinal lumen (Butler et al., 2019). Their development relies on the available data on the composition of luminal fluids, such as the findings from the current study. Fasted state simulated colonic fluid

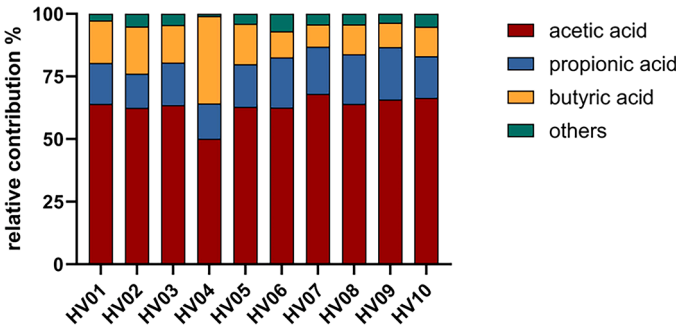

Fig. 7. Relative SCFA composition in colonic fluids from 10 healthy volunteers (red: acetic acid, blue: propionic acid, orange: butyric acid, green: others (valeric acid, caproic acid, isobutyric acid, isovaleric acid)) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

(FaSSCoF) is a commercially available biorelevant medium simulating the conditions in the ascending colon based on the study by Diakidou et al. (Diakidou et al., 2009; Vertzoni et al., 2010). It consists of a TRIS/maleate buffer containing sodium cholate (0.15 mM), lecithin (0.3 mM) and sodium oleate (0.1 mM). The current study, with its minimized disturbance of the physiological conditions in the proximal colon prior to sample collection, identifies features of FaSSCoF which could be refined in order to attain more accurate predictions. Particularly for the pH, there is a clear discrepancy between FaSSCoF and the values we measured in the colonic fluid aspirates. Our findings suggest that a pH of 6.55 would be more appropriate to reflect the conditions in the proximal colon, as opposed to the 7.8 of FaSSCoF. The differences in bile salts, phospholipids and lipids, in relation to the literature data informing the composition of FaSSCoF, have been discussed in previous sections. Although their impact may not be as substantial as for pH, they should still be taken into account when studying drug behavior in the colon. For certain compounds, additional levels of complexity, for example through the addition of different bile salts, proteins and cholesterol, may be necessary for reliable predictions. The significant concentrations of short-chain fatty acids found in the proximal colon are unique for luminal contents in the gastrointestinal tract. Therefore, their possible impact on drug absorption should be elucidated. Apart from solubility and dissolution, the colonic luminal environment also has implications for studying other absorption related processes, including the permeation of certain drugs. The pH, for example, can affect the mucosal permeability for ionizable compounds. It is important to recognize that, like most characterization studies, the current study included only a limited number of subjects and the study population consisted of

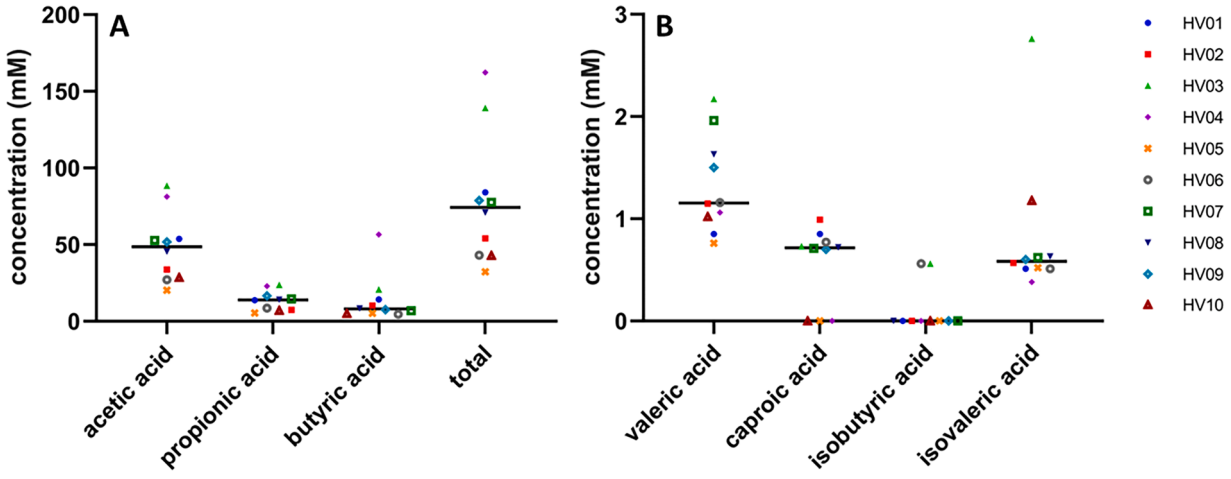

Fig. 6. Individual short-chain fatty acid concentrations in colonic fluids from 10 healthy volunteers; lines represent the median of the observed values.

healthy, young adults.

5. Conclusion

This study provides unique insights into the composition of the luminal colonic contents after sampling from an undisturbed proximal colon in healthy volunteers. The conditions in the fasted colon are distinctly different from the more proximal regions, not only in the abundance of various components, but also in their composition. The presence of microbiota appears to shape the luminal environment, which is especially reflected by colonic pH, bile salt profile and the high concentrations of short-chain fatty acids detected. The obtained results contribute to an advanced understanding of factors affecting drug behavior in the colon and therefore facilitate the development of better predictive tools for colonic drug absorption, addressing the growing demand for more effective local therapies in the colon.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 3.5 in order to check spelling and grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Sebastian Steigert: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Joachim Brouwers:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Data curation, Conceptualization. **Kristin Verbeke:** Writing – review & editing, Methodology, Data curation. **Tim Vanuytsel:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Data curation, Conceptualization. **Patrick Augustijns:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2024.106821.

References

Alatab, S., Sepanlou, S.G., Ikuta, K., Vahedi, H., Bisignano, C., Safiri, S., Sadeghi, A., Nixon, M.R., Abdoli, A., Abolhassani, H., Alipour, V., Almadi, M.A.H., Almasi-Hashiani, A., Anushiravani, A., Arabloo, J., Atique, S., Awasthi, A., Badawi, A., Baig, A.A.A., Bhala, N., Bijani, A., Biondi, A., Borzì, A.M., Burke, K.E., Carvalho, F., Daryani, A., Dubey, M., Eftekhari, A., Fernandes, E., Fernandes, J.C., Fischer, F., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hasanzadeh, A., Hashemian, M., Hay, S.I., Hoang, C.L., Househ, M., Ilesanmi, O.S., Balalami, N.J., James, S.L., Kengne, A.P., Malekzadeh, M.M., Merat, S., Meretoja, T.J., Mestrovic, T., Mirrakhimov, E.M., Mirzaei, H., Mohammad, K.A., Mokdad, A.H., Monasta, L., Negoi, I., Nguyen, T.H., Nguyen, C.T., Pourshams, A., Poustchi, H., Rabiee, M., Rabiee, N., Ramezanzadeh, K., Rawaf, D.L., Rawaf, S., Rezaei, N., Robinson, S.R., Ronfani, L., Saxena, S., Sepehrimanesh, M., Shaikh, M.A., Sharafi, Z., Sharif, M., Siabani, S., Sima, A.R., Singh, J.A., Soheili, A.R., Sotoudehmanesh, Suleria, H.A.R., Tesfay, B.E., Tran, B., Tsoi, D., Vacante, M., Wondmieneh, A.B., Zarghi, A., Zhang, Z.J., Dirac, M., Malekzadeh, R., Naghavi, M., 2020. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol. Hepatol. 5, 17–30. https://doi.org/10.1016/S2468-1253(19)30333-4.

Augustijns, P., Vertzoni, M., Reppas, C., Langguth, P., Lennernäs, H., Abrahamsson, B., Hasler, W.L., Baker, J.R., Vanuytsel, T., Tack, J., Corsetti, M., Bermejo, M., Paixão, P., Amidon, G.L., Hens, B., 2020. Unraveling the behavior of oral drug products inside the human gastrointestinal tract using the aspiration technique: history, methodology and applications. Eur. J. Pharmaceut. Sci. https://doi.org/10.1016/j.ejps.2020.105517.

Bakatselou, V., Oppenheim, R.C., Dressman, J.B., 1991. Solubilization and wetting effects of bile salts on the dissolution of steroids. Pharm. Res. 8, 1461–1469. https://doi.org/10.1023/a:1015877929381.

Billich, C.O., Levitan, R., 1969. Effects of sodium concentration and osmolality on water and electrolyte absorption form the intact human colon. J. Clin. Invest. 48, 1336–1347. https://doi.org/10.1172/JCI106100.

Bown, R.L., Gibson, J.A., Sladen, G.E., Hicks, B., Dawson, A.M., 1974. Effects of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. Gut 15, 999–1004. https://doi.org/10.1136/gut.15.12.999.

Butler, J., Hens, B., Vertzoni, M., Brouwers, J., Berben, P., Dressman, J., Andreas, C.J., Schaefer, K.J., Mann, J., McAllister, M., Jamei, M., Kostewicz, E., Kesisoglou, F., Langguth, P., Minekus, M., Müllertz, A., Schilderink, R., Koziolek, M., Jedamzik, P., Weitschies, W., Reppas, C., Augustijns, P., 2019. In vitro models for the prediction of in vivo performance of oral dosage forms: recent progress from partnership through the IMI OrBiTo collaboration. Eur. J. Pharmaceut. Biopharmaceut. 136, 70–83. https://doi.org/10.1016/j.ejpb.2018.12.010.

Dalile, B., Vervliet, B., Bergonzelli, G., Verbeke, K., Van Oudenhove, L., 2020. Colon-delivered short-chain fatty acids attenuate the cortisol response to psychosocial stress in healthy men: a randomized, placebo-controlled trial. Neuropsychopharmacology 45, 2257–2266. https://doi.org/10.1038/s41386-020-0732-x.

Darwich, A.S., Aslam, U., Ashcroft, D.M., Rostami-Hodjegan, A., 2014. Meta-analysis of the turnover of intestinal epithelia in preclinical animal species and humans. Drug Metabol. Disposit. https://doi.org/10.1124/dmd.114.058404.

de Waal, T., Brouwers, J., Berben, P., Flanagan, T., Tack, J., Vandenberghe, W., Vanuytsel, T., Augustijns, P., 2023a. Characterization of aspirated duodenal fluids from Parkinson's disease patients. Pharmaceutics 15. https://doi.org/10.3390/pharmaceutics15041243.

de Waal, T., Brouwers, J., Mols, R., Hoffman, I., Rayyan, M., Augustijns, P., 2023b. Characterization of neonatal and infant enterostomy fluids. Int. J. Pharm. 639 https://doi.org/10.1016/j.ijpharm.2023.122943.

Den Besten, G., Van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.J., Bakker, B.M., 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J. Lipid Res. 54, 2325–2340. https://doi.org/10.1194/jlr.R036012.

Diakidou, A., Vertzoni, M., Goumas, K., Söderlind, E., Abrahamsson, B., Dressman, J., Reppas, C., 2009. Characterization of the contents of ascending colon to which drugs are exposed after oral administration to healthy adults. Pharm. Res. 26, 2141–2151. https://doi.org/10.1007/s11095-009-9927-x.

Enright, E.F., Joyce, S.A., Gahan, C.G.M., Griffin, B.T., 2017. Impact of gut microbiota-mediated bile acid metabolism on the solubilization capacity of bile salt micelles and drug solubility. Mol. Pharm. 14, 1251–1263. https://doi.org/10.1021/acs.molpharmaceut.6b01155.

Evans, D.F., Pye, G., Bramley, R., Clark, A.G., Dyson, J., Hardcastle, J.D., 1988. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 29, 1035–1041. https://doi.org/10.1136/gut.29.8.1035.

Ewe, K.E., Schwartz, S., Petersen, S., Press, A.G., 1999. Inflammation does not decrease intraluminal pH in chronic inflammatory bowel disease. Dig. Dis. Sci. 44, 1434–1439. https://doi.org/10.1023/a:1026664105112.

Fallingborg, J., Christensen, L.A., Ingeman-Nielsen, M., Jacobsen, B.A., Abildgaard, K., Rasmussen, H.H., 1989. pH-Profile and regional transit fimes of the normal gut

measured by a radiotelemetry device. Alimenf. Pharmacol. Therap 3, 605–613. https://doi.org/10.1111/j.1365-2036.1989.tb00254.x.
Fallingborg, J., Pedersen, U.L., Jacobsen, B.A., 1998. Small intestinal transit time and intraluminal pH in ileocecal resected patients with Crohn's disease. Dig. Dis. Sci. 43, 702–705. https://doi.org/10.1023/a:1018893409596.
Fuchs, A., Dressman, J.B., 2014. Composition and physicochemical properties of fasted-state human duodenal and jejunal fluid: a critical evaluation of the available data. J. Pharm. Sci. https://doi.org/10.1002/jps.24183.
Ibekwe, V.C., Fadda, H.M., McConnell, E.L., Khela, M.K., Evans, D.F., Basit, A.W., 2008. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems. Pharm. Res. 25, 1828–1835. https://doi.org/10.1007/s11095-008-9580-9.
Jantratid, E., Janssen, N., Reppas, C., Dressman, J.B., 2008. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. Pharm. Res. 25, 1663–1676. https://doi.org/10.1007/s11095-008-9569-4.
Koziolek, M., Grimm, M., Becker, D., Iordanov, V., Zou, H., Shimizu, J., Wanke, C., Garbacz, G., Weitschies, W., 2015. Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the intellicap® system. J. Pharm. Sci. 104, 2855–2863. https://doi.org/10.1002/jps.24274.
Lemmens, G., Brouwers, J., Snoeys, J., Augustijns, P., Vanuytsel, T., 2021a. Insight into the colonic disposition of sulindac in humans. J. Pharm. Sci. 110, 259–267. https://doi.org/10.1016/j.xphs.2020.09.034.
Lemmens, G., Brouwers, J., Snoeys, J., Augustijns, P., Vanuytsel, T., 2020. Insight into the colonic disposition of celecoxib in humans. Eur. J. Pharmaceut. Sci. 145 https://doi.org/10.1016/j.ejps.2020.105242.
Lemmens, G., Van Camp, A., Kourula, S., Vanuytsel, T., Augustijns, P., 2021b. Drug disposition in the lower gastrointestinal tract: targeting and monitoring. Pharmaceutics. https://doi.org/10.3390/pharmaceutics13020161.
Luner, P.E., 2000. Wetting properties of bile salt solutions and dissolution media. J. Pharm. Sci. 89, 382–395. https://doi.org/10.1002/(SICI)1520-6017(200003)89:3%3C382::AID-JPS9%3E3.0.CO;2-H.
Maqbool, S., Parkman, H.P., Friedenberg, F.K., 2009. Wireless capsule motility: comparison of the smartPill® GI monitoring system with scintigraphy for measuring whole gut transit. Dig. Dis. Sci. 54, 2167–2174. https://doi.org/10.1007/s10620-009-0899-9.
Monte, M.J., Marin, J.J.G., Antelo, A., Vazquez-Tato, J., 2009. Bile acids: chemistry, physiology, and pathophysiology. World J. Gastroenterol. 15, 804–816. https://doi.org/10.3748/wjg.15.804.
Natalini, B., Sardella, R., Gioiello, A., Ianni, F., Di Michele, A., Marinozzi, M., 2014. Determination of bile salt critical micellization concentration on the road to drug discovery. J. Pharm. Biomed. Anal. https://doi.org/10.1016/j.jpba.2013.06.029.
Press, A.G., Hauptmann, I.A., Hauptmann, L., Fuchs, B., Fuchs, M., Ewe, K., Ramadori, G., 1998. Gastrointestinal pH profiles in patients with inflammatory bowel disease. Aliment. Pharmacol. Ther. 12, 673–678. https://doi.org/10.1046/j.1365-2036.1998.00358.x.
Reppas, C., Karatza, E., Goumas, C., Markopoulos, C., Vertzoni, M., 2015. Characterization of contents of distal ileum and cecum to which drugs/drug products are exposed during bioavailability/bioequivalence studies in healthy adults. Pharm. Res. 32, 3338–3349. https://doi.org/10.1007/s11095-015-1710-6.
Riethorst, D., Mols, R., Duchateau, G., Tack, J., Brouwers, J., Augustijns, P., 2016. Characterization of human duodenal fluids in fasted and fed state conditions. J. Pharm. Sci. 105, 673–681. https://doi.org/10.1002/jps.24603.
Sasaki, Y., Hada, R., Nakajima, H., Fukuda, S., Munakata, A., 1997. Improved localizing method of radiopill in measurement of entire gastrointestinal pH profiles: colonic luminal pH in normal subjects and patients with Crohn's disease. Am. J. Gastroenterol. 92, 114–118.
Schiller, C., Fröhlich, C.P., Giessmann, T., Siegmund, W., Mönnikes, H., Hosten, N., Weitschies, W., 2005. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. Aliment. Pharmacol. Ther. 22, 971–979. https://doi.org/10.1111/j.1365-2036.2005.02683.x.
Shalon, D., Culver, R.N., Grembi, J.A., Folz, J., Treit, P.V., Shi, H., Rosenberger, F.A., Dethlefsen, L., Meng, X., Yaffe, E., Aranda-Díaz, A., Geyer, P.E., Mueller-Reif, J.B., Spencer, S., Patterson, A.D., Triadafilopoulos, G., Holmes, S.P., Mann, M., Fiehn, O., Relman, D.A., Huang, K.C., 2023. Profiling the human intestinal environment under physiological conditions. Nature 617, 581–591. https://doi.org/10.1038/s41586-023-05989-7.
Vertzoni, M., Augustijns, P., Grimm, M., Koziolek, M., Lemmens, G., Parrott, N., Pentafragka, C., Reppas, C., Rubbens, J., Van Den Abeele, J., Vanuytsel, T., Weitschies, W., Wilson, C.G., 2019. Impact of regional differences along the gastrointestinal tract of healthy adults on oral drug absorption: an UNGAP review. Eur. J. Pharmaceut. Sci. 134, 153–175. https://doi.org/10.1016/j.ejps.2019.04.013.
Vertzoni, M., Diakidou, A., Chatzilias, M., Söderlind, E., Abrahamsson, B., Dressman, J. B., Reppas, C., 2010. Biorelevant media to simulate fluids in the ascending colon of humans and their usefulness in predicting intracolonic drug solubility. Pharm. Res. 27, 2187–2196. https://doi.org/10.1007/s11095-010-0223-6.
Vertzoni, M., Sulaiman, S., Goumas, K., Kersten, E., Anlahr, J., Muenster, U., Reppas, C., 2021. Characteristics of contents of lower intestine in the 65–74 years of age range could impact the performance of safe and efficacious modified release products. J. Pharm. Sci. 110, 251–258. https://doi.org/10.1016/j.xphs.2020.10.029.
Vinarov, Z., Abdallah, M., Agundez, J.A.G., Allegaert, K., Basit, A.W., Braeckmans, M., Ceulemans, J., Corsetti, M., Griffin, B.T., Grimm, M., Keszthelyi, D., Koziolek, M., Madla, C.M., Matthys, C., McCoubrey, L.E., Mitra, A., Reppas, C., Stappaerts, J., Steenackers, N., Trevaskis, N.L., Vanuytsel, T., Vertzoni, M., Weitschies, W., Wilson, C., Augustijns, P., 2021. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review. Eur. J. Pharmaceut. Sci. 162 https://doi.org/10.1016/j.ejps.2021.105812.
Wang, S., Zheng, R., Li, J., Zeng, H., Li, L., Chen, R., Sun, K., Han, B., Bray, F., Wei, W., He, J., 2024. Global, regional, and national lifetime risks of developing and dying from gastrointestinal cancers in 185 countries: a population-based systematic analysis of GLOBOCAN. Lancet Gastroenterol. Hepatol. https://doi.org/10.1016/S2468-1253(23)00366-7.
Wang, Y.T., Mohammed, S.D., Farmer, A.D., Wang, D., Zarate, N., Hobson, A.R., Hellström, P.M., Semler, J.R., Kuo, B., Rao, S.S., Hasler, W.L., Camilleri, M., Scott, S. M., 2015. Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: Influence of age, gender, study country and testing protocol. Aliment. Pharmacol. Ther. 42, 761–772. https://doi.org/10.1111/apt.13329.
Wiśniewski, J.R., Gaugaz, F.Z., 2015. Fast and sensitive total protein and peptide assays for proteomic analysis. Anal. Chem. 87, 4110–4116. https://doi.org/10.1021/ac504689z.
Wong, J.M.W., De Souza, R., Kendall, C.W.C., Emam, A., Jenkins, D.J.A., 2006. Colonic health: fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40, 235–243. https://doi.org/10.1097/00004836-200603000-00015.