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Research Paper

Characterization of the Human Upper Gastrointestinal Contents Under Conditions Simulating Bioavailability/Bioequivalence Studies

Lida Kalantzi,[1] Konstantinos Goumas,[2] Vasilios Kalioras,[2] Bertil Abrahamsson,[3] Jennifer B. Dressman,[4] and Christos Reppas[1,5]

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Purpose. This study was conducted to compare the luminal composition of the upper gastrointestinal tract in the fasted and fed states in humans, with a view toward designing *in vitro* studies to explain/predict food effects on dosage form performance.
Methods. Twenty healthy human subjects received 250 mL water or 500 mL Ensure plus® (a complete nutrient drink) through a nasogastric tube and samples were aspirated from the gastric antrum or duodenum for a period up to 3.5 h, depending on location/fluid combination. Samples were analyzed for polyethylene glycol, pH, buffer capacity, osmolality, surface tension, pepsin, total carbohydrates, total protein content, and bile salts.
Results. Following Ensure plus® administration, gastric pH was elevated, buffer capacity ranged from 14 to 28 mmoL L^{-1} ΔpH^{-1} (*vs.* 7–18 mmol L^{-1} ΔpH^{-1}), contents were hyperosmolar, gastric pepsin levels doubled, and surface tension was 30% lower than after administration of water. Post- and preprandial duodenal pH values were initially similar, but slowly decreased to 5.2 postprandially, whereas buffer capacity increased from 5.6 mmol L^{-1} ΔpH^{-1} (fasted) to 18–30 mmol L^{-1} ΔpH^{-1} ($p < 0.05$). Postprandial surface tension in the duodenum decreased by >30%, bile salt levels were two to four times higher, luminal contents were hyperosmotic, and the presence of peptides and sugars was confirmed.
Conclusions. This work shows that, in addition to already well characterized parameters (e.g., pH, and bile salt levels), significant differences in buffer capacity, surface tension, osmolality, and food components are observed pre-/postprandially. These differences should be reflected in test media to predict food effects on intralumenal performance of dosage forms.

KEY WORDS: Ensure plus®; fasted state; fed state; human gastric fluid; human intestinal fluid.

INTRODUCTION

The *in vivo* performance of oral dosage forms is an important issue when a new chemical entity is to be administered orally for first time in humans, when scale-up and postapproval changes to the dosage form are made, and when a generic formulation is to be evaluated for marketing authorization. To date, relevant bioavailability (BA)/bioequivalence (BE) information is obtained mostly with studies performed in healthy humans, making the procedure time-consuming and costly. During the last decade, the Biopharmaceutics Classification System (BCS) has introduced the possibility of obtaining a marketing approval for a generic formulation based on dissolution test results in certain cases (1), whereas the development of biorelevant media for the *in vitro* assessment of intralumenal fate of dosage forms has improved our ability to predict *in vivo* performance (2–4).

The initial compositions of the biorelevant media were based on existing intralumenal data (4–7). For various reasons, those data may not optimally reflect the *in vivo* situation during a standard BA/BE study. First, the buffer capacity, which is of primary importance for the dissolution characteristics of ionizable compounds, has not been well characterized in humans and the buffer capacity of biorelevant media had to be based on canine data (5). Second, in the fasted state, intragastric composition may be highly dependent on the volume of coadministered water and, although in BA or BE studies a standard volume of water is coadministered with the dosage form (8,9), characterization of human gastric environment has in many cases been performed without administration of water, or with unspecified volumes (10–15). Third, although the distribution of nutrients in the meals administered to characterize intralumenal conditions in the fed state was generally similar in previous relevant studies, meal energy content (which will affect gastric residence time) varied dramatically; in most previous studies total energy content was substantially lower [e.g., 158 kcal

[1] Laboratory of Biopharmaceutics and Pharmacokinetics, School of Pharmacy, University of Athens, Panepistimiopolis 157 71, Zografou, Greece.
[2] Red Cross Hospital of Athens, Athens, Greece.
[3] Preformulation and Biopharmaceutics Department, AstraZeneca R&D, Mölndal, Sweden.
[4] Department of Pharmaceutical Technology, JW Goethe University of Frankfurt, Frankfurt, Germany.
[5] To whom correspondence should be addressed. (e-mail: reppas@pharm.uoa.gr

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(10) or 300 kcal (16)] than the 800–1,000 kcal content of the FDA-recommended standard meal (17). Moreover, in studies where the meal composition was similar to the FDA meal, the intralumenal environment was only partly characterized (11,13,18). In addition to the buffer capacity, few or no data for osmolality and surface tension data in the fed state have been reported in the literature. Finally, in some studies, agents that induce the fed state rather than an actual meal have been administered to induce "fed state" conditions (19,20). This would minimize the intralumenal volume generated and, as a result, the intralumenal concentrations may be exaggerated compared to those generated during a BA/BE study.

In this study we characterized the upper gastrointestinal (GI) contents under conditions simulating BA/BE studies both in the fasted and in the fed states with a view toward designing media for the *in vitro* study of food effects on dosage form performance. In doing so, we gave emphasis to parameters that have not been fully characterized in the past and/or which are expected to vary with the composition of the administered meal. Fasted state conditions were simulated by administering 250 mL of water to fasted subjects, whereas fed state conditions were simulated by administration of 500 mL of Ensure plus® (21,22). It has previously been shown that Ensure plus® has a similar composition to that of the FDA meal that is commonly administered to study food effects in BA/BE studies (22).

MATERIALS AND METHODS

Phases of the Study

The study consisted of four phases. Each subject was administered a specific volume of fluid in the stomach and samples were aspirated either from the stomach or from a location lower than the sphincter of Oddi in the duodenum as follows:

Phase 1: Samples were aspirated from the antrum of stomach, after administration of 250 mL of water to the antrum through a nasogastric tube.

Phase 2: Samples were aspirated from the antrum of stomach, after administration of 500 mL of Ensure plus® (21,22) to the antrum through a nasogastric tube.

Phase 3: Samples were aspirated from the duodenum after administration of 250 mL of water to the antrum through a nasogastric tube.

Phase 4: Samples were aspirated from the duodenum after administration of 500 mL of Ensure plus® (21) to the antrum through a nasogastric tube.

Subjects

Twenty healthy nonsmokers (16 males and 4 females) with a mean age of 25 years (range 20–32 years) gave informed consent and participated in the study. One subject was 24% heavier than his ideal body weight [as determined from the Metropolitan Life Tables (23)]. Body weights of all other subjects deviated from the ideal weights by less than 10%. None of the participants had a history or any clinical evidence of gastrointestinal disease. The health status of each subject was confirmed by physical examination and screening of blood parameters for renal and hepatic functions.

The study was held in the Red Cross Hospital of Athens after receiving approval by the Scientific and the Executive Committee of the Hospital. The study followed the tenets of the Declarations of Helsinki promulgated in 1964.

From the 80 phases initially planned (20 subjects × 4 phases per subject), a total of 62 phases were successfully completed.

Four phases were incomplete for the following reasons:

– subject vomited during the aspiration period (two phases; one 45 min and another 33 min after administration of Ensure Plus®);

– movement of the tube toward the stomach (two phases; one 40 min and another 100 min after administration of Ensure Plus®).

Fourteen phases were not been performed for the following reasons:

– failure to position the duodenal lumen within a reasonable period (approximately 15 min) (7 phases);

– failure to aspirate samples from the duodenum partly due to creation of a vacuum in the duodenum (two phases);

– subject's decision to terminate his/her participation in the study (five phases).

Study Protocol

The study was performed on two separate experimental days in each subject. Alcohol and any over-the-counter medication were discontinued 3 days prior to and throughout each experimental day, whereas food intake was discontinued for at least 12 h prior to the start of each experimental day and water was restricted on the morning of the experimental day. At about 8 AM on the experimental day, the subject arrived at the clinic and, after a brief screening of his/her health status by a physician, the upper throat was sprayed with lidocaine.

Experimental Day A

The subject was intubated nasally using a sterile disposable tube (Levin #14). The tube is approximately 120 cm long with an external diameter of 4.9 mm. The tube was placed in the antrum of the stomach (under fluoroscopic guidance) and used for both manual administration of meals and manual aspiration of samples. Two hundred and fifty milliliters of mineral water containing 10 mg mL^{-1} PEG 4000 as a nonabsorbable marker were administered through the tube and ~20-mL samples were drawn and placed immediately on ice every 20 min for 60 min. Ninety minutes after water administration, 500 mL of Ensure Plus® containing 10 mg mL^{-1} PEG 4000 were administered to the antrum through the tube, and ~20-mL samples were drawn and placed immediately on ice every 30 min for 210 min. Immediately after each sample was taken, 20 mL of air was pumped into the sampling tube to clear the contents back into the lumen (total internal volume of the tube was estimated to be ~13 mL). After the last sample and before removing the tube/discharging the subject, the position of the tube was confirmed fluoroscopically.

Experimental Day B

The subject was intubated nasally using a sterile two lumen duodenal tube (model 455400 ch.15.0 Ruesch, Stuttgart, Germany). In contrast to some previous aspiration studies [e.g., (14)], no attempt was made to isolate the aspiration segment from the rest of the GI contents. The two lumen tubes were approximately 150 cm long with an external diameter of 4.7 mm and a metal tip at its distal end. A series of holes 27–36 cm proximal to the metal tip was used to access the antrum of the stomach. A further series of holes 0–10 cm proximally to the metal tip was used to aspirate samples from the duodenum (near the ligament of Treitz). Insertion of the tube was assisted by a hydrophilic guiding wire and its position was monitored fluoroscopically. After reaching its final position and removing the wire, 250 mL of mineral water containing 10 mg mL^{-1} PEG 4000 as a nonabsorbable marker were administered using 60-mL (capacity) syringes to the antrum. Thirty minutes after administration of water a ~20-mL sample from the duodenum was aspirated over ice. One hour after administration of water, 500 mL of Ensure Plus® containing 10 mg mL^{-1} PEG 4000 were administered to the antrum using 60-mL syringes over a period of 8–10 min. Samples of up to 20 mL were aspirated over ice from the duodenum every 30 min for 210 min after completion of administration of Ensure plus®. Immediately after each sample was taken, 20 mL of air was pumped into the tube to clear its contents back into the lumen (total internal volume of this sampling tube was estimated to be ~18 mL). At the end of the experimental day and before removing the tube/discharging the subject, the final position of the tube was confirmed fluoroscopically.

Handling and Analysis of Samples

Each aspirated sample was immediately divided into several subsamples and each subsample was used for measuring just one parameter.

pH and buffer capacity measurements were performed on the first subsample immediately upon aspiration. pH values were measured by a pH electrode (ER350B, Metrohm, Herisau, Switzerland). Because of subsample volume restrictions, buffer capacities were measured in just one pH direction, by dropwise addition of either NaOH (samples from fasted stomach) or HCl (samples from fed stomach, fasted duodenum, and fed duodenum). It is worth mentioning that titrating FaSSIF or FeSSIF with HCl has indeed proven to be more appropriate than titrating with NaOH (24). Buffer capacity was calculated according to the following definition: the sample has a buffer capacity value of 1 when one equivalent of strong acid or alkali is required to change the pH value of 1 L by one pH unit (25,26).

Effect of Sample Handling on pH Results. The pH of some subsamples was also measured after maintaining the sample at room temperature without stirring for up to 20 min, to determine whether any drift in the value with time/exposure to open air occurs.

Protein Content: Immediately upon aspiration and before storage at −70°C, gastric subsamples in which total protein content was to be measured were titrated to pH 1 to inhibit proteolytic activity of pepsin (27). Similarly, in duodenal aspirates phenylmethylsulfonyl fluoride (PMSF) was added to inhibit trypsin activity by achieving an end concentration of 1 mM (28). Total protein content was determined using a commercially available kit (BCA, Protein Assay Reagent Kit; Pierce, Rockford, IL, USA) and albumin as a standard. The quantification limit was calculated (29) every analytical day and it was always less than 0.300 mg mL^{-1}.

Pepsin Activity: Immediately upon collection and before storage at −70°C, gastric subsamples in which pepsin activity was to be measured were titrated to pH 6 (27). Pepsin activity was measured by a modification of the method described by Anson (30), and quantification was based on hog pepsin as a standard. The quantification limit was calculated (29) to be 0.010 mg mL^{-1}.

All the remaining subsamples were stored at −70°C immediately after collection. Surface tension was measured using the DeNouy ring method (Sigma70, KSV Instruments, Monroe, CT, USA). Osmolality was measured by using the freezing point depression technique (semimicro osmometer Typ Dig L; Knauer, Berlin, Germany). Total 3α-hydroxy bile acid levels were determined using a commercially available kit (Enzabile; Nycomed, Lidingö, Sweden) and the quantification limit (29) was 500 μM. This kit should only be used for assaying 3α-hydroxy bile salts in simple aqueous samples and/or nonprotein based media after appropriate dilution of the sample with equine serum (which does not contain any bile salts) [e.g., (31)]. PEG 4000 was determined by the method described by Malawer and Powell (32) and modified by Buxton *et al.* (33). The quantification limit (29) of the polyethylene glycol (PEG) assay method was 3.33 mg mL^{-1}. Total carbohydrate content was determined by a modification of the method described by Galanos and Kapoulas (34), using glucose as a standard. The quantification limit (29) was 0.800 mg mL^{-1}.

The physicochemical characteristics of the administered meals, determined using the analytical techniques described above and information from the manufacturers, are presented in Table I.

Data Analysis

Data are presented as box plots showing the median value, the 10th, 25th, 75th, and 90th percentiles, and the

Table I. Physicochemical Characteristics of Administered Liquid Meals

	Water containing 10 mg mL^{-1} PEG	Ensure Plus® containing 10 mg mL^{-1} PEG
Volume (mL)	250	500
Calories (kcal)	0	750
Osmolality (mOsm kg^{-1})	16	610
pH	7.8	6.6
Buffer capacity (mmol L^{-1} ΔpH^{-1})	1	24
Surface tension (mN m^{-1})	62.0	42.4
Total proteins (mg mL^{-1})	–	62
Carbohydrates (mg mL^{-1})	–	202
Fat (mg mL^{-1})	–	49.2

outlier data points, with triangles indicating the mean value. The number of subjects that contributed to a specific box plot is indicated in parentheses above/below each box. Data from a minimum of four subjects was used as the basis for constructing a box plot. Only the data exceeding the quantification limit (LOQ) have been included in the box plots. Data biased to higher values because the number of samples less than LOQ was equal to or greater than the number of samples with greater than LOQ are clearly designated in the text. For each parameter, differences between times were evaluated with one-way ANOVA or the Kruskal–Wallis test. When data did not vary with time, differences between pooled fasted data and pooled fed data were performed with the unpaired *t* test or the Mann–Whitney test. Decision on the use of a parametric or a distribution-free test was made on the basis of the normality and the equal variance tests. Comparisons of pH data were always made with distribution-free tests. All statistical comparisons were performed using Sigmastat 2.03 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Fasted Stomach

Data measured in the fasted stomach are presented in Fig. 1.

Polyethylene Glycol: Median PEG concentration 20 min after administration of water was only 40% of the input value (4.0 mg mL^{-1}). Because the number of samples with PEG concentrations <LOQ was the same as the number of samples having values >LOQ (nine, Fig. 1), data are biased to higher values. Therefore, at 20 min, at least 60% of the contents must have consisted of secretions. Because resting volumes are of the order of 25 mL (35), this rather substantial dilution is attributed to secretions by the gastric mucosa, incoming saliva, and, possibly, incoming nasal secretions generated by the presence of the tube into the nostrils and/or the pharynx (36). Due to sample volume limitations, data at later time points were not collected. The dilution of contents

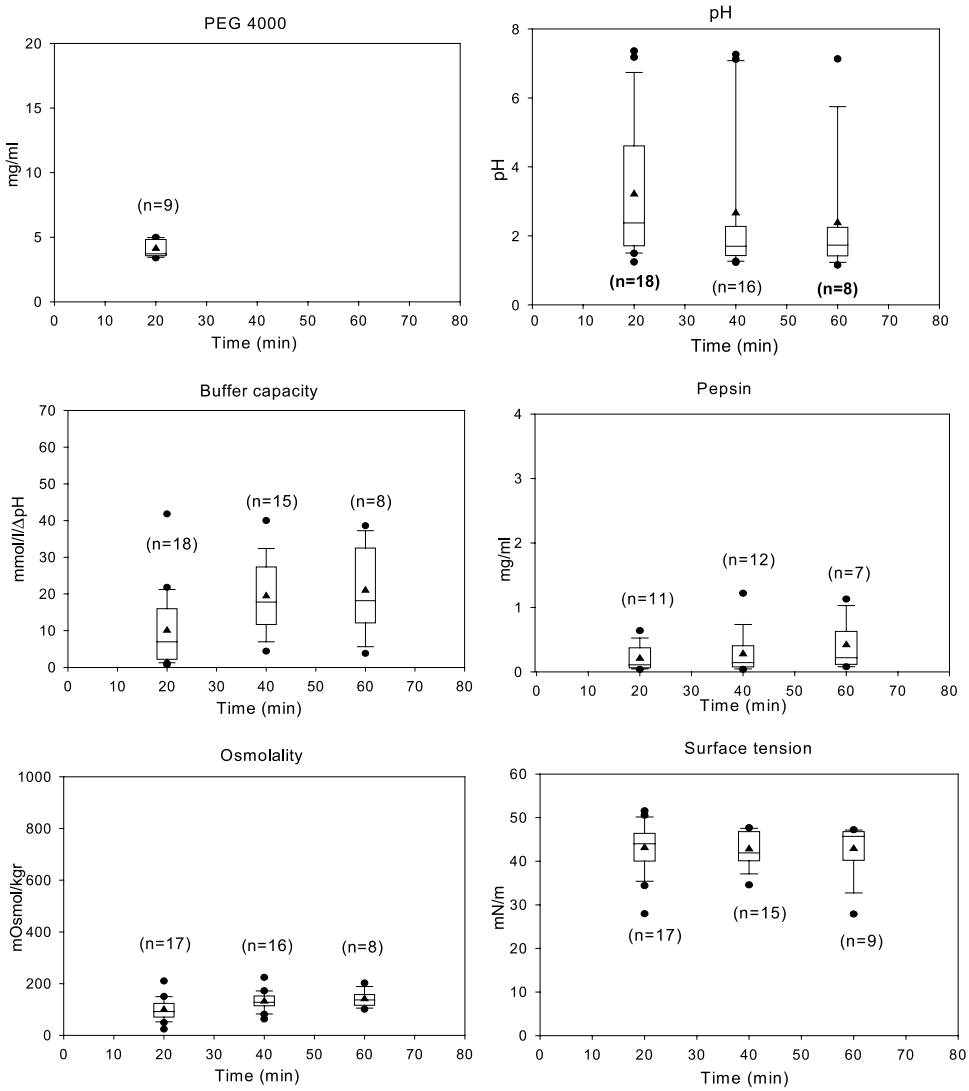


Fig. 1. Box plots for the samples aspirated from the antrum of fasted healthy subjects after administration of 250 mL water containing 10 mg mL^{-1} PEG 4000 into the antrum.

within the 20-min period after administration of water had an impact on pH, buffer capacity, pepsin levels, and osmolality.

pH: Intersubject variation was high (range of individual pH values was 1.23–7.36). Extreme high pH values may in some cases reflect an underlying hypochlorhydria [two subjects in our study consistently showed (at all sampling times) pH values close to neutral], but in most cases they probably reflect the dilution of gastric contents with saliva and/or nasal secretions [the baseline pH of saliva ranges from 5.45 to 6.06 and upon stimulation, the pH rises by about two pH units to a maximum of 7.8 (37)]. Median pH value was 2.4 twenty minutes after administration of water and stabilized to 1.7 at later time points. However, the decline over time did not achieve statistical significance ($p = 0.223$). pH values of 1.7 at late time points are in agreement with the generally accepted value for fasting gastric pH, which is usually measured to be

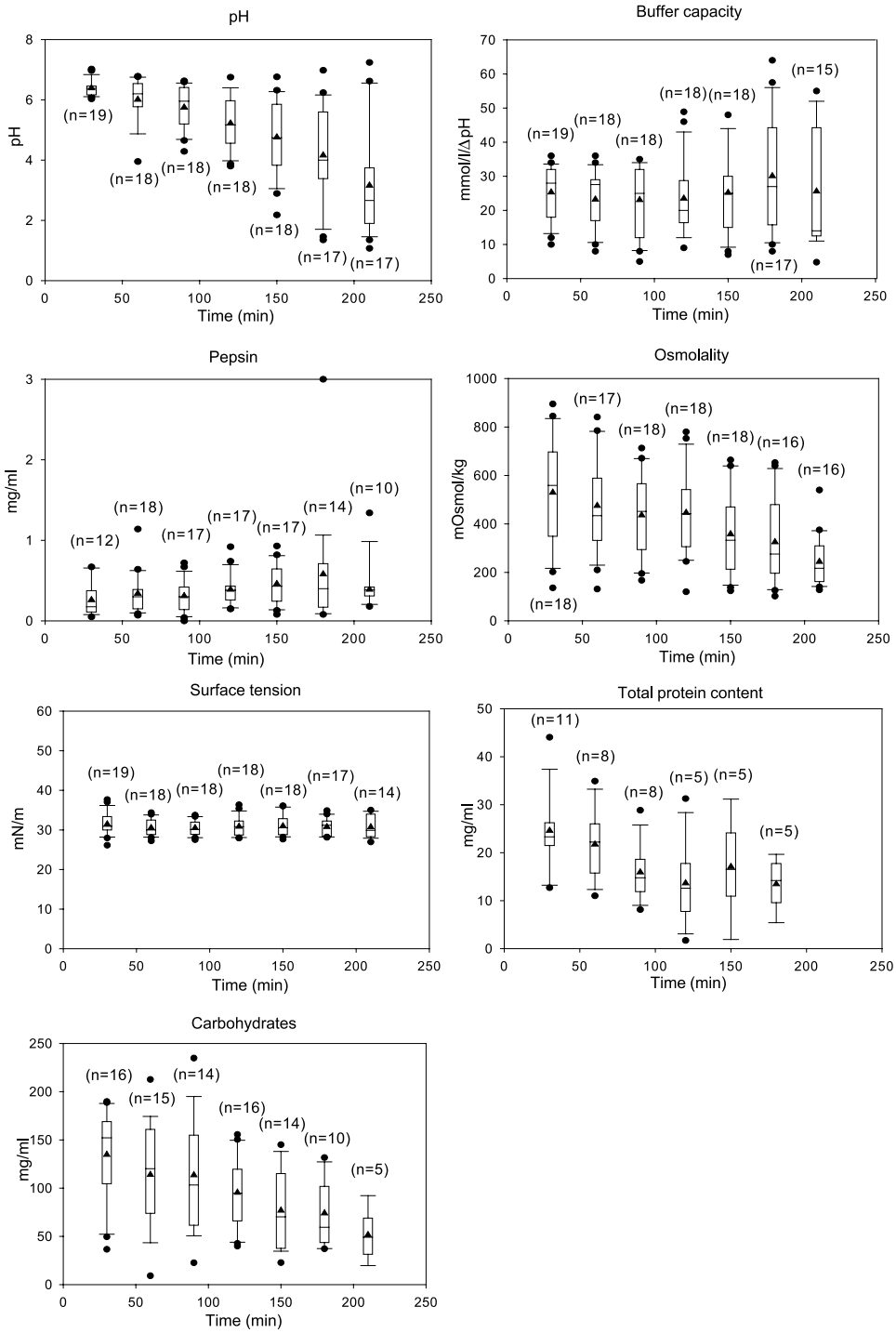


Fig. 2. Box plots for the samples aspirated from the antrum of fasted healthy subjects after administration of 500 mL Ensure Plus® containing 10 mg mL^{-1} PEG 4000 into the antrum.

about 2 or slightly lower (12–17). The pH of fasting gastric aspirates remained unchanged when the samples were maintained at room temperature for up to 20 min.

Buffer Capacity: The median value for buffer capacity 20 min after administration of water was 7 mmol L^{-1} ΔpH^{-1} and increased to about 18 mmol L^{-1} ΔpH^{-1} at later time points ($p < 0.001$).

Pepsin: Median values ranged from 0.11 mg mL^{-1} at 20 min to 0.22 mg mL^{-1} at 60 min (NS, $p > 0.05$). In the literature, values reported for pepsin concentration are higher. Schmidt *et al.* (19) report a value of 0.87 mg mL^{-1} (determined by hydrolysis of hemoglobin at pH 1.7 and by using hog pepsin as standard) and Lambert *et al.* (38) reported values of 0.83–1.27 mg mL^{-1} (determined with an analytical technique similar to that used in this study). An important methodological difference between the present and the previous studies is that no water seems to have been administered prior to collecting aspirates in the previous studies (19,38).

Osmolality: Although gastric contents were clearly hyposmotic, osmolality was lower at early time points (98 mOsm kg^{-1} at 20 min) and plateaued to about 140 mOsm kg^{-1} at later times ($p = 0.026$). These values are consistent with those reported earlier by Gisolfi *et al.* (39) (mean of 29 mOsm kg^{-1} over an 85-min exercise period during which 1,850 mL water was concurrently administered), by Lindahl *et al.* (14) (191 mOsm kg^{-1}, without prior water administration), and by Davenport (37) (171–276 mOsm kg^{-1}, no indication of whether water was preadministered).

Surface Tension: Surface tension was practically unaffected by water administration, with median values ranging from 41.9 to 45.7 mN m^{-1} during the first hour after the administration of water. These values are similar to previously reported results (40,41). Some investigators have attributed the low surface tension of gastric contents (pure water has a surface tension of 72 mN m^{-1}) to a reflux of duodenal contents (42,43). However, others have shown that this cannot be the reason in all subjects, as in many of them the bile salt levels in gastric aspirates are below the limits of detection (41,44–46). It is interesting to note that pepsin alone (at physiological relevant concentrations) is able to decrease the surface tension of water to about 57 mN m^{-1} (4).

Bile Acids: Bile acids, if any, were present at concentrations below the quantification limit of analytical method used in this study (i.e., less than 500 μM). However, bile salts at concentrations up to 1 mM (refluxed from the duodenum) have been quantified by other research groups in the fasted stomach (14,41,43,47). It is worth mentioning that in some of the earlier studies, samples were aspirated from the resting gastric contents rather than during gastric emptying of water from the stomach. From convectional considerations, one might reasonably expect that duodenal reflux would be more pronounced during resting conditions than during active gastric emptying of a liquid.

Fed Stomach

Data for the fed stomach are presented in Fig. 2. In agreement with literature data (33), the analytical method employed in our study for measuring PEG levels was not reliable in the presence of high nutrient concentrations. Therefore, an accurate picture of dilution or concentration of gastric contents over time in the fed state was not possible.

pH: Thirty minutes post-Ensure Plus® administration, the median gastric pH was 6.4 and intersubject variability was low. This value is close to the pH value of Ensure Plus® (6.6). Although intersubject variability increased with time, median pH values gradually decreased ($p < 0.001$) to reach 2.7 at 210 min, indicating that the meal effects on intragastric pH were still apparent 3 h and 30 min after the meal was given. The time required to restore the fasting pH levels depends mainly on the composition and the quantity of the meal, whereas the input pH value seems to be of secondary importance. For example, the time for gastric pH to return to fasting levels after administration of 580 mL of a pH 5.6 meal (651 mOsm, 1,000 kcal) was about 2 h (13), whereas after administration of 400 mL of a pH 6 meal (540 mOsm, 458 kcal) it was about 1 h (11). pH of gastric aspirates drifted in both directions by 5–25% within 15 min when the samples were kept at room temperature.

Buffer Capacity: As with pH, variability increased with time. Unlike pH, no trend in the median value was apparent. During the 30- to 210-min sampling period, median values of buffer capacity ranged from 14 to 28 mmol L^{-1} ΔpH^{-1}, close to input value (Table I). These values are significantly higher than the values measured 20 min after administration of water ($p < 0.001$), but are not different from values measured at times longer then 20 min after the administration of water. Higher total buffer content of gastric contents in the fed compared to the fasting state has also been reported by others; when 10 mL of homogenized meal (500 mL, 546 kcal, ~50% from lipids) was incubated with 20 mL of fresh gastric juice, a 33% increase in buffer capacity in 2 h was generated (48).

Pepsin: Both median values and intersubject variability remained fairly constant with time. During the 30- to 210-min sampling period, pepsin levels ranged from 0.26 to 0.58 mg mL^{-1}. These values are significantly different ($p = 0.006$) and up to twice as high as those measured in fasted state (Fig. 1). However, they are lower than the values found in the study of Lambert *et al.* (38), where pepsin levels in gastric aspirates after intravenous administration of insulin or betazole (histalog) were reported to be 0.56–1.72 mg mL^{-1} and also lower than the values found in the study of Schmidt *et al.* (19), where values of 1.25 and 1.68 mg mL^{-1} were reported after stimulation with histamine or with insulin, respectively. Again, these differences can be attributed to differences in the study protocols; in the earlier studies, which recruited subjects hospitalized for various disorders, induction of the fed state was performed pharmacologically rather than by administration of a meal, leading to substantially lower intragastric volumes and thus, higher pepsin concentration.

Osmolality: Both the median value and intersubject variability decreased with time after ingestion of the meal. The median value 30 min after the administration of the Ensure Plus® was 559 mOsm kg^{-1}, whereas at 210 min it decreased to 217 mOsm kg^{-1} ($p = 0.001$). Mertz and Poppe (20) reported a range of 262–306 mOsm kg^{-1} for osmolality after intravenous infusion of betazole (an analog of histamine); therefore gastric secretions under fed simulating conditions are isoosmotic or only slightly hyperosmotic and the high osmolality of fed aspirates in this study can be attributed to the hyperosmolarity of the administered meal.

Surface Tension: Surface tension values showed remarkable reproducibility and during the entire aspiration period median values ranged from 30 to 31 mN m^{-1}, i.e., they were 30% lower than in the fasting state ($p < 0.001$).

Bile Salts: Only one sample had a bile content above the quantification limit (i.e., higher than 500 μM). In the literature trace levels of bile salts have been reported to be present in the fed stomach [mean value = 60 μM (43)]. Because of the high limit of quantification of our method, it is not surprising that no bile salts could be detected in the stomach either fasted or fed and it is not possible from our results to say whether bile salts are refluxed or not.

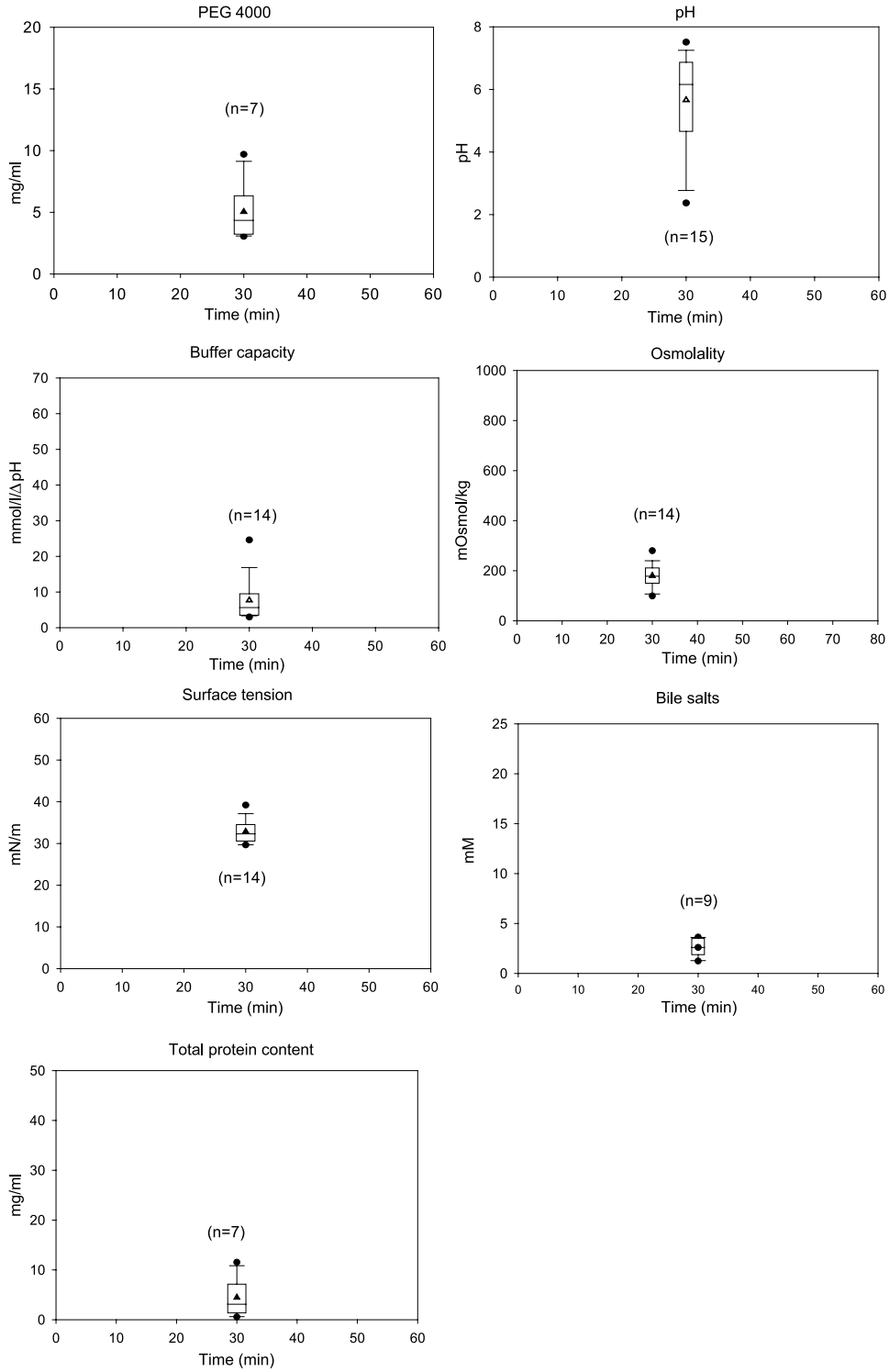


Fig. 3. Box plots for the samples aspirated from the distal duodenum of fasted healthy subjects after administration of 250 mL water containing 10 mg mL^{-1} PEG 4000 into the antrum.

Total Protein and Carbohydrate Content: The median values for total protein and total carbohydrate content decreased gradually from 23.3 and 152.1 mg mL^{-1}, respectively, at 30 min to 11.2 and 49.1 mg mL^{-1}, respectively, at 210 min after the meal's administration. The substantial presence of nutrients 210 min after administration of the meal is in accordance with the higher than baseline pH level discussed earlier. However, it should be noted that, in Fig. 2, total protein data are biased to higher values because the total number of samples with values <LOQ (sixty one) was higher

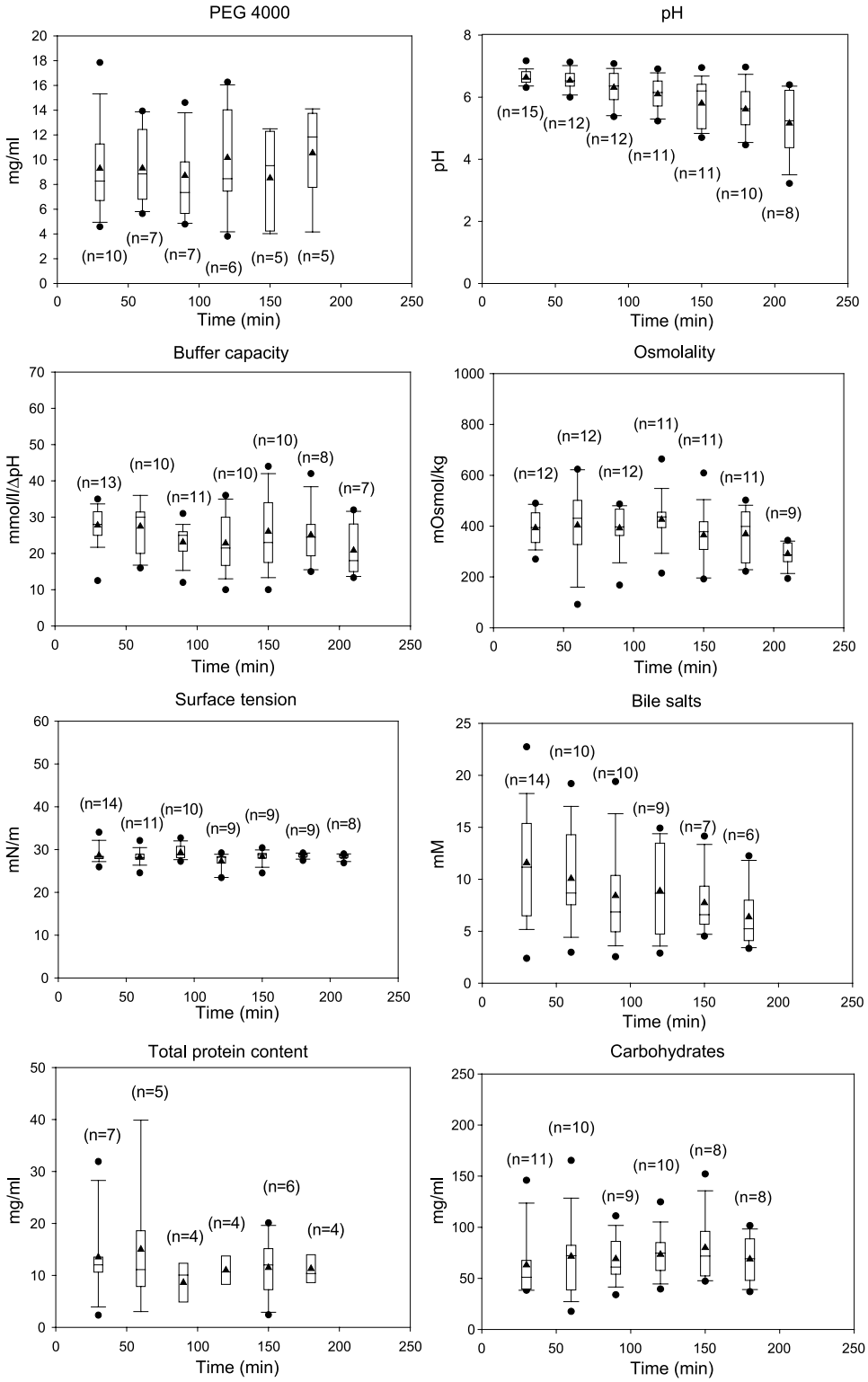


Fig. 4. Box plots for the samples aspirated from the distal duodenum of fasted healthy subjects after administration of 500 mL Ensure Plus® containing 10 mg mL^{-1} PEG 4000 into the antrum.

than the total number of samples with total protein values >LOQ (forty five, Fig. 2).

Fasted Duodenum

Data obtained from duodenal aspirates after administration of water are presented in Fig. 3.

Polyethylene glycol: Median PEG value was 4.3 mg mL^{-1}, similar to the gastric value 20 min after administration of water. These results provide an indication that any water absorption across the duodenal wall is balanced by the baseline bile and pancreatic secretions. However, it should be noted that, in Fig. 3, PEG data are biased to higher values because the total number of samples with values <LOQ (eight) was higher than the total number of samples with total protein values >LOQ (seven, Fig. 3).

pH: As in the fasting stomach, pH values were highly variable. However, the variability observed in the fasted duodenum may be related to reasons other than those speculated for the fasting stomach. According to Woodtli and Owyang (49), intersubject variations of the pH are related to the different phases of interdigestive motility. Moreover, in our study it was confirmed that the two subjects with the lowest intraduodenal pH values were those for which the tube was slightly moved upwards during the experimentation period and, therefore, aspirated sample corresponded to the descending part of the duodenum (i.e., close to or even proximal to the sphincter of Oddi). At the other extreme end, the subject with a slightly alkaline pH in the duodenum was the one that showed almost neutral gastric pH in the fasting state. The median pH value was 6.2. This is in agreement with median fasting duodenal pH values reported in the literature, which vary from 5.95 to 6.72 (10–13,15,50,51). pH of duodenal aspirates drifted to higher values, increasing by up to 6% within 20 min at room temperature. This could be related to a slow transformation of bicarbonates to carbon dioxide under zero-convection conditions (52).

Buffer Capacity: Median buffer capacity was 5.6 mmol L^{-1} ΔpH^{-1}, i.e., much lower than the median gastric value. To the best of our knowledge, there is only one relevant study in which the concentration of bicarbonates immediately next to the duodenal bulb was (albeit indirectly) measured. By measuring pH and partial pressure of carbon dioxide (53), the concentration was found to be about 6.7 mM.

Osmolality: Contents were hypoosmotic (median value = 178 mOsm kg^{-1}). Data are in accordance with those reported by Gisolfi *et al.* (39) (142 mOsm kg^{-1} in intestinal fluids aspirated 25 cm from pylorus) and, as would be expected, lower than the value reported by Lindahl *et al.* (14) for jejunal aspirates (271 mOsm kg^{-1}).

Surface Tension: As in the fasted stomach, this parameter showed the least variability. However, the median value (32.3 mN m^{-1}) was much lower than the gastric value, presumably as a result of the higher level of surface active agents such as bile salts and enzymes.

Bile Salts: The median value was 2.6 mM, similar to the value reported by Lindahl *et al.* (14) for the concentration of bile salts in jejunum during fasted state conditions (average 2.9 mM), but lower than the values found by other investigators for fasted duodenal contents [4.3–6.4 mM (5)].

Total Protein Content: The median value was 3.1 mg mL^{-1} and corresponds to enzymes arriving into duodenum from the stomach (e.g., pepsin, data not shown), the pancreas, and the bile duct [10 g of protein are secreted by the liver into the bile every day in a 70-kg man (54)]. However, this value probably overestimates the actual average intralumenal total protein content because the number of samples with values >LOQ (seven, Fig. 3) was only slightly higher than the number samples with total protein values <LOQ (six). It should be noted that the average total protein content of fasted jejunum has been reported to be 2.1 mg mL^{-1} (14).

Fed Duodenum

Data for the characterization of the luminal contents in the fed duodenum are presented in Fig. 4.

Polyethylene Glycol: Although median values were close to input PEG concentrations, data were extremely variable, suggesting that in some cases there was significant water absorption whereas in others there was significant water secretion. Postprandial values were on the average significantly higher than those 20 min after water administration ($p = 0.006$).

pH: Data were less variable than in the fasting state. The median duodenal pH 30 min after meal administration was 6.6, somewhat higher than the fasting state value, but it fell ($p < 0.001$) slowly to 5.2 at 210 min after the administration of Ensure Plus®. Although the pH decrease with time in the fed upper small intestine is known (10,11), earlier data had suggested that it occurs faster than in the present study and that perhaps the return to the higher pH levels of the fasted duodenal lumen was also faster (11). In the latter study, the energy content of the meal was 458 kcal, with 40% of calories coming from carbohydrates, 20% from proteins, and 40% from fats (11). The meal administered in the present study had similar percentage of calories coming from proteins (the major buffering species among nutrients), but contained much higher total energy content (Table I). Therefore, the buffer capacity is expected to be higher in this study and this could possibly account for the different timescale of progression of the pH value. The pH of duodenal aspirates drifted slightly by up to 3% to lower values within 10 min of storage at room temperature. This is speculated to be related to the creation of digestion products with acidic properties (e.g., digestion of triglycerides).

Buffer Capacity: Median values were between 18 and 30 mmol L^{-1} ΔpH^{-1} without showing a specific trend over time. These values are significantly higher than those measured after water administration ($p < 0.001$). Based on medians, the picture is similar to the corresponding gastric data in the fed state (Fig. 2). It is interesting to note that the extremes in buffer capacity results (high and low) corresponded with the extreme PEG values, i.e., with extremes in net water flux behavior. However, variability in net water flux did not impact the intraduodenal pH (as discussed above), presumably because intraduodenal pH values, the pH of the meal, and the pH of secretions are all close to neutral. Literature data on buffer capacity in the fed duodenum are very limited. Rune (55) measured the pH and the partial

pressure of carbon dioxide in samples aspirated 10 cm lower than the pylorus 3 and 3.75 h after administration of a meal (393 kcal with 39% fats and 51% carbohydrates); estimated bicarbonate concentrations were 10 and 23 mEq L^{-1} (55).

Osmolality: As with both the PEG and the buffer capacity data, osmolality data showed increased variability. The increased variability may be related to the extremes in net water flux observed in some cases and would support the diverse clinical data with regard to the effects of input osmolality on intralumenal water absorption and secretion (39,56–60) that have been reported in the literature. Based on median values, duodenal contents were hyperosmotic over most of the aspiration period but achieved isoosmolality (287 mOsm kg^{-1}) first at 210 min. Values were significantly higher than those after water administration ($p < 0.001$). Ensure plus® contains a disaccharide (25% of total carbohydrate is sucrose), and, like the FDA meal, complex carbohydrates (34% of total carbohydrate is maltodextrin). Gradual hydrolysis of carbohydrates and, perhaps, increased intestinal residence prior to their absorption [41% of total carbohydrates is corn syrup that contains mainly fructose; fructose is absorbed three to six times slower than glucose from the gut (36)] generate higher luminal osmolality (54).

Surface Tension: Surface tension, as in all previous phases, showed the least variability. Medians were very low and ranged between 28.1 and 28.8 mN m^{-1}. Values were significantly different from those measured after water administration ($p < 0.001$).

Bile Salts: Data showed higher variability in the fed state, but results tended to decrease and become more consistent with time. Extreme low values were associated with sampling from the upper-middle duodenum, i.e., close to or even proximal to the sphincter of Oddi. Medians dropped from 11.2 to 5.2 mM at 180 min postdosing. Armand *et al.* (61) reported mean values of 6.7–13.4 mM up to 4 h after a 960-kcal meal (67.5% of calories were from lipids). Fausa (62) reported a mean concentration of 14.5 mM for bile salts at 30 min after administration of the meal (300 mL) and 5.2 mM between 30 and 60 min after administration of the meal.

Total Protein and Carbohydrate Content: Total protein content and total carbohydrate content were variable but much lower than input values over the entire aspiration period. However, data for total protein content are probably biased to higher values because the total number of samples with values <LOQ (twenty nine) was only slightly less than the number of samples with values >LOQ (thirty, Fig. 4). The high total protein content even 180 min after administration of the meal, significantly different than the content measured after water administration ($p = 0.005$), can be attributed partly to the increased presence of enzymes and partly to the presence of proteins in Ensure plus®. Caseinates, as well as other phosphopeptides that are present both in Ensure plus® and in the meal administered in BA/BE studies, are known to be relatively resistant to enzymatic digestion, and their digestibility may be affected by the presence of starch (54). With regard to carbohydrates, significant amounts were still present 180 min after administration of the meal. Although some carbohydrates may be contributed by the bile (37,53), a part of the carbohydrates measured would have been contributed by the maltodextrins [i.e., degradation products of starch; 2–20% of dietary starch and, perhaps, fructose escape absorption in the small bowel (64–66)]. It is worth mentioning that the FDA meal, which is often administered in BA/BE studies, also contains fructose (in orange juice) and starch.

CONCLUSION

The foregoing results confirm that there are very substantial differences in well-characterized parameters such as pH and bile salt concentrations between the fasted and fed states. Furthermore, substantial differences were established in less well-characterized, but pharmaceutically important, parameters such as buffer capacity, osmolality, and volume of luminal contents. A key difference between this study and previous studies was the attempt to simulate usual dosing conditions in a bioavailability/bioequivalence study. For experimental reasons (potential for clogging of aspiration tubes), it was not possible to aspirate after administration of the standard FDA meal. However, a fluid "total nutrition drink" with very similar carbohydrate/protein/fat ratios as well as most other physical chemical properties to the standard meal was substituted, making it possible to aspirate and still at least approximate the conditions usually adopted in bioavailability/bioequivalence studies. In addition, there have been very few studies published that have attempted to characterize luminal conditions in the duodenum after the administration of a meal. The data reported here suggest that, although the current biorelevant media better simulate the luminal environment much more nearly than standard compendial media, there is still some room for improvement. These results, coupled with a separate set of results characterizing the lipids in the GI tract in the fed state, will be used to design a "second generation" of biorelevant media. Potential uses of these media would be for characterization of solubility, dissolution, and permeability properties of drugs and dosage forms.

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