Characterization of Contents of Distal Ileum and Cecum to Which Drugs/Drug Products are Exposed During Bioavailability/Bioequivalence Studies in Healthy Adults

Christos Reppas [1] · Eleni Karatza [1] · Constantinos Goumas [2] · Constantinos Markopoulos [1] · Maria Vertzoni [1]

ABSTRACT

Purpose Characterize the contents of distal ileum and cecum in healthy adults under conditions simulating the bioavailability/bioequivalance studies of drug products in fasted and fed state.

Methods Twelve males participated in a two-phase crossover study. Phase I: subjects remained fasted overnight plus 4.5 h in the morning prior to colonoscopy. Phase II: subjects remained fasted overnight, consumed breakfast in the morning, and abstain from food until colonoscopy, 4.5 h after breakfast. Upon sampling, volume, pH and buffer capacity were measured; after ultracentrifugation, supernatant was physicochemically characterized and non-liquid particles diameter was measured.

Results In distal ileum, pH is ~8.1 and size of non-liquid particles is ~200 μm, regardless of dosing conditions; in fed state, liquid fraction was lower whereas osmolality and carbohydrate content were higher. In cecum, the environment was similar with previously characterized environment in the ascending colon; in fasted state, size of non-liquid particles is smaller than in distal ileum (~70 μm). Fluid composition in distal ileum is different from cecum, especially in fasted state.

Conclusion Differences in luminal environment between distal ileum and cecum may impact the performance of orally administered products which deliver drug during residence in lower intestine. Dosing conditions affect cecal environment more than in distal ileum.

✉ Maria Vertzoni
vertzoni@pharm.uoa.gr

[1] Faculty of Pharmacy, School of Health Sciences, National & Kapodistrian University of Athens, Panepistimiopolis, 157 71 Zografou, Greece

[2] Red Cross Hospital of Athens, Athens, Greece

ABBREVIATIONS

AA	Acetic acid
API	Active pharmaceutical ingredient
BA	n-Butyric acid
BA/BE	Bioavailability/Bioequivalence
BIS	Bisacodyl
C	Cholic acid
CA	Caproic acid
CDC	Chenodeoxycholic acid
CHO	Cholesterol
DC	Deoxycholic acid
DG	Diglyceride
FA	Fatty acid
GC	Glycocholic acid
GCDC	Glycochenodeoxycholic acid
GDC	Glycodeoxycholic acid
GI	Gastrointestinal
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
IBA	Iso-butyric acid
IVA	Iso-valeric acid
LC	Lithocholic acid
Lyso-PC	Lyso-phosphatidylcholine
MG	Monoglyceride
PA	Propionic acid
PC	Phosphatidylcholine
SCFA	Short chain fatty acid
SD	Standard deviation
TC	Taurocholic acid
TCDC	Taurochenodeoxycholic acid

TG   Triglyceride
UDC   Ursodeoxycholic acid
VA   Valeric acid

INTRODUCTION

Luminal environment in the lower intestine is important for the performance of orally administered active pharmaceutical ingredients (APIs) with extended and/or delayed absorption kinetics. Examples are immediate release products of highly dosed APIs and modified release drug products. Due to the limited free water volume in the transverse colon (1) regions of primary interest in regard to drug/dosage form performance in the lower gut are the distal small intestine, the cecum, and the ascending colon.

To date, performance of dosage forms is assessed with *in vivo* bioavailability (BA) / bioequivalence (BE) studies in healthy adults which are time consuming and costly. One way to reduce the likelihood of failing an *in vivo* BA/BE study, it would be to screen the performance *in vitro* in a simulated environment. In recent years our knowledge on the environment in healthy adult gastrointestinal (GI) tract under conditions simulating BA/BE studies has been improved substantially, for the upper GI lumen and to a limited extent for the lower gut (e.g. 2). It is interesting to note that physicochemical characteristics of contents of ascending colon differ from those in upper intestinal lumen under both fasted and fed state conditions to an extent that may affect API and/or dosage form performance (3–5). Although in clinical practice a distinction between fasted and fed state may not be relevant, in BA/BE studies food effects are of primary concern. Studies have shown that solubility of lipophilic APIs in the lumen of the ascending colon can be different than in the contents of stomach and/or upper intestine during fasting state (6) and such differences together with subsequent difference in luminal dissolution rates may be crucial for the prediction of intraluminal behaviour of highly dosed APIs (7).

In the present investigation the environment in distal ileum was evaluated *versus* the environment in cecum, two regions in the lower intestine which have been poorly characterized to date. Although bacteria may also affect the fate of drugs and dosage forms, in the present study we characterized the contents on a physicochemical basis without specifically considering the microbes.

MATERIALS AND METHODS

Materials

Dulcolax® (disacodyl tablets, 5 mg/tablet) was from Boehringer Ingelheim (Athens, Greece). All chemicals were of analytical grade and purchased from Sigma Aldrich Chemie GmbH (Germany), except for egg phosphatidylcholine (for constructing standard curves), which was donated by Lipoid GmbH (Germany). All solvents were of HPLC grade. Total protein content was determined using a commercially available kit (BCA®, Thermo Scientific, Rockford, USA using bovine serum albumin as reference) whereas samples were pretreated with Compact-Able™ Protein Assay Preparation Reagent set (Pierce, Rockford, USA).

Human Study

The study was conducted in the Red Cross Hospital of Athens after receiving approvals by the Scientific and the Executive Committee of the Hospital (AP 30799, 06 December 2012). The study was performed according to the Declarations of Helsinki for biomedical research involving human subjects.

Inclusion Criteria

Willingness of the subject to participate as indicated by his signed informed consent, age 18–60 years, weight within 20% of ideal body weight as determined by Metropolitan Life Tables, verification of suitability by a general physical examination and ability to abstain from cigarette smoking, alcohol, and over-the-counter and prescription medication(s) for 3 days prior to and throughout the Colonoscopy Day. In addition, a blood sample was taken to assess electrolyte balance, kidney and liver function, blood morphologic characteristics, and lipid levels, and the subject had to be found healthy in all these examinations to qualify.

Exclusion Criteria

Existence of a major health problem (cardiovascular, pancreatic, hepatic, thyroid etc.) and/or existence of any condition requiring prescription drug therapy, recent history of gastrointestinal symptom regardless of the severity (e.g. heartburn, constipation, haemorrhoids, etc.), receipt of an investigational agent (new or generic) within 30 days prior to the initiation of study, presence of antibodies indicating active acute or chronic HIV, HBV, or HCV infection, use of medication which may affect GI function (including antibiotics) within 30 days of the study, and irregular bowel habits.

Subjects

Twelve healthy males with a mean age of 28 (range: 22–42 years) gave informed consent and participated in this two-phase crossover study.

Study Protocol

Subject work-up prior to either Phase involved the administration of 10 mg of bisacodyl 50 h prior to colonoscopy plus 10 mg of bisacodyl 44 h prior to each colonoscopy. With this regimen, bisacodyl effects on the intracolonic environment at the time of colonoscopy have been shown to be non-significant (4). One subject received an additional 5 mg bisacodyl tablet 30 h prior to each colonoscopy. From the time of first bisacodyl administration until the night prior to each colonoscopy day, subjects ate only liquified food (e.g. fish/chicken/ rice soups, fruit juices) and white bread *ad libitum*. From the night prior to colonoscopy day until arrival to the clinic, i.e. for at least 12 h, subjects remained fasted (Fig. 1). Subject workup prior to colonoscopy did not have major effect on stools consistency. Based on Bristol Stool Chart (8) stools consistency at distal colon during colonoscopy varied from Type 3 (like a sausage or snake, but with cracks on its surface) to Type 6 (fluffy pieces with ragged edges, a mushy stool).

In Phase I, subjects reported to the clinic at 8:00 am and then continued fasting for an extra 4.5 h. Colonoscopy was started at ≈12:30 pm (fasted state). In Phase II, subjects were fasted overnight, reported to the clinic at 8:00 am, and were given a standard breakfast (8:00–8:30 am). Four and a half hours after breakfast, colonoscopy was started (fed state). Breakfast consisted of two eggs fried in butter, two strips of bacon, two slices of toasted bread with butter, four ounces of fried potatos, and eight ounces of milk and 150 ml of water (960 kcal: approximately 150, 250 and 560 cal of protein, carbohydrate and fat, respectively) (4,9). In order to control water consumption on Colonoscopy Day non-carbonated water was administerd to subjects according to the schedule described in Fig. 1. Total amount of water from arrival to the clinic until colonoscopy was 900 ml (Fig. 1).

A few minutes prior to colonoscopy, 0.3 mg alphentanyl hydrochloride (0.5 mg/ml, Rapifen®) and 2.0 mg of midazolam (5 mg/ml, Dormicum®) were administered intravenously to induce conscious sedation. Since conscious sedation was induced 10–40 min prior to sample collection, its potential impact on intestinal transit times requires consideration. In preclinical species the effect of opiates on intestinal transit times is unclear (10) whereas the effect of alfentanyl, specifically, is dose-dependent (11). In cats, midazolam administered at 0.1 mg/kg dose level, i.e. much higher than the dose used in the present study on mg/kg basis, increased significantly orocaecal transit times (12), i.e. the impact on intestinal transit specifically cannot be evaluated with certainty. These data suggest that the impact on intestinal transit of alfentanyl and midazolam, administered in the present study at low doses to induce conscious sedation, is not clear.

In both Phases, after placement of the colonoscope, collection of contents from the distal ileum and cecum was performed under anaerobic conditions and lasted approximately 20 min in total; approximately 5 min and 15 min were sufficient to collect practically all of the contents from terminal ileum and cecum, respectively. Ileal samples were collected from the distal ileum approximately 20–30 cm from the ileocecal valve and after completion of collection of ileal samples, and discarding all the ileal contents from the colonoscope, cecal samples, i.e. contents from the region between the ileocecal valve up to the end of the cecum (length of about 8–9 cm (13) were collected. Sampling from each region aimed
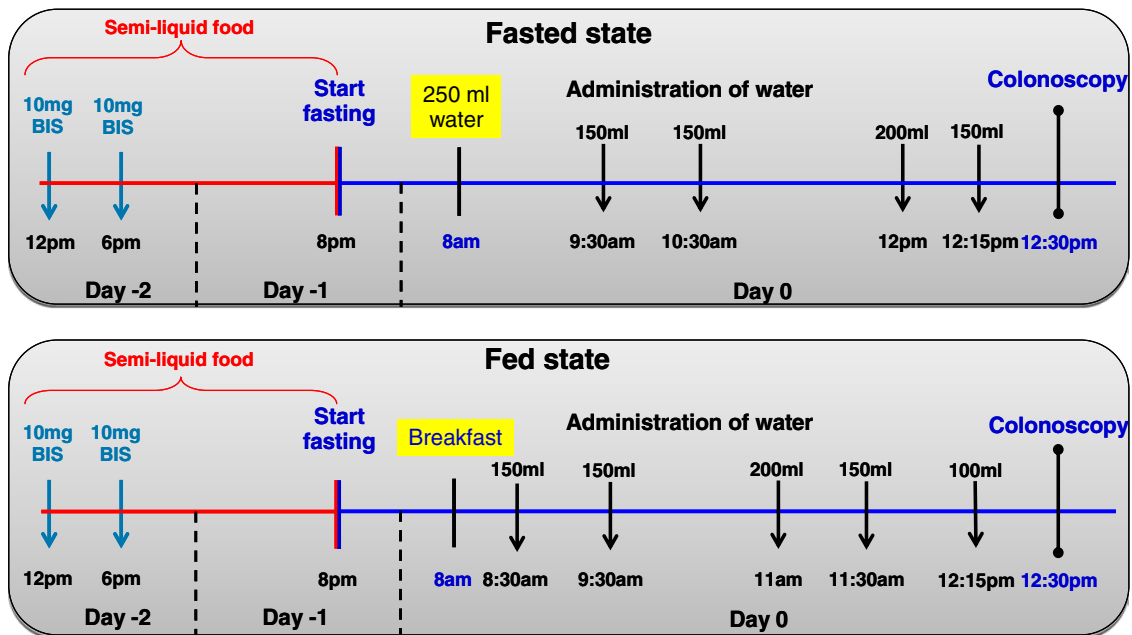
Fig. 1 Subject work-up prior to colonoscopy and administration of water and/or food during the colonoscopy day.

at collecting the entire volume of contents. After removal of the colonoscope, 2 mg of flumazenil (0.1 mg/ml, Anexate®) was administered as antidote to midazolam. In all cases, colonoscopy lasted for about 1 h in total.

Handling of Samples

Sample volume, pH and buffer capacity were measured immediately upon collection. After adding a methanolic cocktail of 50 mM diisopropylfluorophosphate, 50 mM diethyl(p-nitrophenyl)phosphate, 50 mM acetophenone, and 250 mM phenylboronic acid [2% (v:v)] to terminate the supernatant's lipolytic and proteolytic activity (14), the sample was ultracentrifuged (30,000 g, 15 min, 25°C) under anaerobic conditions and the % aqueous content was measured. Ultracentrifugation conditions were appropriate for eliminating both solids and bacteria (15,16) without affecting the structure of bacteria, i.e. without liberating intracellular components which could contribute to degradation in the supernatant (17). To avoid second freeze—thawing cycle of samples, the supernatant was divided in 6 portions that were kept at −70°C until analysis of each of the following six parameters was performed: osmolality, total protein content, total carbohydrate content, short chain fatty acids, ten individual bile acids and lipids. Precipitate was stored under anaerobic conditions at −70°C and in a part of this material non-liquid particles diameter was measured.

Analysis of Samples

pH values were measured by a pH electrode (Schott, model CG842, Mainz, Germany). Buffer capacity was measured by dropwise addition of HCl solution and was calculated as described previously (3).

Osmolality, total protein content and total carbohydrate content, were measured as described previously (3,4).

Individual bile acids, i.e., taurocholic acid (TC), glycocholic acid (GC), taurochenodeoxycholic acid (TCDC), ursodeoxycholic acid (UDC), glycochenodeoxycholic acid (GCDC), cholic acid (C), glycodeoxycholic acid (GDC), chenodeoxycholic (CDC), deoxycholic (DC) and and lithocholic acid (LC) were quantified by a gradient HPLC method using a Charged Aerosol Detector (4).

Lipids, i.e. triglycerides (TGs), diglycerides (DGs), mono-glycerides (MG), fatty acids (FAs), phosphatidylcholine (PC), lyso-phosphatidylcholine (Lyso-PC) and cholesterol (CHO) were assayed by using a previously developed gradient HPLC method using a Charged Aerosol Detector (4). Glycerol trioleate was used for quantifying TGs, 1,2 dioleoyl-rac-glycerol and dipalmitin and dioleylglycerol were used for quantifying DGs, and 1-monooleyl-rac-glycerol was used for quantifying MGs. Oleic acid, linoleic acid and stearic acid were used for quantifying FAs.

Seven short chain fatty acids (SCFAs), i.e. acetic acid (AA), propionic acid (PA), iso-butyric acid (IBA), n-butyric acid (BA), iso-valeric acid (IVA), valeric acid (VA), and caproic acid (CA) were quantified by using a previously developed gas chromatography method (4,18).

Size of non-liquid luminal components was measured using the Malvern Mastersizer apparatus (Malvern Instruments Ltd. Uk). After ultracentrifugation of the luminal samples, a sample from the precipitate was transferred in an Eppendorf tube, 1 mL of water was added, and after vortexing, the sample was transferred in the Malvern Mastersizer volume sample dispersion unit (water volume in the unit: 120 ml, stirrer speed: 2000 rpm), and the volume mean diameter (D[4,3]) was measured. Due to volume availability restrictions, precipitates from five volunteers in the fasted state and six volunteers in the fed state were used; one to four samples from each precipitate were subjected to measurements. A preliminary experiment had showed that same results are obtained if a sample from the precipitate (volunteer #8, cecum sample, fed state, D[4,3] = 271 ± 65 μm) or 1 ml of total luminal sample is used (volunteer #8, cecum sample, fed state, D[4,3] = 269 ± 38 μm). Because of the small volume of collected luminal samples, the use of precipitates was preferred.

Data Analysis

Data are presented as Box-Whisker plots showing the median value, the 10th, 25th, 75th and 90th percentiles, and the individual outlying data points, with dotted lines indicating the mean value.

For each parameter, differences between fasting and fed state and between distal ileum and cecum were evaluated with the paired t-test or the Wilcoxon test, depending on the results of normality and equal variance tests. Type I error was set to 0.05. All statistical comparisons were performed using SigmaStat 3.5 (SPSS Science Inc., New York, USA).

RESULTS AND DISCUSSION

Characteristics of Contents of Distal Ileum and Cecum

Total Volume

The volume of contents recovered from distal ileum and from cecum varied significantly with the prandial state ($p = 0.007$ and $p = 0.010$ for ileal and cecal contents, respectively, Fig. 2). Mean(SD) volumes of samples collected from distal ileum were 3.8(2.3) ml and 7.3(3.3) ml in the fasted and in the fed state, respectively, whereas the corresponding volumes collected from the cecum were 5.0(2.1) ml and 8.0(2.7) ml, respectively (Fig. 2). There was no statistical difference between ileal and cecal volumes at the same prandial state

Fig. 2 Volume, non-liquid particles diameter, pH, and buffer capacity of the contents of distal ileum (*light yellow boxes*) and of cecum (*dark green boxes*) of healthy volunteers measured in the fasted and in the fed state on a crossover basis. n is the number of subjects contributed to the construction of box plots on a crossover basis.
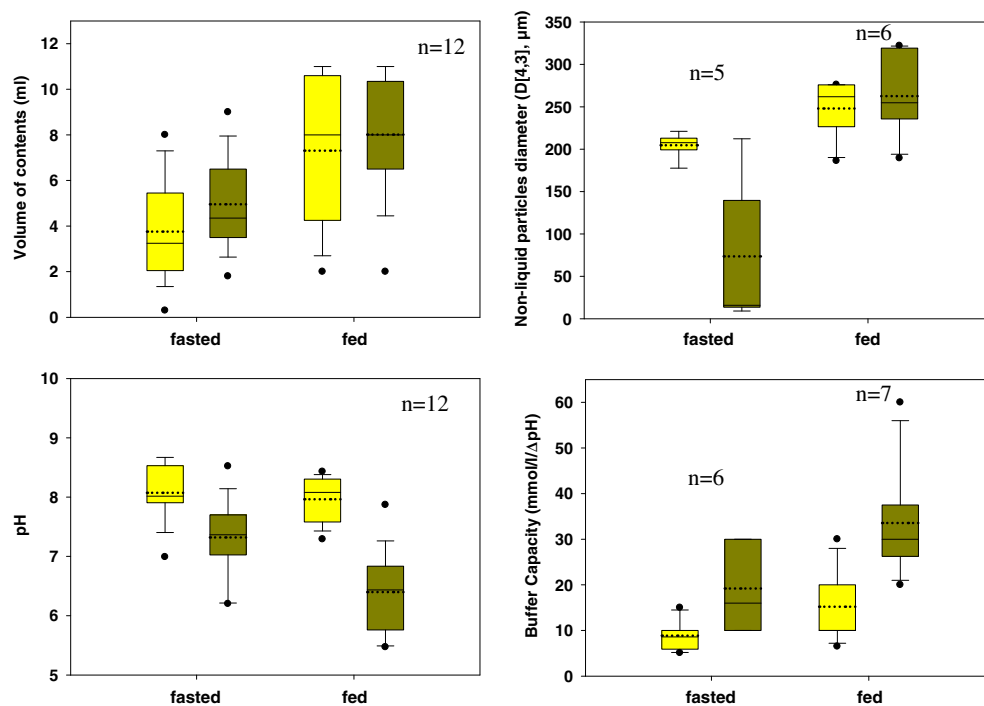
($p=0.058$ and 0.485 in the fasted and in the fed state, respectively). As the collection of ileal contents lasted approximately 5 min, the values of collected volumes from distal ileum are in line with Ladas *et al.* (19) where the ileal flow was measured in 5 healthy persons in fasted state and after a long chain triglyceride rich meal and it was found to be 62.4 ± 24.7 ml/h and 96.1 ± 20.2 ml/h. It should be noted that the estimated daily endogenous ileal flow in healthy adults under normal dietary intakes is approximately 2 l per day (20). Compared to volumes recovered from the ascending colon of healthy volunteers [22.3(7.7) ml and 30.8(10.6) ml, in fasted and fed state, respectively (4)], cecal volumes were statistically lower in both dosing conditions ($p<0.001$). It is interesting to note that, based on mean values, total volume of contents in the ascending colon is 5.9 and 4.2 times bigger than the total volume of contents in distal ileum.

pH and Buffer Capacity

Median pH value of ileal contents was 8.1 regardless the dosing conditions (Fig. 2). Information regarding ileal luminal pH is limited and has been collected either from patients with ileostomy or from healthy adults using the radiotelemetric capsules. The median values estimated from data in the present study are slightly higher than literature data, according to which ileal pH varies between 7 and 8, but in line with literature data indicating no effect of dosing conditions (21). Furthermore, these data confirm the trend of increasing pH values along the small intestine (2,22). pH of the duodenal contents has been measured by direct sampling in several

studies and ranges between 6 and 7 (2,3,21,23) whereas in the jejunum it ranges from 6.5 to 7.8 (2,21) in the fasted state. In the fed state, pH values have been reported to be 5.0–6.5 both in the duodenum (2,3,24,25) and in the jejunum (2,21). Using a radiotelemetry device, pH gradually increases along the small intestine with reported median values being 6.6 in the proximal part, 7.0 in the mid part and 7.3 in the distal part of the small intestine (22).

In cecum, pH of contents varied significantly with prandial state ($p=0.003$) (Fig. 2). Median(range) pH values of cecal contents were 7.4 (range: 6.2–8.5) and 6.4 (range: 5.5–7.9) in the fasted and in the fed state, respectively, and these values differ from pH values of ileal contents ($p<0.001$ at corresponding prandial states) (Fig. 2). Using a similar protocol for collection of contents from the ascending colon, Diakidou *et al.* (4) have reported values [pH 7.6, fasted state and pH 6.2, fed state] which are not statistically different from values measured in cecum in the present study ($p>0.245$). The trend of decreasing pH values from small intestine to large intestine is in line with pH data obtained with radiotelemetry device (22).

As with pH, buffer capacity also did not differ significantly between prandial states in distal ileum [8.9(3.6) mmol/l/ΔpH in the fasted state and 15.2(8.4) mmol/l/ΔpH in the fed state, $p=0.230$] but it differed in cecum [19.2(10.2) mmol/l/ΔpH in the fasted state and 33.6(13.1) mmol/l/ΔpH in the fed state, $p=0.025$] (Fig. 2). It is interesting to note that buffer capacity in distal ileum is similar with values previously reported for duodenal and jejunal contents in the fasted state [2.8 mmol/l/ΔpH (19) to 13 mmol/l/ΔpH (3,26)] and similar with the

value in ileal contents of a patient with polyposis [6.4 mmol/l/ΔpH (27)]. Compared to cecal contents, ileal contents had significantly lower buffer capacity only in the fed state (*p*=0.031). On the other hand, cecal contents had similar buffer capacity with contents in ascending colon in the fasted state [21.5(7.9) mmol/l/ΔpH, *p*=0.246] and in the fed state [37.7(15.5) mmol/l/ΔpH, *p*=0.563] (4).

Liquid Fraction

The liquid fraction of contents in distal ileum was significantly lower (*p*=0.005) in the fed state [68.6(13.2)%] compared to the fasted state [89.9(10.0)%] but in cecum the trend did not reach significance [(63.7(13.1) % in the fed state *vs.* 69.7(19.2) % in the fasted state, *p*=0.436] (Fig. 3). However, the liquid fraction in the fed ascending colon was significantly lower than in the fasted ascending colon (4). Liquid fractions in conjuction with the very small total volumes in the distal ileum and cecum show that liquid volumes in the region are extremely small. In the fasted state, ileal contents had significantly higher proportion of fluids than cecal contents (*p*<0.001), but similar in the fed state (*p*=0.352). It should be noted that the liquid fraction of cecal contents was not statistically different from previously measured values in contents of ascending colon [(70.3(17.0) % and 56.0(9.0) % in the fasted state and in the fed state, respectively (4)] but statistical power of relevant comparisons was low (0.050 for the fasted state and 0.239 for the fed state comparisons). It is interesting to note that, based on mean values, liquid volume in the ascending colon is 15.6 ml in the fasted state and 18.5 ml in the fed state whereas

corresponding liquid volumes in distal ileum are 3.4 and 5.1 ml, respectively. Therefore, ileal liquid volumes are increased 4.6 and 3.6 times upon arrival to the ascending colon in the fasted and in the fed state, respectively. It should also be clarified that liquid volumes reported in this section refer to specific regions at the intestinal lumen, i.e. 20–30 cm of distal ileum, cecum or ascending colon, and, therefore, they are lower than liquid volumes expected to be present in the entire small intestine or colon.

Physicochemical Characteristics of Supernatant After Ultracentrifugation of Contents of Distal Ileum and Cecum

Size of Non-liquid Particles (Solids and Bacteria)

Volume mean diameter (D[4,3]) of non-liquid particles in distal ileum was not statitistically different between the fasted state [205(16) µm] and fed state [248(35) µm, *p*=0.052]. On the other hand, D[4,3] of non-liquid particles in the cecum was statistically lower in the fasted state [74(89) µm] compared with the fed state [263(52) µm, *p*=0.002]. In the fasted state, the diameter of non-liquid particles in distal ileum was significantly higher than in the cecum (*p*=0.012) but it did not differ between the two regions in the fed state (*p*=0.581).

Osmolality

After ultracentrifugation of ileal contents, supernatants were generally hypoosmotic and values in the fasted state were

Fig. 3 Percent liquid fraction of the contents, and osmolality, total protein content and total carbohydrate content of supernatant after ultracentrifugation of contents of distal ileum (*light yellow boxes*) and of cecum (*dark green boxes*) of 12 healthy volunteers in the fasted state and in the fed state on a crossover basis.
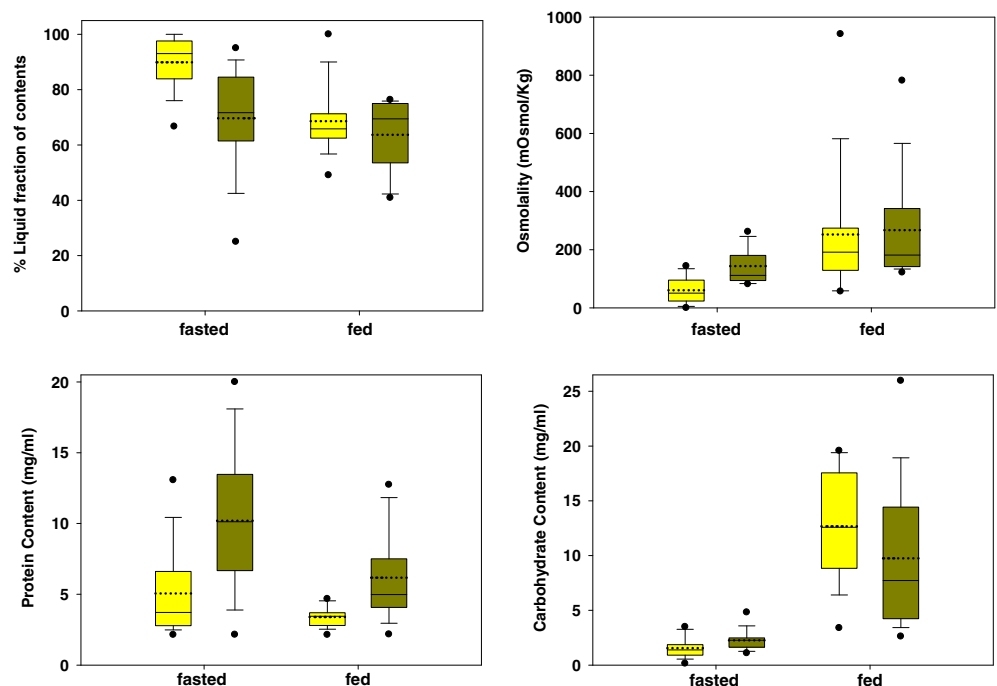
significantly lower than in the fed state [60(50) mOsmol/Kg] *vs.* [252(245) mOsmol/Kg, $p=0.034$] (Fig. 3). Ileal osmolality was lower than that measured in upper small intestine at corresponding dosing conditions (3,26). Data from this study are in agreement with earlier data by Fodtran and Locklear (28) who have reported osmolality values in the mid and lower intestine, after a steak meal to healthy volunteers, between 270 and 310 mOsmol/kg.

Supernatants of ultracentrifuged cecal contents collected in the fasted and in the fed state did not have statistically different osmolalities [144(65) mOsmol/Kg *vs* 267(197) mOsmol/Kg, $p=0.112$] (Fig. 3). Osmolality differed significantly between cecum and distal ileum in the fasted state ($p=0.023$) but not in the fed state ($p=0.775$). Osmolality in the supernatants of ultracentrifuged cecal contents (this study) is similar with values in ascending colon (4) at corresponding dosing conditions.

Protein Content

Protein content in supernatants of ultracentrifuged ileal samples did not differ significantly between fasted state [5.1(3.3) mg/ml] and fed state [3.39(0.74) mg/ml] state (Fig. 3). In supernatants of ultracentrifuged cecal samples, protein content was higher in the fasted state [10.2(2.2) mg/ml] than in the fed state [6.2(3.2) mg/ml] ($p=0.023$, Fig. 2). These data are in line with protein content measured in ultracentrifuged samples collected from ascending colon of healthy volunteers in the fasted [9.8(4.6) mg/ml] and in the fed [6.9(2.4) mg/ml] state (4). Trend for decreased protein content in fed state in ascending colon has been related to the fact that in the fed state digestion and absorption of peptides and proteins administered with the meal are almost complete by the end of the small intestine and the liquid that arrives in the colon in the fed state primarily dilutes existing proteins in the ascending colon (4). It is interesting to note that in the fasted state, protein content in samples collected from the duodenum is approximately 1 mg/ml (23,25), rises in distal ileum and, then, it is almost doubled in cecal contents ($p=0.030$). The increase in protein concentration in cecum compared to distal ileum is also observed in the fed state ($p=0.020$).

Carbohydrate Content

Mean(SD) carbohydrate content of ileal samples was significantly lower in the fasted state [1.55(0.99) mg/ml] than in the fed state [12.7(5.3) mg/ml] ($p<0.001$, Fig. 3). Similar trend was observed in cecal contents; mean(SD) carbohydrate levels were 2.3(1.0) mg/ml and 9.8(7.0) mg/ml, in the fasted and in the fed state, respectively (Fig. 3). In the fasted state, there is statistically significant increase in carbohydrate content from distal ileum to cecum ($p=0.042$, Fig. 3) and from cecum to the

ascending colon where the concentration of soluble carbohydrates was 8.1(8.6) mg/ml [$p=0.042$ (4)]. This trend is not observed in the fed state where mean level of soluble carbohydrates in cecal contents was similar with that observed in the supernatant of colonic contents of fed healthy adults [14.0(7.4) mg/ml] ($p=0.174$ (4)].

Short Chain Fatty acids

Total SCFA concentration was statistically higher in cecum than in distal ileum both in the fasted state ($p<0.001$) and in the fed state ($p<0.001$) (Fig. 4). This is in line with the significantly lower pH values in the cecum compared to the distal ileum. The higher buffer capacity of cecal contents compared to ileal contents could also be attributed (at least partly) to the higher levels of SCFAs.

Mean(SD) total SCFAs levels in distal ileum were 8.6(6.6) mM and 5.8(4.7) mM in the fasted and in the fed state, respectively, whereas corresponding concentrations in cecal contents were 32.2(17.6) mM and 29.3(15.4) mM indicating that the presence of SCFAs did not differ significantly with the dosing conditions in the two regions (Fig. 4). This is in contrast with the singnificant food effect observed previously in contents of ascending colon (4), and may be attributed to a smaller efficiency of bacteria in distal ileum and cecum to digest food remnants, compared with bacteria in the ascending colon where mean(SD) concentration of total SCFAs has been measured to be 30.9(15.4) mM and 48.1(23.8) mM in fasted state and fed state, respectively (4).

Acetate dominated (69–83%) and it was followed by propionate and butyrate whereas caproate was found only in trace amounts (Fig. 4). Similar observations have been made also in fluids collected from the ascending colon of healthy volunteers (4,29).

Bile Acids

In the supernatants of ultracentrifuged contents of distal ileum, cholic acid and chenodeoxycholic acid were the most abundant (mean values are 26 and 13 μM, respectively) in the fasted state, and glycocholic and glycochenodeoxycholic in fed state (mean values are 51 and 28 μM, respectively) (Fig. 5). In cecum, the most abundant bile acids were cholic acid, chenodeoxycholic acid and deoxycholic acid both in the fasted state (mean values are 54, 32, and 51 μM, respectively) and in the fed state (mean values are 100, 70, and 41 μM, respectively) (Fig. 5). These data indicate that deconjugation of bile acids is almost complete in the fasted distal ileum and confirm previous data implying that deconjugation of bile acids is almost complete by entering the large intestine, regardless of dosing conditions (4).

In distal ileum, mean(SD) total bile acid concentration in the fasted state and in the fed state was 71(151) μM and

Fig. 4 Concentrations of individual short chain fatty acids in the supernatant after ultracentrifugation of contents of distal ileum (*light yellow boxes*) and from cecum (*dark green boxes*) of 12 healthy volunteers in the fasted state (**a**) and in the fed state (**b**) on a crossover basis. Asterisk (*) indicates that the difference between ileal and cecal data is significant. Key: AA, acetic acid; PA, propionic acid; IBA, iso-butyric acid; BA, n-butyric acid; IVA, iso-valeric acid; VA, valeric acid; CA, caproic acid.
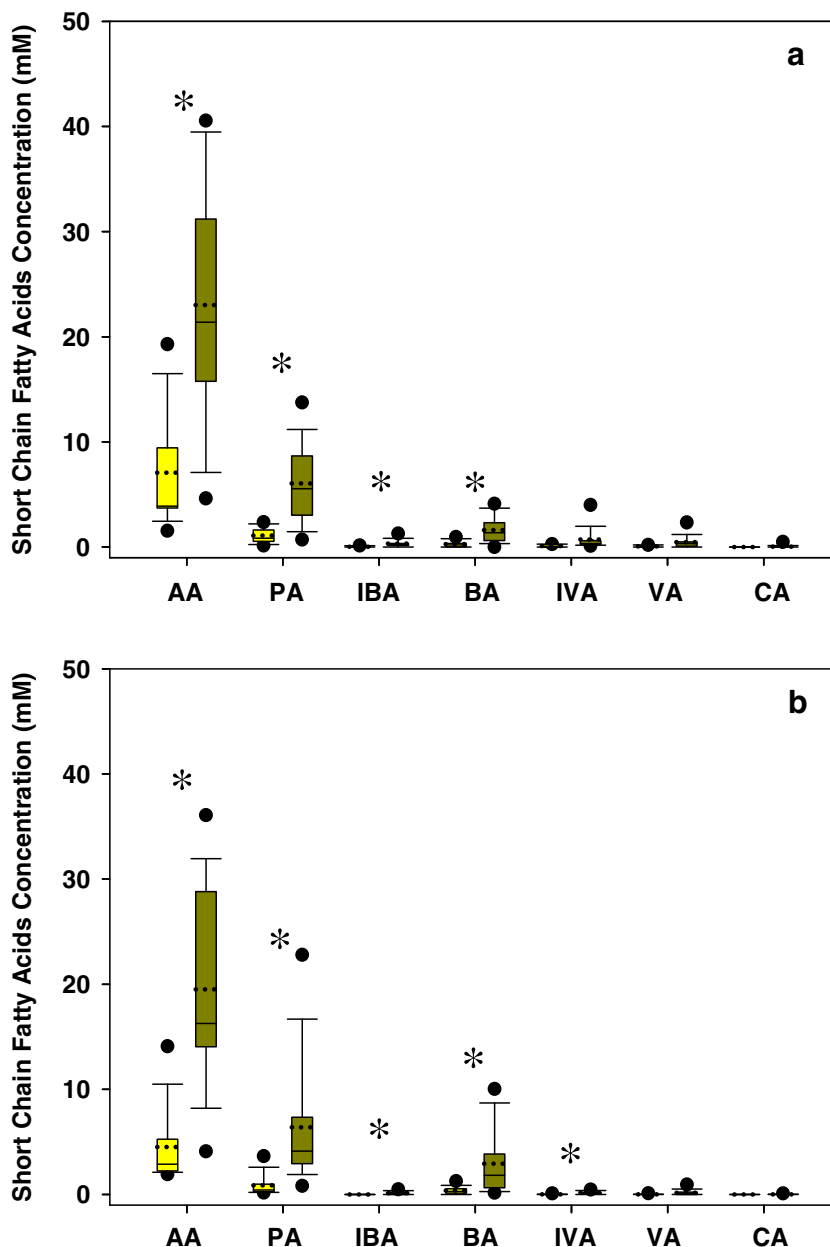
182(132) μM, respectively; this difference did not reach significance ($p=0.078$), possibly due to the low power of the test (power=0.320). In the cecal contents, mean(SD) total bile acid concentration was 183(221) μM and 280(305) μM in the fasted state and in the fed state, respectively, again with the difference not being significant ($p=0.419$ / power=0.050).

Total bile acid concentration in fasted cecum is similar to that in ascending colon [117(123) μM (4), $p=0.373$] but significantly higher than that measured in distal ileum ($p=0.004$). In contrast, in the fed state, total bile acid concentration in cecal contents is similar to ileal contents ($p=0.470$) but lower to recently reported values in colonic contents from healthy volunteers in which the same

meal with the present study was administered [587(413) μM (4), $p=0.023$].

It has been reported that the concentration of bile acids in the distal ileum is influenced by the type of administered meal. Ladas *et al.* (30) found that after a long chain triglyceride meal the bile acid concentrations in distal ileum were higher (median=1.0 mM) than after a medium chain triglyceride meal (median=0.5 mM) (30). As expected, due to bile acid reabsorption in distal ileum (31) these values are significantly lower than in the upper small intestine where concentrations in the fasted state are 2–5 mM (2,3,21,23,25,26) and in the fed state are 8–20 mM (2,3,21,24,25).
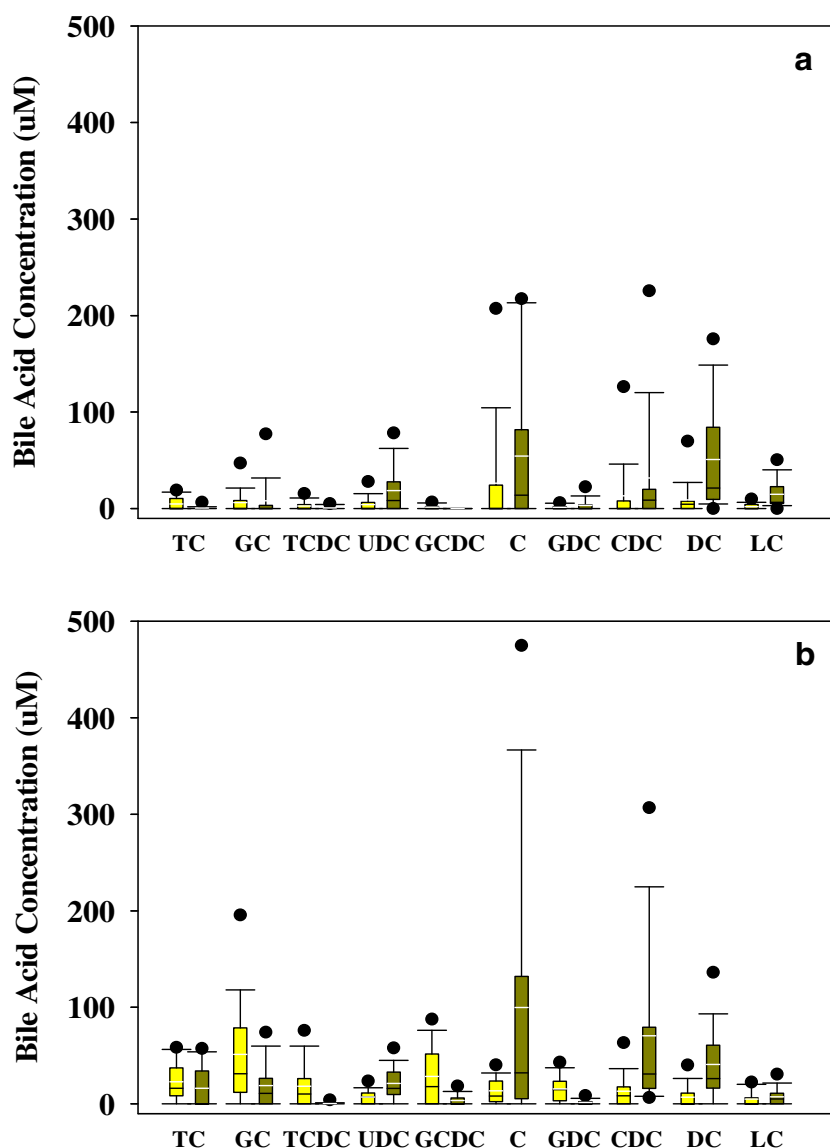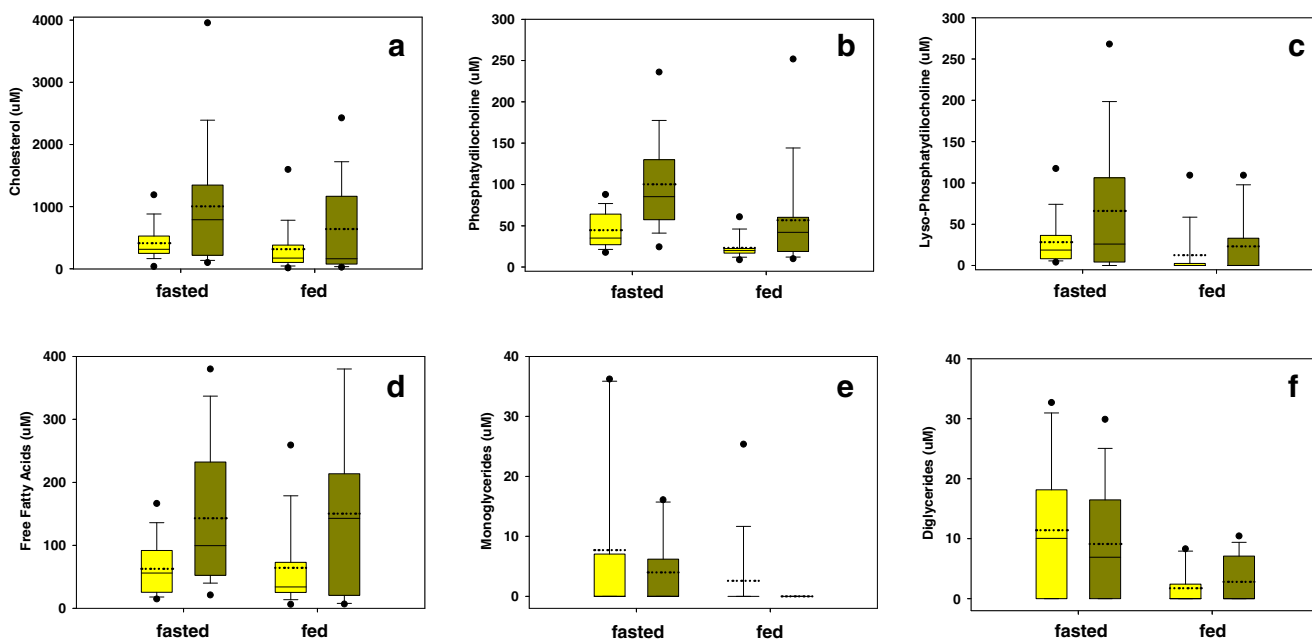
Cholesterol, Phosphatidylcholine, Free Fatty Acids and Glycerides

Mean(SD) concentration of cholesterol in distal ileum was 413(309) μM and 317(426) μM in the fasted and in the fed state, respectively, whereas corresponding concentrations in cecum were 1004(1072) μM and 640(771) μM (Fig. 6a). Within each region concentration of cholesterol does not differ significantly with the dosing conditions. Cholesterol concentration was higher in cecum compared with distal ileum both in the fasted state ($p = 0.036$) and in the fed state ($p = 0.044$). This is in agreement with the higher concentration of SCFAs, and mainly of acetates, in cecum compared to distal ileum, as acetate is the primary substrate for cholesterol synthesis (32). Although cholesterol levels were similar in cecum and ascending colon (4) in the fasted state ($p = 0.260$), in the fed state cholesterol concentration in the

ascending colon was higher [1882(1502) μM, (4)] than in the cecum ($p = 0.006$).

Phosphatidylchloline was quantified in all supernatants after ultracentrifugation collected from distal ileum and cecum (Fig. 6b). Lyso-phosphatidylchloline was quantified in all supernatants in the fasted state but in only 3 out 12 ileal and in 5 out 12 cecal supernatants in the fed state (Fig. 6c). Concentration of total phospholipids in distal ileum did not differ in the fasted state compared to the fed state [73(41) μM *vs* 40(51) μM, $p = 0.170$]. In cecum, the total concentration of phospholipids was higher in the fasted state compared to the fed state [166(110) μM *vs* 82(77) μM, $p = 0.033$]. In the fasted state, total concentration of phospholipids increases significantly from distal ileum to cecum ($p < 0.001$) and, as previous data indicate from cecum to ascending colon [362(210) μM, (4)]. In the fed state, there is no difference in phospholipids

Fig. 6 Concentrations of cholesterol (**a**), phosphatidylcholine (**b**), lyso- phosphatidylcholine (**c**), long chain free fatty acids (**d**), monoglycerides (**e**) and diglycerides (**f**) in the supernatant after ultracentrifugation of contents of distal ileum (*light yellow boxes*) and of cecum (*dark green boxes*) of 12 healthy volunteers measured in the fasted state and in the fed state on a crossover basis.

concentration between distal ileum and cecum ($p=0.110$) but there is an increase in the ascending colon [539(393) μM, (4)]. It should be noted that no lyso-phosphatidylchloline could be identified in supernatants of samples collected from the ascending colon (4) and its absence indicated that phosphatidylchloline was not of pancreatic origin as phosphatidylchloline is converted to lyso-phosphatidylchloline by pancreatic phospholipase A in the upper small intestine (4). Data from the present study confirm this hypothesis for the fed state, but it is unclear if in the fasted state lyso-phospahtidylcholine in ileum and cecum is partly of pancreatic origin.

Free fatty acids were quantified in the supernatants of collected contents as the sum of oleic, linoleic and steatic acid and there was no statistically significant difference between prandial states. Mean(SD) concentrations in distal ileum were 63(47) μM and 64(72) μM, in the fasted and fed state. In cecum, corresponding concentrations were 143(118) μM and 150(141) μM (Fig. 6d). Supernatants from cecal contents contained almost double concentration of free fatty acids than ileal contents both in fasted state ($p=0.030$) and in the fed state ($p=0.044$) with concentrations being similar with those in supernatants from ascending colon (4).

Regardless of the dosing conditions, triglycerides were below detection limit in the supernatant after ultracentrifugation of ileal and cecal contents and were found in traces only in ileal supernatant in 3 out of 12 fed subjects. Minimal concentrations of monoglycerides and diglycerides were observed in the fasted state both in the distal ileum and in the cecum, especially in the fed state (Fig. 6e and f). Similar observations have been made previously for the contents of the ascending colon (4).

CONCLUDING REMARKS

In the present investigation we characterized the contents of distal ileum and cecum in healthy adults under conditions simulating the bioavailability/bioequivelance studies of drug products in fasted and fed state.

Fluid composition in distal ileum is different from cecum, especially in the fasted state.

In distal ileum, pH seems to be approximately 8.1 and size of non-liquid particles approximately 200 μm, regardless of dosing conditions. Liquid fraction is lower whereas osmolality and carbohydrate content is higher in fed state. Other components exist at low concentrations, not significantly affected by dosing conditions; therefore, they are unlikely to affect drug product performance in the region.

In cecum, dosing conditions affect the environment more than in distal ileum, i.e. pH, protein content and phospholipids are lower in the fed state whereas buffer capacity is higher in the fed state. Relevant data are qualitatively and quantitatively in line with previously reported data for the conditions in the ascending colon (4). In the fasted state, size of non-liquid particles is much smaller than in distal ileum (approximately 70 μm).

Data collected in this study provide a basis for developing *in vitro* methods to evaluate the impact of luminal environment in distal ileum and in cecum on the performance of orally administered highly dosed active pharmaceutical ingredients and modified release drug products.

ACKNOWLEDGMENTS AND DISCLOSURES

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