

Characteristics of the Human Upper Gastrointestinal Contents in the Fasted State Under Hypo- and A-chlorhydric Gastric Conditions Under Conditions of Typical Drug – Drug Interaction Studies

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ABSTRACT

Objective Evaluate the impact of reduced gastric acid secretion after administration of two acid-reducing agents on the physicochemical characteristics of contents of upper gastrointestinal lumen of fasted adults.

Materials and Methods Eight healthy male adults, fasted from food for 12 h, participated in a three-phase crossover study. *Phase 1:* No drug treatment prior to aspirations. *Phase 2:* Oral administration of 40 mg pantoprazole at ~9 am the last 3 days prior to aspirations and at ~7 am on aspiration day. *Phase 3:* Oral administration of 20 mg famotidine at ~7 pm prior to aspirations and at ~7 am on aspiration day. Samples from the contents of upper gastrointestinal lumen were aspirated for 50 min, after administration of 240 ml table water at ~9 am.

Results Reduction of gastric acid secretion was accompanied by reduced buffer capacity, chloride ion concentration, osmolality and surface tension in stomach and by increased pH (up to ~0.7 units) in upper small intestine during the first 50 min post-water administration. The mechanism of reduction of acid secretion seems to be important for the buffer capacity in stomach and for the surface tension in upper gastrointestinal lumen.

Conclusions Apart from gastric pH, reduced acid secretion affects physicochemical characteristics of contents of upper

gastrointestinal lumen which may be important for the performance of certain drugs/products in the fasted state.

KEY WORDS achlorhydria · duodenal composition · fasted state · gastric composition · hypochlorhydria · upper gastrointestinal lumen

ABBREVIATIONS

API	Active pharmaceutical ingredient
BIC	Bayesian information criterion
DDI	Drug-drug interaction
FaSSIF	Fasted state simulating intestinal fluid
FMT	Famotidine
GC	Glycocholic acid
GCDC	Glycochenodeoxycholic acid
GDC	Glycodeoxycholic acid
GI	Gastrointestinal
H ₂ -RA	Histamine-2 receptor antagonist
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
Lyso-PC	Lyso-phosphatidylcholine
MG	Monoglyceride
PC	Phosphatidylcholine
PNT	Pantoprazole
PPI	Proton pump inhibitor
SD	Standard deviation
TC	Taurocholic acid
TCDC	Taurochenodeoxycholic acid

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INTRODUCTION

Reduced acid secretion in stomach has been shown to significantly impair absorption of poorly soluble, weakly alkaline

drugs (1). As of the end of 2006, 38% of active pharmaceutical ingredients (APIs) approved in the U.S.A. after 1981 for oral administration were basic molecules (2). It is estimated that 50% of recently approved molecular targeted oral cancer therapies are weak bases and display solubility-limited dissolution properties that are known to impact drug absorption (3). For this reason, the effects of acid reducing agents on cancer therapeutics will be challenging for drug-development scientists, oncologists and regulatory agencies in ensuring that patients achieve safe and efficacious exposures of their cancer therapeutics and thus optimal patient outcomes (3). Indicative of the severity of the issue are recent proposals for gastric re-acidification of gastric contents in order the efficacy of certain APIs to be maintained (4).

Various strategies are being considered for mitigating the effects of increased gastric pH and for enabling the development of effective and safe pharmaceutical products of poorly soluble weak bases (5). However, to date there is no systematic study of potential changes in the characteristics of contents of upper gastrointestinal (GI) lumen that may accompany the increased gastric pH and affect the intraluminal performance of poorly soluble weak bases in the fasted state.

The primary objective of this study was to evaluate the impact of hypochlorhydria (i.e. partially impaired acid secretion) and achlorhydria (i.e. no acid secretion) in stomach on the physicochemical characteristics of contents of stomach and upper small intestine of fasted adults under conditions typically employed for conducting such drug – drug interaction (DDI) studies with stomach pH altering medications during clinical development. A secondary objective was to evaluate whether the medication class employed results in differential effect on these characteristics. More specifically, hypochlorhydria and achlorhydria were achieved by treating healthy adults with a proton pump inhibitor (PPI), pantoprazole, and a histamine-2 receptor antagonist (H₂-RA), famotidine, respectively. Both agents are routinely used for typical DDI studies this study was intended to mimic. Compared with other members in their class, these acid reducing agents are much less likely to induce or inhibit metabolic processes (6). Famotidine has also been shown not to affect pancreatic exocrine or gastrointestinal motility functions (7).

Pantoprazole was selected as the PPI for this study, as it has high and not highly variable bioavailability (bioavailability is approximately 77%) which does not change on multiple dosing, so that maximum blood levels are achieved after the first dose (8,9). It has been documented that the potency of pantoprazole is similar to that of omeprazole, another commonly used PPI, on mg for mg basis (9). From 6 to 8 h after a single oral dose of 30 mg omeprazole, there is a 66% reduction in basal acid output and 71.2% reduction in pentagastrin stimulated acid output (10). In the present investigation, 40 mg pantoprazole per day for 3 days prior to and in the

morning of each aspiration day were administered to each volunteer. A similar scheme has been previously used by others with esomeprazole (the S-enantiomer of omeprazole) to simulate the effects of acid reducing agents in stomach (11,12).

With famotidine, dose related suppression of basal and stimulated gastric acid output has been shown with oral doses of 5–40 mg; within this dose range the oral absorption is dose independent (7,13). In the present investigation, each volunteer was administered 20 mg famotidine on the day before and in the morning of each aspiration day.

EXPERIMENTAL

Materials

Pantoprazole (PNT) film-coated tablets (Controloc[®], 40 mg, Takeda Hellas, Greece) and famotidine (FMT) film-coated tablets (Peptan[®] 20 mg, MSD/Vianex, Athens, Greece) were purchased locally. 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.

Human Study

The study was held in the Red Cross Hospital of Athens after receiving approval by the Scientific and the Executive Committee of the Hospital (AP 10117–3 April 2014). The study followed the tenets of the Declarations of Helsinki for biomedical research involving human subjects.

Inclusion Criteria

Willingness to participate as indicated by subject's signed informed consent; age 18–50 years; body weight within 20% of ideal body weight as determined by Metropolitan Life Tables; verification of suitability by a general physical examination; ability to abstain from cigarette smoking, alcohol, and over-the-counter and prescription medication(s) for 3 days prior to Aspiration Day until the end of the Aspiration Day. 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 deemed healthy in all these examinations to qualify.

Exclusion Criteria

Existence of a major health problem (cardiovascular, pancreatic, hepatic, thyroid etc.); existence of any condition requiring prescription drug therapy; recent history of gastrointestinal

symptom regardless of the severity (e.g. heartburn, constipation etc.); receipt of an investigational agent (new or generic) within 30 days prior to the initiation of the study; presence of antibodies indicating active acute or chronic HIV, HBV, or HCV infection; use of medication which may affect GI function (including antacids, PPIs, H₂-RAs, and laxatives) within 30 days of the study.

Subjects

Eight healthy male adults with a mean age of 27 years (range 23–34 years) gave informed consent and participated in the study. Body weights of all subjects deviated from the ideal weights by less than 12%. Health status of each subject was confirmed by physical examination and screening of blood parameters for renal and hepatic functions, as described above.

Study Protocol

The study consisted of three phases and was performed on a crossover basis (Fig. 1).

Phase 1: Subject was fasted from food (water *ad libitum*) for at least 12 h prior to the initiation of aspirations.

Phase 2: Subject was administered 40 mg of pantoprazole (Controloc[®], 40 mg/tab) *per os* at about 9 am for 3 days prior to the Aspiration Day. On the

Aspiration Day at about 7 am subject was administered 40 mg of pantoprazole. Subject was fasted from food (water *ad libitum*) for at least 12 h prior to the initiation of aspirations.

Phase 3: Subject was administered 20 mg of famotidine (Peptan[®], 20 mg/tab) *per os* at 7 pm on the day before the Aspiration Day. On the Aspiration Day at 7 am subject was administered 20 mg of famotidine. Subject was fasted from food (water *ad libitum*) for at least 12 h prior to the initiation of aspirations.

At about 8 am on each Aspiration Day, the subject arrived at the clinic and received about 150–200 mL of table water. At about 8:30 am the upper throat was sprayed with lidocaine and the subject was intubated nasally using a sterile two-lumen tube (Freka[®] Trelumina, CH/FR 16/9, 150 cm, Fresenius Kabi Deutschland GmbH, Bad Hombourg, Germany). The double bore tube was 150 cm long with an external diameter of 5.3 mm in stomach, an external diameter of 2.9 mm at the pylorus and in the small intestine, and a plastic tip at its distal end. A series of holes 55–65 cm proximal to the tip were used to access the antrum of the stomach. A further series of hand-made holes 13.5–23.5 cm proximal to the tip were used to aspirate samples from the ligament of Treitz (14). Insertion of the tube was assisted by a guiding wire, and its position was monitored fluoroscopically. After reaching its final position and removing the wire, the subject

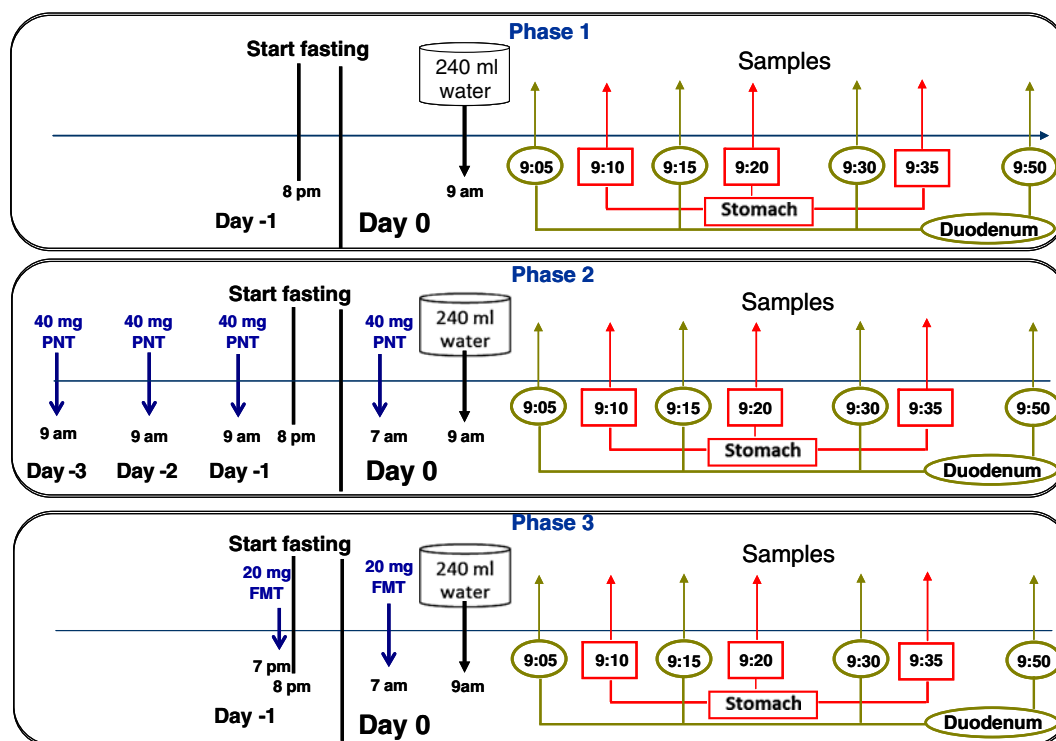


Fig. 1 Schematic of the three Phases of the clinical study. Key: PNT, pantoprazole; FMT, famotidine.

laid semisupine, secretions accumulated in stomach during the intubation process were removed and a sample was aspirated to measure baseline gastric pH. After collecting a sample from the upper small intestine and baseline pH in upper small intestine was recorded, 240 mL of table water were administered via the tube through the gastric series of holes. The administration lasted for about a minute. Samples (up to 8 mL) were aspirated from the stomach at 10, 20, 35 min and from the end of duodenum at 5, 15, 30, 50 min, after the administration of water. At the end of each aspiration and before removing the tube / discharging the subject, the final position of the tube was confirmed fluoroscopically.

Handling of Aspirated Samples

pH and buffer capacity were measured immediately upon sample aspiration.

The remaining of each aspirated sample was immediately divided into eight subsamples for measuring chloride ion concentration, osmolality, total protein content, six individual bile acids, lipids, buffer capacity, surface tension and kinematic viscosity of aspirated samples, after one freeze-thaw cycle. All measurements, apart from surface tension and kinematic viscosity, were performed in individual subsamples. Each subsample was kept at -70°C until a relevant measurement was performed. Surface tension and kinematic viscosity were performed in pooled aspirates. Pools were created by using all subsamples collected during a specific phase from the antrum and from the end of duodenum, i.e. 2 pools per phase were created. Each pool was created just prior to measuring the surface tension and viscosity.

Analysis of Samples

pH values were measured with a pH electrode (Schott, model CG842, Mainz, Germany). Buffer capacities that were measured upon sample collection were measured in just one pH direction by dropwise addition of either NaOH (for samples aspirated from the stomach) or HCl (for samples aspirated from duodenum). Buffer capacities that were measured after storage at -70°C and thawing were measured in both pH directions by dropwise addition of NaOH and HCl solutions. Concentration of chloride ions was measured using a Chloride Ion-Selective Electrode with built in reference electrode (LIS-146CLCM Micro Chloride Ion Electrode, Lazaar Research Laboratories, Inc., Los Angeles, USA). Osmolality was measured by using the freezing point depression technique (semi-micro osmometer Typ Dig L; Knauer, Berlin, Germany). Total protein content was determined using a commercially available kit (BCA[®], Protein Assay Reagent Kit, Thermo SCIENTIFIC, Rockford, IL, USA) and bovine serum albumin as standard whereas samples were pretreated with Compact-AbleTM Protein Assay Preparation Reagent set

(Pierce, Rockford, IL, USA). Taurocholic acid (TC), glycocholic acid (GC), taurochenodeoxycholic acid (TCDC), ursodeoxycholic acid (UDC), glycochenodeoxycholic acid (GCDC) and glycodeoxycholic acid (GDC) were quantified by HPLC using a Charged Aerosol Detector (15). Monoglycerides (MGs), free fatty acids (linoleic, palmitic and stearic acids), phosphatidylcholine (PC), lysophosphatidylcholine (Lyso-PC) and cholesterol were assayed by using a previously developed gradient HPLC method using a Charged Aerosol Detector (16). Data are presented on molar basis. Surface tension was measured according to the Du Nouy Ring method (KSV Sigma 70, KSV Instruments, U.S.A) at 37°C ; mean(SD) values of 10–12 measurements that the instrument performs for each sample were reported. Kinematic viscosity was measured using a direct-flow gravimetric capillary (Cannon-Fenske type) at 37°C , using as reference the kinematic viscosity of water at 37°C ($0.6959\text{ mm}^2/\text{s}$, www.viscopedia.com), in order to calibrate the capillary and calculate the capillary factor.

Data Analysis

Mean(SD) values are reported in the text, after confirming normality. Alternatively, median(range) values are reported. For certain parameters, overall mean or median values were also estimated by using all individual data collected within each Phase in stomach or in upper small intestine.

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

For each parameter, data from different treatments and time points were obtained from the same subject and, therefore, were assumed to be correlated. The MIXED procedure of SPSS v 17.0.0 was used to model the correlation between treatments and/or between aspiration time points. This was accomplished by including random effects based on subjects, on location (stomach-duodenum) within subject, on treatments within subject or on the interaction of treatment and location within subject. The choice of the best model for the covariance was based on the lowest value of the Schwarz's Bayesian Information Criterion (BIC). Fixed effects variables like treatments, locations, and their interactions with aspiration time were included and chosen using the F-test. Type I error was set at 0.05. For bile acids in stomach, differences between treatments were analyzed using Wilcoxon test as they presented an excess of zeros. In this case, type I error was set at 0.025. For bile acids in upper small intestine, mean values are typically reported in literature but, since distribution of values is skewed (14), in this paper both mean and median values are presented.

In order to evaluate the effect of one freeze-thaw cycle on buffer capacity, differences between values obtained upon

collections and after one freeze-thaw cycle 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.

RESULTS

Effects of Pantoprazole and Famotidine Treatments on Fasting pH Values in Upper Gastrointestinal Lumen

Fasting gastric pH values in the morning prior to water administration in Phase 2 (pantoprazole) and in Phase 3 (famotidine) were significantly higher than in Phase 1 (control); median(range) values were 1.4(1.2–2.7) in Phase 1, 2.4(1.5–7.3) in Phase 2, and 7.0(2.6–7.6) in Phase 3 ($p \leq 0.009$). These data confirm that the famotidine treatment regimen selected resulted in maximal effect of increasing basal gastric pH. Pantoprazole under the regimen selected resulted in hypochlorhydric conditions. The hypochlorhydric conditions achieved with the pantoprazole treatment resemble the values which are observed in certain populations groups, such as the Japanese (17), the elderly (18) and occasional users of proton pump inhibitors.

Fasting pH values in upper small intestine in the morning prior to water administration in Phase 2 and in Phase 3 were not significantly different from those in Phase 1 ($p = 0.120$ for the Phase 1 *vs.* Phase 2 comparison and $p = 0.101$ for the Phase 1 *vs.* Phase 3 comparison); median(range) values were 6.4(2.6–7.7) in Phase 1, 7.2(6.5–7.6) in Phase 2, and 7.3(7.0–7.6) in Phase 3.

Effect of Reduced Acid Secretion in Stomach on the Characteristics of Gastric Contents in the Fasted State After Water Administration

pH

During the first 35 min post water administration, intragastric pH values in Phase 1 (control) were significantly lower than in Phase 2 (pantoprazole) or in Phase 3 (famotidine) at all sampling time points ($p \leq 0.001$). In Phase 1, median(range) pH values of samples aspirated 10, 25 and 35 min post water administration were 2.9(1.8–3.9), 1.7(1.2–2.0) and 1.6(1.1–2.4), respectively (Fig. 2). pH values post water administration in Phase 1 are in line with previous data (19). Corresponding values in Phase 2 and in Phase 3 were 6.4(2.0–7.2), 5.2(1.4–7.3) and 2.4(1.3–7.6), and 7.2(6.9–7.3), 7.1(6.0–7.2) and 7.1(5.0–7.3), respectively (Fig. 2). Unlike with Phase 3, in Phase 1 and in Phase 2 intragastric pH decreased significantly as a function of time post water administration ($p \leq 0.014$). The decreasing intragastric pH with time in Phase 2 is in contrast with the steady and higher intragastric pH values, after similar

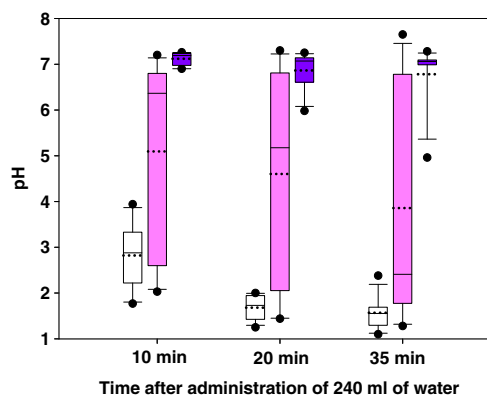


Fig. 2 pH in the stomach of fasted healthy adults as a function of time, after administration of 240 ml table water into the antrum of the stomach. Key: White boxes, Phase 1; Light pink boxes, Phase 2; Dark blue boxes, Phase 3. Each box was constructed by using 7–8 individual values.

treatment of healthy adults with esomeprazole (12). Overall median values for gastric pH during the first 35 min post water administration in Phase 1, in Phase 2 and in Phase 3 were 1.8, 5.1, and 7.1, respectively.

Buffer Capacity

During the first 35 min post water administration, buffer capacity measured immediately upon aspiration with NaOH in Phase 1 was higher than in Phase 2 or Phase 3 but the difference reached significance only *versus* Phase 3 (Fig. 3, empty boxes, $p < 0.001$). In Phase 1, mean(SD) buffer capacity values of samples aspirated 10, 25 and 35 min after water administration were 4.7(4.6), 21.3(11.4) and 27.6(15.7) mmol/L/ Δ pH, respectively, i.e. in line with previous data (Kalantzi *et al.* 2006). Corresponding values in Phase 2 and Phase 3 were 1.7(2.3), 6.3(10.6), 12.4(15.5) and 0.49(0.21), 0.69(0.21), 1.29(0.65) mmol/L/ Δ pH, respectively. Buffer capacity values increase between 10 and 35 min post water administration in all Phases, but the trend reached significance only in Phase 1 (Fig. 3, empty boxes, $p = 0.002$).

One freeze-thaw cycle affected buffer capacity measured with NaOH in Phase 1 and in Phase 3 significantly (Fig. 3, empty *vs.* lined boxes within Phase 1 and within Phase 3, $p < 0.02$) but not in Phase 2 (Fig. 3, empty *vs.* lined boxes within Phase 2, $p > 0.06$). Also, compared with the values estimated after titration with NaOH, buffer capacities estimated with HCl were significantly higher in Phase 1 and in Phase 3 (Fig. 3, lined *vs.* dotted boxes within Phase 1 and within Phase 3, $p < 0.03$) but not in Phase 2 (Fig. 3, lined *vs.* dotted boxes within each Phase, $p > 0.05$). These data suggest that the smaller decrease of buffer capacity in the pantoprazole Phase (Phase 2) may not be entirely due to the incomplete reduction of gastric acid secretion.

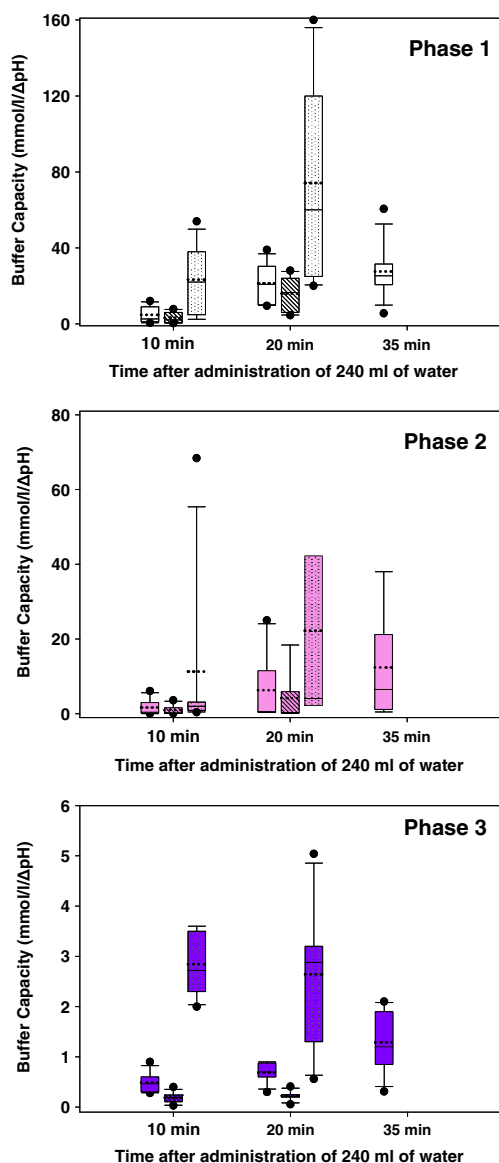


Fig. 3 Buffer capacity of contents of the stomach of fasted healthy adults as a function of time, after administration of 240 ml table water into the antrum of the stomach measured in Phase 1 (White boxes, $n = 6-8$), in Phase 2 (Light pink boxes, $n = 4-8$), and in Phase 3 (Dark blue boxes, $n = 6-8$) immediately upon collection using NaOH (empty boxes), after one freeze-thaw cycle using NaOH (lined boxes) and after one freeze-thaw cycle using HCl (dotted boxes). Due to limited available volumes, data after one freeze-thaw cycle in samples aspirated 35 min post water administration could not be collected.

Chloride ion Concentration

During the first 35 min post water administration, concentration of chloride ions in Phase 2 and in Phase 3 was lower than in Phase 1 (Fig. 4a) but the difference reached significance only at 20 and 35 min post water administration ($p \leq 0.003$). In Phase 1, mean(SD) values in samples aspirated 10, 20 and 35 min post water administration were 41.0(25.3), 110.1(43.8), and 176.3(84.9) mM, respectively, i.e. in line with previous data (20). Corresponding values in Phase 2 and in

Phase 3 were 18.2(10.5), 54.2(49.0), 88.2(86.1) and 10.1(3.2), 20.3(7.7), 47.9(28.5) mM, respectively. Chloride ion concentration increased between 10 and 35 min post water administration in all three phases but the trend reached significance only in Phase 1 and in Phase 2 ($p < 0.001$). These data are in line with the gradual emptying of gastric contents and the minimal hydrochloric acid secretion in Phase 3.

Osmolality

During the first 35 min post water administration, osmolality in Phase 2 and in Phase 3 was lower than in Phase 1 (Fig. 4b) but the difference reached significance only versus Phase 3 ($p \leq 0.002$ at 20 min and 35 min). In Phase 1, mean(SD) osmolality values of samples aspirated 10, 25 and 35 min after water administration were 44.9(22.6), 103.6(41.5) and 144.0(44.0) mOsmol/kg, respectively, i.e. in line with previous data (19). Corresponding values in Phase 2 and Phase 3 were 29.1(14.1), 61.9(32.8), 98.2(53.3) and 22.9(4.1), 39.6(15.7), 91.2(54.0) mOsmol/kg, respectively. Osmolality values increased significantly between 10 and 35 min post water administration but remained hypo-osmotic in all Phases (Fig. 4b).

Total Protein Content

Total protein content was not significantly different among the three Phases (Fig. 4c). This is in line with previous data in humans showing that pepsin is not affected by long term administration of omeprazole (10,21). The trend for increased protein content with time post water administration did not reach significance, in all Phases. Mean(SD) values for protein content at 10, 20 and 35 min post water administration were 0.27(0.14), 0.53(0.18) and 0.71(0.35) mg/ml in Phase 1, 0.40(0.15), 0.87(0.66) and 0.88(0.61) mg/ml Phase 2 and 0.45(0.32), 0.60(0.21) and 0.92(0.32) mg/ml Phase 3, respectively (Fig. 4c). Values are in line with previous data in healthy adults (19).

Bile Acids

Median(range) values for total bile salt content in Phase 1 (Fig. 4d) 10, 20 and 35 min post water administration were 0(0–145), 0(0–114) and 54.0(0–620) μ M, respectively. Values are in line with previous data (15). Similar median(range) values were estimated for total bile acid content in Phase 2 [4.8(0–262), 0(0–604) and 0(0–1166) μ M, respectively] and in Phase 3 [0(0–83.0), 15.0(0–971) and 256(0–1975) μ M, respectively]. In all three Phases, glycocholate and glycochenodeoxycholate were the most prevalent bile acids (Table I), in line with previous studies (22). The trend for increased total bile acids content under reduced gastric acid conditions at late times post water administration could be related with the small basal intragastric volumes under such conditions (23).

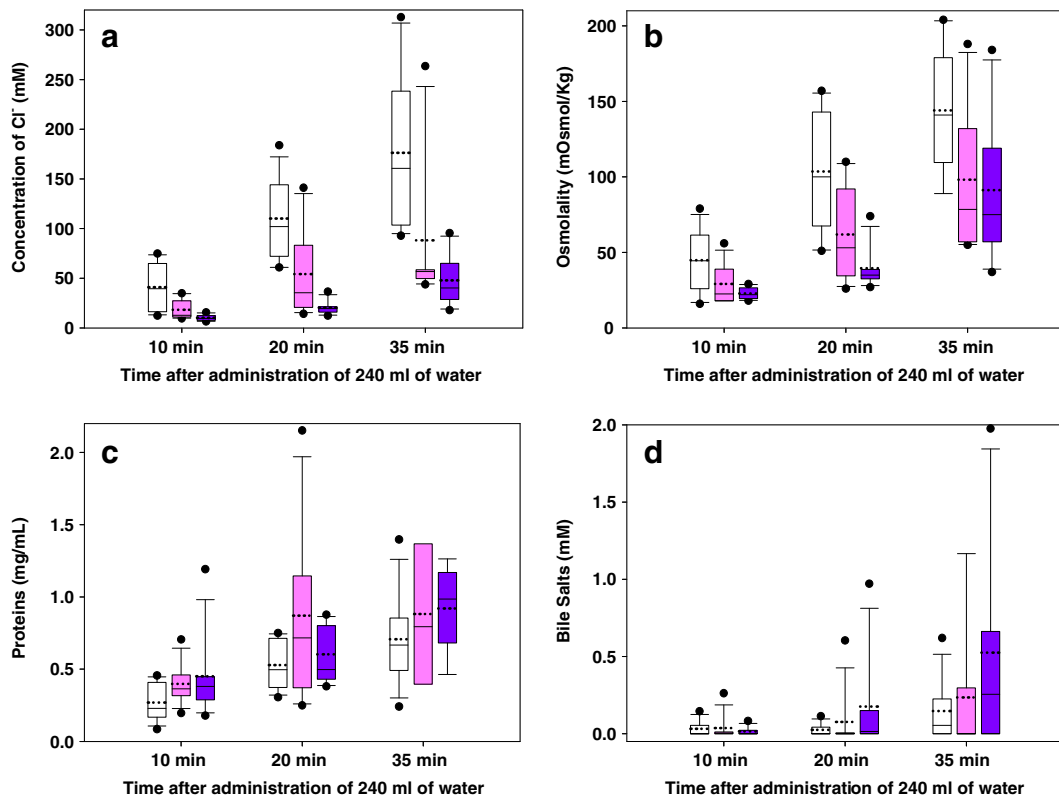


Fig. 4 Concentration of chloride ions (a), osmolality (b), total protein content (c), and total bile salt content (d) in the stomach of fasted healthy adults as a function of time, after administration of 240 ml table water into the antrum of the stomach. Key: White boxes, Phase 1; Light pink boxes, Phase 2; Dark blue boxes, Phase 3. Each box was constructed by using 4–8 individual values.

Surface Tension

Mean(SD) values for the surface tension of gastric contents after administration of 240 ml water to fasted males in Phase 1, Phase 2 and Phase 3 were 43.22(0.74), 34.29(0.51) and 37.35(0.99) mN/m, respectively. Data in Phase 1 are in line with previously published data in healthy adults using individual aspirates (19). The lowest surface tension was observed in Phase 2, i.e. under conditions moderate reduction of gastric acid reduction, indicating that the mechanism for reducing gastric acid secretion is important for the surface tension of gastric contents.

Effect of Reduced Acid Secretion in Stomach on the Characteristics of Contents of Upper Small Intestine in the Fasted State After Water Administration

pH

During the first 50 min post water administration, pH values in upper small intestine in Phase 1 were lower than in Phase 2 or in Phase 3 (Fig. 5), but the difference reached significance only in Phase 3 for the period 15–30 min post water administration ($p \leq 0.014$). In Phase 1, median pH values of samples

aspirated 5, 15, 30 and 50 min post water administration were 6.8, 6.2, 6.3, and 6.5, respectively (Fig. 5), in line with previous data (19). Corresponding median values in Phase 2 and in Phase 3 were 6.9, 6.9, 6.4, 7.3 and 7.2, 7.2, 7.2, 7.3, respectively (Fig. 5). Increased pH in upper small intestine when achlorhydric conditions prevail in stomach has been also observed previously when achlorhydric conditions were achieved in stomach by administering repeated doses of esomeprazole (12). In all Phases, pH did not change significantly with time after water administration. Similar observations have also been made previously (12,24). However, unlike with Phase 1, in Phases 2 and 3, occasional excursions to highly acidic pH values were not observed (Fig. 5), i.e. pH in upper small intestine was much less variable. Overall median values for pH during the first 50 min. post water administration in Phase 1, Phase 2 and Phase 3 were 6.5, 7.0, and 7.2, respectively.

Buffer Capacity

During the first 50 min. post water administration, buffer capacity was not statistically different among the three Phases (Fig. 6, empty boxes). Mean(SD) buffer capacity values of samples aspirated 5, 15, 30 and 50 min after water administration were 8.4(2.9), 19.2(33.7),

Table 1 Mean \pm SD ($n = 6-8$) Concentrations of Individual Bile Acids (μM) in Contents of Stomach and Upper Small Intestine at Various Times After Administration of Water to Fasted Male Adults Participated in this Study*

Minutes after water (240 ml) administration	Phase	TC	GC	TCDC	UDC	GCDC	GDC
Stomach							
10	1	3.6 \pm 6.7	10.5 \pm 21.5	5.1 \pm 10.8	1.3 \pm 3.6	7.8 \pm 12.3	3.6 \pm 6.8
	2	8 \pm 16	14 \pm 35	5 \pm 14	<LOQ	9 \pm 26	<LOQ
	3	3.2 \pm 7.2	7 \pm 10	2.0 \pm 5.8	<LOQ	4.0 \pm 8.4	<LOQ
20	1	2.7 \pm 5.4	9.8 \pm 17.9	2.2 \pm 4.8	<LOQ	6.3 \pm 11.5	3.7 \pm 10.4
	2	14 \pm 39	27 \pm 74	14 \pm 39	<LOQ	22 \pm 62	<LOQ
	3	23 \pm 42	70 \pm 154	15 \pm 25	2.6 \pm 4.9	48 \pm 99	17 \pm 36
35	1	21.9 \pm 31.1	46 \pm 63	14 \pm 15	9.3 \pm 16.5	29 \pm 44	28 \pm 57
	2	46 \pm 103	76 \pm 166	40 \pm 89	2.7 \pm 6.1	66 \pm 148	3.6 \pm 8.1
	3	64 \pm 93	204 \pm 332	38 \pm 45	12 \pm 15	136 \pm 200	71 \pm 90
Upper Small Intestine							
5	1	178 \pm 179	260 \pm 230	170 \pm 159	76 \pm 100	242 \pm 167	201 \pm 321
	2	317 \pm 527	1069 \pm 1731	280 \pm 429	90 \pm 100	1148 \pm 1887	456 \pm 607
	3	82 \pm 77	222 \pm 238	73 \pm 72	38 \pm 69	187 \pm 223	160 \pm 271
15	1	662 \pm 786	1541 \pm 1746	539 \pm 480	188 \pm 244	1174 \pm 1278	689 \pm 827
	2	738 \pm 492	1745 \pm 1389	1118 \pm 1901	243 \pm 266	1733 \pm 1249	901 \pm 918
	3	469 \pm 448	1391 \pm 1809	372 \pm 378	202 \pm 265	1569 \pm 1730	581 \pm 644
30	1	1337 \pm 1476	2463 \pm 2710	806 \pm 784	436 \pm 430	1488 \pm 1439	1241 \pm 1156
	2	716 \pm 588	2312 \pm 2123	632 \pm 624	289 \pm 428	1914 \pm 1767	1138 \pm 1766
	3	537 \pm 427	1921 \pm 1994	517 \pm 419	231 \pm 316	2046 \pm 1746	976 \pm 1451
50	1	558 \pm 605	1023 \pm 930	377 \pm 412	255 \pm 339	719 \pm 665	816 \pm 1116
	2	242 \pm 265	1240 \pm 1685	214 \pm 186	74 \pm 111	994 \pm 1306	468 \pm 613
	3	467 \pm 420	1728 \pm 1871	386 \pm 343	216 \pm 311	2196 \pm 2262	556 \pm 708

* Limit of quantification (LOQ); TC: 1.26 μM , GC: 0.71 μM , TCDC: 0.20 μM ; UDC: 1.08 μM ; GCDC: 1.55 μM ; GDC: 1.80 μM

9.0(3.8), and 14.2(10.5) mmol/L/ ΔpH in Phase 1, 6.6(3.2), 9.4(4.2), 8.2(3.7), and 35.7(57.2) mmol/L/ ΔpH in Phase 2, and 6.1(0.8), 9.0(3.8), 7.7(2.8), and 6.9(2.7) mmol/L/ ΔpH in Phase 3, respectively, i.e. in line with previous data collected in healthy adults (19).

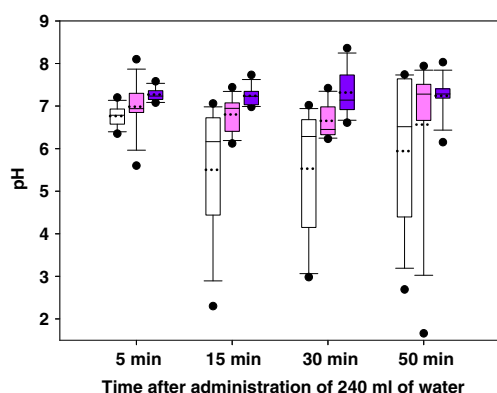


Fig. 5 pH in the upper small intestine of fasted healthy adults as a function of time, after administration of 240 ml table water into the antrum of the stomach. Key: White boxes, Phase 1; Light pink boxes, Phase 2; Dark blue boxes, Phase 3. Each box was constructed by using 7–8 individual values.

One freeze-thaw cycle affected buffer capacity measured with HCl in all Phases significantly (Fig. 6, empty *vs.* lined boxes within each Phase, $p < 0.02$). Compared with the values estimated after titration with NaOH, buffer capacities estimated with HCl were significantly higher in all Phases (Fig. 6, lined *vs.* dotted boxes within each Phase, $p < 0.01$).

Chloride ion Concentration

During the first 50 min post water administration, concentration of chloride ions was not statistically different among the three Phases (Fig. 7a). Mean(SD) values in samples aspirated 5, 15, 30 and 50 min. post water administration were 42.1(11.7), 70.3(33.0), 106.6(30.0), and 121.6(16.1) mM in Phase 1, 44.0(16.1), 58.1(34.4), 91.5(30.7), and 135.9(35.7) mM in Phase 2, and 30.7(6.5), 55.1(29.1), 84.3(46.7), and 96.7(36.8) mM in Phase 3, respectively, i.e. in line with previous data collected in healthy adults (20). In all Phases, chloride ion concentration increased significantly during the 5 to 50 min period post water administration (Fig. 7a).

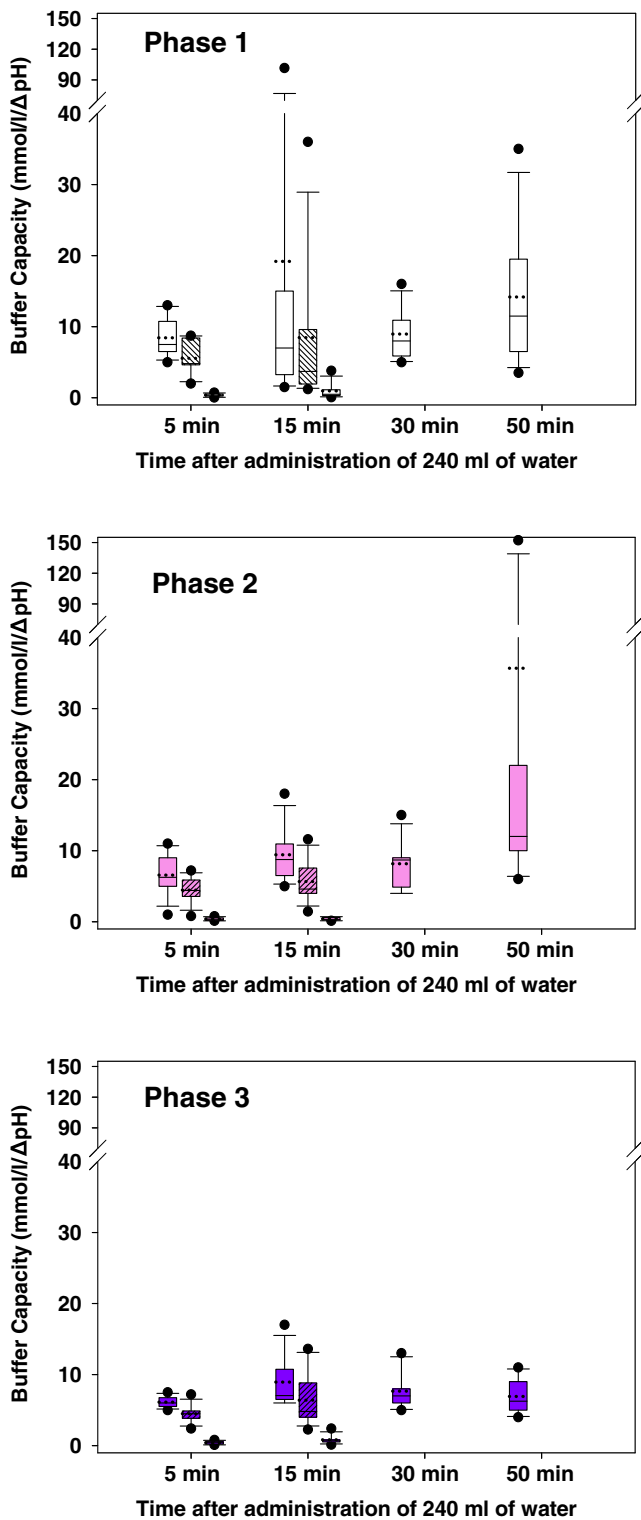


Fig. 6 Buffer capacity of contents of upper small intestine of fasted healthy adults as a function of time, after administration of 240 ml table water into the antrum of the stomach measured in Phase 1 (White boxes, $n = 6-8$), in Phase 2 (Light pink boxes, $n = 6-8$), and in Phase 3 (Dark blue boxes, $n = 7-8$) immediately upon collection using HCl (empty boxes), after one freeze-thaw cycle using HCl (lined boxes) and after one freeze-thaw cycle using NaOH (dotted boxes). Due to limited available volumes, data after one freeze-thaw cycle in samples aspirated 30 and 50 min post water administration could not be collected.

Osmolality

During the first 50 min post water administration, osmolality was not statistically different among the three Phases (Fig. 7b). Mean(SD) osmolality values of samples aspirated 5, 15, 30 and 50 min after water administration were 92.5(17.9), 126.9(49.2), 207.4(31.6), and 217.4(44.5) mOsmol/kg in Phase 1, 78.4(27.1), 96.5(30.7), 170.0(57.6), and 222.8(27.2) mOsmol/kg in Phase 2, and 65.0(15.7), 111.3(51.6), 154.5(77.0), and 187.4(56.7) mOsmol/kg in Phase 3, respectively, i.e. in line with previous data (19). In all Phases, osmolality increased significantly during the 5 to 50 min period post water administration (Fig. 7b).

Total Protein Content

During the first 50 min post water administration, total protein content was not significantly different among the three Phases (Fig. 7c). In all Phases, total protein content increases significantly during the 5 to 50 min period post water administration. Mean(SD) values for total protein content 5, 15, 30 and 50 min post water administration were 1.00(0.37), 1.8(1.2), 2.7(1.7) and 3.7(1.1) mg/ml in Phase 1, 1.5(1.4), 1.6(1.1), 2.3(1.3) and 3.2(1.1) mg/ml in Phase 2, and 0.90(0.52), 2.1(1.3), 2.6(1.5), 3.3(2.0) mg/ml in Phase 3. Data are in line with previous values in healthy adults (19).

Bile Acids

During the first 50 min post water administration, total bile salt content was not significantly different among the three Phases (Fig. 7d). In all Phases, total bile acids content did not change significantly with time, after water administration (Fig. 7d). Mean(Median) values for total bile salt content in samples aspirated 5, 15, 30 and 50 min. after water administration were 1.1(1.1), 4.8(2.3), 7.7(5.0), and 3.3(1.9) mM in Phase 1, 3.4(1.3), 6.5(6.7), 7.0(6.2), and 3.2(1.7) mM in Phase 2, and 0.8(0.5), 4.6(3.4), 6.2(3.7), and 5.5(5.4) mM in Phase 3, respectively, i.e. in line with previous data (14,22,24,25). As in stomach, in all three Phases, glycocholate and glycochenodeoxycholate were the most prevalent bile acids (Table I), in line with previous studies (24,25).

Free Fatty Acids

During the first 50 min post water administration, free fatty acid content was not significantly different among the three Phases (Fig. 8a). In all Phases, free fatty acid content did not change significantly with time, after water administration (Fig. 8a). Mean(SD) values for the free acid content 5, 15, 30 and 50 min post water administration were 401(273),

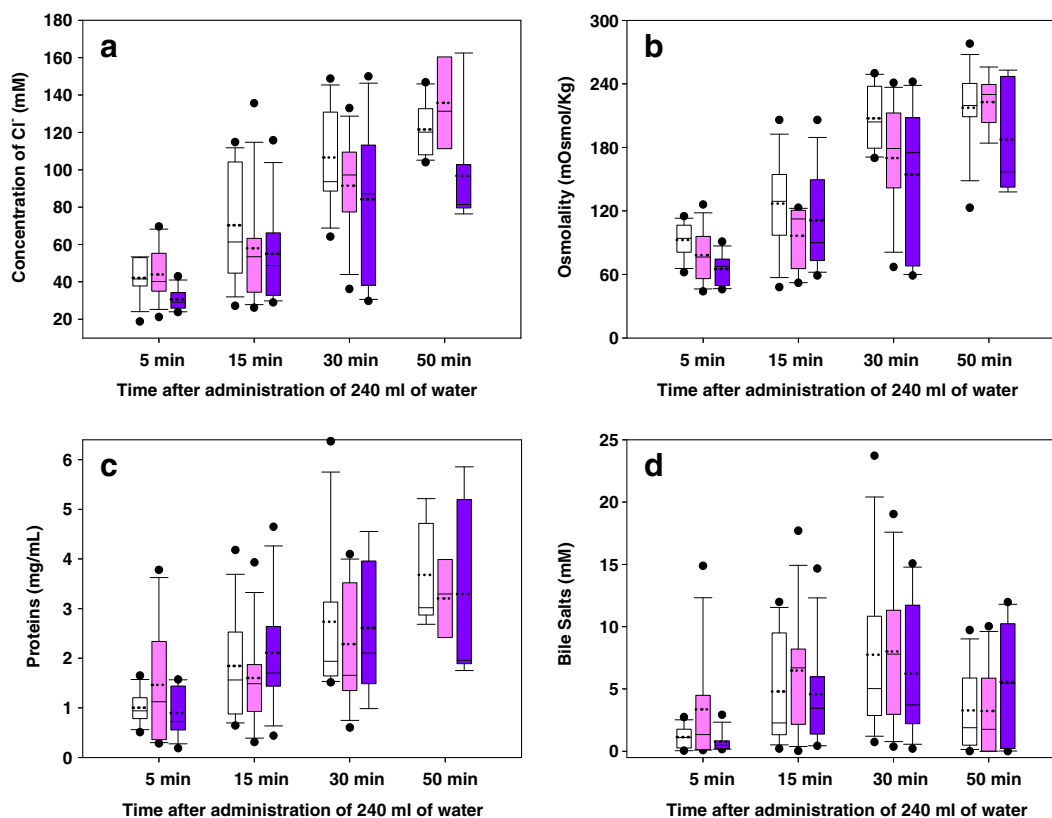


Fig. 7 Concentration of chloride ions (a), osmolality (b), total protein content (c), and total bile salt content (d) in the upper small intestine of fasted healthy adults as a function of time, after administration of 240 ml table water into the antrum of the stomach. Key: White boxes, Phase 1; Light pink boxes, Phase 2; Dark blue boxes, Phase 3. Each box was constructed by using 4–8 individual values.

576(559), 946(1105), and 280(260) μM in Phase 1, 617(671), 832(474), 994(837), and 531(356) μM in Phase 2, and 425(409), 800(527), 709(565) and 625(614) μM in Phase 3. These data are in line with previous data (22,24,25).

Total Phosphatidylcholine

During the first 50 min post water administration, total phosphatidylcholine content was not significantly different among the three Phases (Fig. 8b). In all Phases, total phosphatidylcholine content did not change significantly with time, after water administration (Fig. 8b). Mean(SD) values at 5, 15, 30 and 50 min post water administration were 116(109), 574(564), 605(857), and 314(300) μM in Phase 1, 340(640), 679(552), 752(654), and 314(236) μM in Phase 2, and 181(287), 701(961), 570(568) and 314(319) μM in Phase 3. These data are slightly higher than previously reported data (14,22,24). These data suggest that the ratio [total bile acid content/total phosphatidylcholine content] in the fasted upper small intestinal lumen after administration of glass of water is about 10/1 in all Phases, i.e. close to the ratio recently suggested to be used in fasted state simulating intestinal fluids [FaSSIF-V3, (26)]. Previously suggested simulated intestinal fluids had lower ratios (to balance the lack of simulation of cholesterol, fatty acids

and monoglycerides, FaSSIF, 4/1) or simply because the lack of simulation of cholesterol, fatty acids and monoglycerides were not taken into account, FaSSIF-V2, 15/1) (27,28).

Cholesterol

Cholesterol concentration in the upper small intestine is not affected by the reduced gastric acid secretion (Fig. 8c). In all Phases cholesterol concentration did not change significantly with time post water administration (Fig. 8c). Mean(SD) values from data collected during the first 50 min. post water administration 117(119), 273(225), 435(408), and 169(103) μM in Phase 1, 225(291), 363(256), 478(379) and 269(234) μM in Phase 2, and 117(100), 334(219), 380(309), and 200(228) μM in Phase 3, in line with previously reported values (14).

Temporal trends of molar concentrations of bile acids, free fatty acids, total PC and cholesterol are similar, with an apparent peak at 30 min in all cases. This is in line with the fact that all these components are present in the same colloidal species.

Surface Tension

Mean(SD) values for the surface tension of contents in upper small intestine, after administration of 240 ml water to the

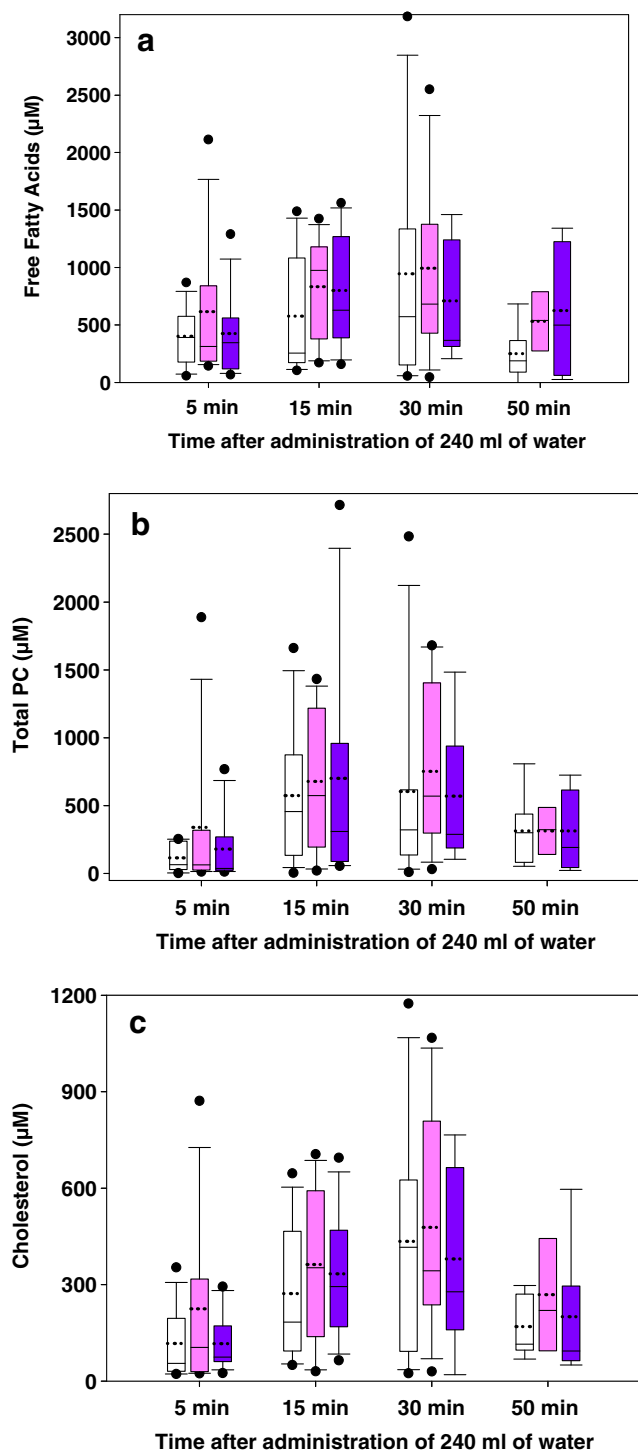


Fig. 8 Free fatty acid content (a), total phosphatidylcholine content (b) and cholesterol content (c) in the upper small intestine of fasted healthy adults as a function of time after administration of 240 ml table water into the antrum of the stomach. Key: White boxes, Phase 1; Light pink boxes, Phase 2; Dark blue boxes, Phase 3. Each box was constructed by using 4–8 individual values.

stomach of fasted males in Phase 1 (control phase), Phase 2 (pantoprazole phase) and Phase 3 (famotidine phase) were 32.70(0.51), 29.81(0.65) and 35.20(1.28) mN/m, respectively. Data in Phase 1 are in line with previously published data in

healthy adults (19). The lowest surface tension in Phase 2 is in line with the lowest surface tension in stomach in the same Phase.

Kinematic Viscosity

Mean(SD) values for the kinematic viscosity of contents in upper small intestine, after administration of 240 ml water to the stomach of fasted males in Phase 1, Phase 2, and Phase 3 were 0.7602(0.0129), 0.7919(0.0021) and 0.7816(0.0011) mm²/s, respectively. These data suggest that viscosity of contents in upper small intestine in the fasting state is not affected by the reduced gastric acid secretion and values are slightly higher than that of water (0.6959 mm²/s, www.viscopedia.com).

DISCUSSION

Data from this study show that, apart from the pH change in stomach, additional physiological changes should be considered when evaluating the luminal performance of APIs (especially of weak bases) and dosage forms in individuals on gastric acid reducing treatment. The focus of this study was to understand these changes that occur after administration of the agents in a typical DDI study design. However, more data are needed for justifying the usefulness of all data collected in the present investigation when evaluating performance of APIs/drug products in individuals with chronic hypochlorhydria. For example, it has been documented that long term hypochlorhydria results to bacterial overgrowth in gastric and jejunal fluids and some of the relevant species promote deconjugation of bile acids (29). Deconjugation may have an impact on surface tension (wettability), micellar structure and solubilization capacity in the upper small intestine.

With the focus on a DDI study setting in mind, based on the data from the present study, physiological changes in stomach that accompany the reduced gastric acid secretion include the reduced buffer capacity, chloride ion concentration, osmolality and surface tension. These changes are or can be related to the altered gastric pH. For surface tension, however, other mechanisms could also be involved. Intravenous administration of famotidine (4 mg/kg) results in increased thickness of the adherent mucus gel in rats (30). The impaired quality of mucus and the weakened mucus – bicarbonate barrier, after prolonged administration of famotidine has been observed also in the human stomach (31,32). As far as PPIs are concerned, there are no published data on the effect of pantoprazole on mucosal function in humans. Rabeprazole enhances gastrin mucin content (33) within 1 week of administration. Composition of gastric contents is also affected by the enhanced esophageal mucin secretion, after rabeprazole administration (34). Based on these data part of the increased mucus may be released into the bulk of the gastric contents and decrease surface tension.

Based on data from the present study, physiological changes in upper small intestine which accompany the reduced gastric acid secretion include the increased pH in upper small intestine by about 0.7 units (from ~6.5 in Phase 1 to ~7.2 in Phase 3), despite the unaltered buffer capacity. Since in Phase 2 hypo- and not a-chlorhydric conditions were established, it is not clear from this study if the increased pH in upper small intestine is due to the a-chlorhydric conditions induced in Phase 3 or it is related also to the mechanism of inhibition of gastric acid secretion. The relevance of this change to absorption of compounds requires further investigation. In principle compounds with solubility sensitive to pH in that region may be affected (for example lipophilic weak bases with pKa values between 6 and 7 and high doses that stomach solubilization may not be sufficient) although the net effect will need to be evaluated on a compound basis.

Two findings of the present investigation that are valid regardless of the extent of gastric acid secretion and are reported for first time in the literature deserve to be briefly highlighted. The first relates to the ability of contents of stomach and of upper small intestine to resist in changes to their pH in the fasted state. Specifically, buffer capacity in both locations and regardless of the extent of acid secretion in stomach is much higher when changes to more acidic values are attempted, i.e. contents are more powerful in resisting to lowering the pH than to increasing the pH in upper GI lumen. The second relates to the viscosity of contents of the upper small intestine. Contents are more viscous than de-ionized water. To date the impact of viscosity on drug/drug product performance has been considered only for events in the stomach (35,36). The impact of increased viscosity in upper small intestine on various events relating to drug/drug product performance remains to be evaluated.

The data from this study can help to better design such studies during clinical development of new chemical entities. As discussed previously, the design employed in this study represents a typical study design for studying DDIs with PPIs or H2-RAs during clinical development for compounds where solubility changes as a function of stomach pH are anticipated (most typically weak bases). Under current development paradigms, typically compounds would need to be tested with both regimens for the interactions against both agents to be described in the drug label. In this study, both of the treatment regimens selected proved effective in achieving the intended effect of shifting the stomach pH, supporting the use of such design for DDI studies. The significantly higher pH achieved with the famotidine treatment, relative to the pantoprazole treatment, raises the possibility that, at least for weak bases, testing with famotidine could serve as a worst case scenario in terms of solubilization capacity. Thus, if no effect on pharmacokinetics is seen after the famotidine treatment, perhaps

testing with a PPI, which effect on solubilization would be less pronounced, does not need to be pursued for weak bases, thus reducing the number for unnecessary additional clinical trials. Recently, a preliminary conceptual framework has been recommended that the worst case scenario should be tested by using a PPI (37). Such recommendation was based on the fact that PPIs generally have a longer duration of suppression effect on gastric acid secretion than do H2-RAs and antacids, and are expected to interfere with the intestinal absorption of weak bases to greater extent (37). Such recommendation may be, therefore, logical to make under regular clinical practice. However, data from this study clearly show that, if the objective is to evaluate the effect of reduced gastric acid secretion on drug absorption, under a controlled study, the protocol applied in the present study with the H2-RA agent (including the spacing of doses) is perfectly adequate for achieving the maximal effect; if a positive DDI is observed, potential new DDI study to explore staggered dosing for mitigating the pH effect could be considered with a PPI, following the protocol (and the spacing of doses) applied in the present study.

Finally, although the focus of attention of this manuscript is free base compounds, the higher intestinal pH achieved by famotidine may also translate into a larger difference in absorption for weak acids with pKas between 6 and 7.

CONCLUDING REMARKS

Based on data from this study, reduction of gastric acid secretion in humans is accompanied by additional physicochemical changes which could additionally impact the luminal performance of orally administered drugs/drug products in the fasted state, especially of lipophilic weak bases. Relevant changes include the reduced buffer capacity, chloride ion concentration, osmolality and surface tension in stomach and the increased pH in upper small intestine. The mechanism of reduction of gastric acid secretion (PPIs vs. H2-RAs) seems to be important for the buffer capacity in stomach and surface tension of contents in upper gastrointestinal lumen. The effect of famotidine on stomach pH was much more pronounced relative to pantoprazole, raising the possibility that famotidine can be used as the worst case scenario in assessing interactions of weak bases with stomach pH altering medications.

The impact of these changes in upper gastrointestinal lumen on intraluminal performance of model active pharmaceutical ingredients is currently being evaluated in pooled aspirates collected in the present study and in biorelevant media prepared based on data from the present study.

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