Characterization of Fluids from the Stomach and Proximal Jejunum in Men and Women

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Purpose. To chemically characterize the fluids available for drug dissolution in the upper gastrointestinal tract during the fasted state in humans, and to examine variations and potential gender differences regarding the physico-chemical properties of these fluids.

Methods. Twenty-four healthy volunteers, 12 females and 12 males, were intubated, and fluids from the stomach and upper jejunum were collected separately. Bulk pH, osmolality, electrolytes and total concentrations of bile acids and proteins were assessed. To study intraindividual variations, eleven of the individuals were studied on more than one occasion.

Results. The stomach and upper jejunal fluids varied significantly in all the measured entities, except the total concentration of proteins. The intraindividual variability was pronounced in some of the individuals, both in the stomach and the upper jejunum. We did not, however, observe any gender differences.

Conclusions. This study demonstrates the complex nature of the fluids available for drug dissolution in the stomach and the upper small intestine in humans. The results can be used when designing a more physiological *in vitro* dissolution media representative for the fasted state. When designing such a medium, we suggest that gender differences not be taken into account.

KEY WORDS: drug dissolution; gastrointestinal fluids; *in vitro/in vivo* correlation; variability; gender differences; absorption.

INTRODUCTION

A prerequisite for drug absorption and clinical response for all drugs given orally in a solid dosage form is drug dissolution in the gastrointestinal tract, which, in many cases, can be the rate-limiting step in the *in vivo* overall absorption process. The rate of dissolution can be described by a Noyes-Whitney (1) type of expression:

$$\frac{dX}{dt} = \frac{A \times D}{h}(Cs - Ct) \quad (1)$$

where the dissolution rate of a substance is a function of the surface area available for dissolution, A, the diffusion coefficient of the compound in the dissolution media, D, the boundary layer thickness, h, and the difference between the concentration of the drug's saturated solution, Cs and its concentration in the bulk of the dissolution media at time t, Ct.

These variables are influenced by different components of the complex *in vivo* dissolution medium, such as electrolytes, enzymes, bile acids and a wide range of other lipids. The physical chemistry of these components is complex and varies considerably depending on the nutritional status. In spite of the obvious recognition of this, the most commonly used *in vitro* dissolution media are water and 0.1 N hydrochloric acid (2). For instance, it has been reported that the dissolution rate of phenobarbital is significantly higher in diluted gastric fluid than in 0.1 N hydrochloric acid, most likely due to an increased wetting effect (i.e. an increase in the surface area) (3). In the intestine, bile acids are well known to increase the dissolution rate of different drugs by increasing the solubility and/or the wetting effect (4). Because of these unpredictable interferences in the *in vivo* situation, a more physiological understanding of the complex *in vivo* drug dissolution is required. Variability in the constituents of the gastrointestinal fluids will affect the dissolution and consequently the absorption and bioavailability of different drugs. Our long-term goal is to achieve a better correlation between *in vitro* and *in vivo* dissolution of drugs.

The major aim of the present study was to investigate how gastrointestinal factors such as bulk pH, osmolality, and the concentrations of electrolytes, proteins and bile acids vary in the upper gastrointestinal tract during the fasted state in humans. We also examined potential gender differences regarding these physicochemical properties of the gastrointestinal fluids. Finally, eleven subjects were investigated on two or three separate occasions in order to estimate the intraindividual variability of the selected physicochemical properties.

MATERIAL AND METHODS

Subjects

Twentyfour healthy volunteers, 12 females and 12 males, with a mean age of 28 years (range 19–37 years) all gave informed consent to participate in the study. Six of the individuals (4 males, 2 females) were studied on three separate occasions and five (3 males, 2 females) on two different occasions. The time intervals between the occasions ranged between 2 and 24 weeks (average 6 weeks). In total, 41 gastrointestinal intubation experiments were performed. The study was approved by the Ethics Committee of the Medical Faculty, Uppsala University and followed the convictions of the Declaration of Helsinki.

Experimental Design

Following a ten hours overnight fast and local anaesthesia (lidocain) of the upper throat, the subjects were intubated orally using a sterile and disposable perfusion tube designed for segmental intestinal perfusion (Loc-I-Gut®, Synectics Medical, Sweden) (Fig. 1) (5). The multi-channel tube is 175 cm long with an external diameter of 5.3 mm. Two of the channels are connected to two 40 mm long latex balloons, placed 10 cm apart at the distal part of the tube. When the proximal balloon had passed the ligament of Treitz, both balloons were inflated

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ABBREVIATIONS: dX/dt, change in amount per unit time; A, surface area; D, diffusion coefficient; h, boundary layer thickness; Cs, saturation solubility; Ct, concentration at time t; I, ionic strength; C, molar concentration; Z, valence.

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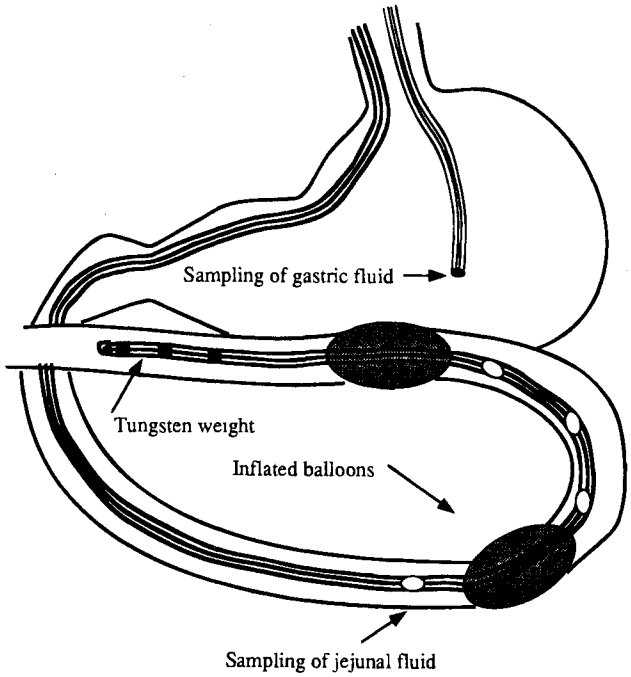

Fig. 1. The tube system used for the collection of gastric and jejunal fluids in healthy human volunteers. The balloons were filled with air when the proximal balloon had passed the ligament of Treitz. Small-intestinal fluid was continuously collected from the region just above the proximal balloon using vacuum drainage. Gastric fluid was collected separately from a tube placed in the antrum region of the stomach.

with 25–30 ml air in order to prevent the tube from migrating down the intestine. A channel with its inlet placed just above the proximal balloon was used for the collection of fluids from the small intestine by continuous vacuum drainage (Ameda suction pump type 23, Ameda Ag, Switzerland). The sampling site of the jejunal fluid was approximately 60 cm distally of the pylorus. The perfusion tube has been validated for absorption studies of drugs and nutrients in humans (6). A separate tube, 120 cm in length (Salem sump tube, Sherwood Medical, UK) with an external diameter of 4.0 mm was placed in the antrum region of the ventricle and used for the manual suction of gastric fluid. The positioning of the tubes was checked by fluoroscopic control (Philips BV 21-S). Jejunal and gastric fluids were collected separately during the 150 minute experiment period, and thereafter kept at −20°C until analysis.

Chemical Assays

The samples were thawed and well-mixed before any analytical determination. The pH values were measured by a pH electrode (632 pH Meter, Metrohm, Switzerland). Sodium and potassium concentrations were determined by flame photometry (FLM 3, Radiometer, Denmark), using lithium as an internal standard. The concentrations of the other electrolytes were obtained using commercially available reagent kits and by colorimetric determination at a suitable wave length (Hitachi spectrophotometer model U-1100, LabKemi, Sweden). Calcium and chloride concentrations were obtained using kits from Sigma Diagnostics (procedures 587 and 461 respectively, LabKemi, Sweden). Total bile acids were determined using Sterognost (Nycomed AB, Sweden). The total protein content of the gastrointestinal fluids was quantified using Pierce BCA protein assay (Tecator AB, Sweden), and albumin as a standard. The osmolality of the fluids was obtained by measuring the vapor pressure (Vapor Pressure Osmometer 5500, Wescor Inc., Logan, USA). The ionic strength, I, was estimated by the following equation:

$$I = \frac{1}{2} \sum CZ^2 \qquad (2)$$

where C and Z are the molarities and the valencies of each ion, respectively.

Statistics

All results are presented as mean values and standard deviation (±SD) unless otherwise stated. Student's unpaired t-test was used to test the difference between the two kinds of gastrointestinal fluids, as well as the potential gender difference. A p-value less than 0.05 was considered significant.

RESULTS

A total of 41 intubations was performed in the present study, but in a few cases it was not possible to collect sufficient volumes of fluids either from the stomach or the jejunum, leaving a total of 36 gastric and 37 jejunal samples to assay.

The concentrations of potassium, sodium, chloride, calcium, total bile acids and proteins, as well as the pH, ionic strength and osmolality of the gastric and jejunal fluids respectively, are given in Table I. There was a significant difference in the concentrations of all the measured constituents, except total protein, between the stomach and the proximal jejunum. The intraindividual variations of the content of gastric and jejunal fluids are given in Figures 2 and 3, respectively.

The mean gastric pH during the fasted state was 2.9 ± 1.97 (median 1.8, range pH 1.4–7.5), with seven samples above pH 5. Interestingly, one of the individuals with elevated gastric pH had a gastric pH of 1.5 on a second investigation (Fig. 2). The samples collected from the proximal part of the jejunum had a pH of 7.1 ± 0.60 (median 7.2, range pH 5.3–8.1). The osmolality of the fluids in the stomach and the jejunum was 191 ± 36 mOsm/kg (median 196, range 114–230 mOsm/kg) and 271 ± 15 mOsm/kg (median 273, range 218–292 mOsm/kg), respectively. The average concentration of sodium in the stomach was 68 ± 29 mM (median 67, range 19–122 mM) and in the jejunum 142 ± 13 mM (median 145, range 111–165 mM), making sodium the dominating ion in the jejunal fluid. The dominating ion in the gastric fluid was chloride, with a mean concentration of 102 ± 28 mM (median 100, range 48–173 mM). A slightly higher concentration of chloride was found in the jejunum, 126 ± 19 mM (median 126, range 92–181 mM). The intraindividual variation of chloride concentration was larger in the jejunum than in the stomach (Figs. 2 and 3). The concentration of potassium was 13.4 ± 3.0 (median 13.9, range 8.4–19.3 mM) and 5.4 ± 2.1 (median 5.2, range 1.7–11.6 mM) in the gastric and jejunal fluids, respectively. A low concentration of calcium ions with a high intraindividual variation was found in fluids from both sampling sites (Figs. 2 and 3). In the stomach the concentration was 0.6 ± 0.2 mM (median 0.6, range 0.3–1.2 mM) and in the jejunum it was 0.5 ± 0.3 mM (median 0.3, range 0.1–1.3 mM). The mean protein content in the gastric and jejunal fluids was similar, i.e., 1.8 ± 0.7

Table I. Osmolality, Ionic Strength and Concentrations of Different Constituents of Gastric and Jejunal Fluids, Collected from the Study Population During the Fasted State

		Ventricle (n = 36)		Jejunum (n = 37)		
		Average	±S.D.	Average	±S.D.	p
Osmolality	mOsm/kg	191	36	271	15	<0.0001
Ionic Strength		0.100	0.025	0.139	0.014	<0.0001
pH		2.9	1.97	7.1	0.60	<0.0001
Na+	mM	68	29	142	13	<0.0001
K+	mM	13.4	3.0	5.4	2.1	<0.0001
Cl−	mM	102	28	126	19	<0.0001
Ca2+	mM	0.6	0.2	0.5	0.3	0.01
Bile Acids	mM	0.2	0.5	2.9	2.9	<0.0001
Proteins	g/l	1.8	0.7	2.1	1.2	NS

Note: NS: Not Significant ($p > 0.05$); n: number of samples.

g/l (median 1.6, range 0.7–3.9 g/l) and 2.1 ± 1.2 g/l (median 1.7, range 0.7–5.3 g/l), respectively. In the gastric fluid we found a low concentration of bile acids in 16 out of 36 samples, providing a mean concentration of 0.2 ± 0.5 mM (median 0.1, range 0.0–2.5 mM). A higher concentration of bile acids was found in the jejunum, 2.9 ± 2.9 mM (median 2.1, range 0.1–13.3 mM). The individual with the highest concentration of bile acids in the stomach also had the highest concentration of bile acids in the jejunum on the same occasion (Figs. 2 and 3). The ionic strength of the gastric and jejunal fluids was estimated at 0.100 ± 0.025 (median 0.095, range 0.051–0.151) and 0.139 ± 0.014 (median 0.137, range 0.105–0.175), respectively.

There were no significant differences in any of the measured constituents between males and females, either in the fluids from the stomach or the jejunum (Table II).

DISCUSSION

This study shows that even in the fasted state in humans, the *in vivo* dissolution media is a complex and highly variable milieu consisting of various bile salts, electrolytes, proteins, cholesterol and other lipids. For instance, we observed a median gastric pH of 1.8 in the fasting state with a range between pH 1.4 and pH 7.5 which agrees with other reports (7). In this study, 7 subjects had an elevated gastric pH (>pH 5), which might be due to a higher incidence of achlorhydria/hypochlorhydria in this population. However, the incidence of achlorhydria/hypochlorhydria in a population under the age of 50 is reported to be less than 1% (8). Given also that one of the individuals with elevated gastric pH (pH 6.3) had a lower value (pH 1.5) on another occasion (Fig. 2), it seems less likely that a loss of, or a low capacity for hydrochloric acid secretion could explain the higher incidence. It might instead be due to swallowed saliva (pH about 7.5), collected from the antrum region of the stomach by manual suction. The low concentration of chloride ions found in the stomach of these subjects also supports this hypothesis. The gastric chloride concentration increases with the secretion rate, with a minimum concentration of approximately 120 mM at the lowest secretion rate (9). A chloride concentration below 120 mM is an indicaton of "contamination" of the gastric fluid with saliva, since the chloride concentration in saliva is approximately 25 mM (10). Five of the seven individuals with elevated gastric pH also had a significantly lower gastric chloride concentration ($p < 0.05$, compared to the average), which supports this hypothesis. However, since the majority of the subjects (25 of 36) had a gastric chloride concentration of less than 120 mM, the gastric fluid probably always contains some saliva.

The elevated gastric pH might also be due to reflux from the duodenum into the antrum region. This is supported by the finding that one subject with a pH of 6.1 simultaneously had a high level of bile acids in the stomach (2.5 mM). Gastric fluids have, however, previously been reported to contain small amounts of bile acids in the fasted state, albeit at lower concentrations than we have found in this study (11). The presence of saliva and/or bile acids in the gastric fluid may indicate that the pH and/or the wetting capacity (i.e. a lower surface tension) of the gastric fluid is higher than expected. This may affect the dissolution rate of a drug *in vivo,* resulting in a poor *in vitro/in vivo* correlation.

The pH in the proximal part of the jejunum was 7.1 ± 0.60 (Table I), which is higher than the jejunal pH in the fasted state reported by Mahé et al. in 1992. They used a double-lumen tube to collect jejunal fluids from seven healthy volunteers and reported a pH of 5.1 ± 0.6 (12). Our results agree more with radiotelemetry data (pH 6.63 ± 0.53) reported by Evans *et al.* in 1988 (13).

The potassium concentration in the jejunal samples was about 5.4 mM, which agrees well with the concentration in both fed and fasted state reported by others (12,14). The sodium concentration in the jejunum was about 140 mM, which also seems to be valid in the fed state (14). The jejunal concentration of chloride, about 125 mM, was on average slightly higher than the average concentration reported by Mahé and coworkers (12). In the fed state, however, Fordtran and coworkers reported similar concentrations of chloride in the mid jejunum, to those that we found in the proximal jejunum in the fasted state (14). To summarize, the jejunal concentrations of the dominating ions: potassium, sodium and chloride, appear to be rather constant between different populations participating in different studies. This may indicate that external factors such as food do not influence the ion concentration, probably because the intestinal membrane secretes either water or ions in order to maintain a steady state level of these ions. While this is valid for the jejunum, the chemical composition of the stomach fluid

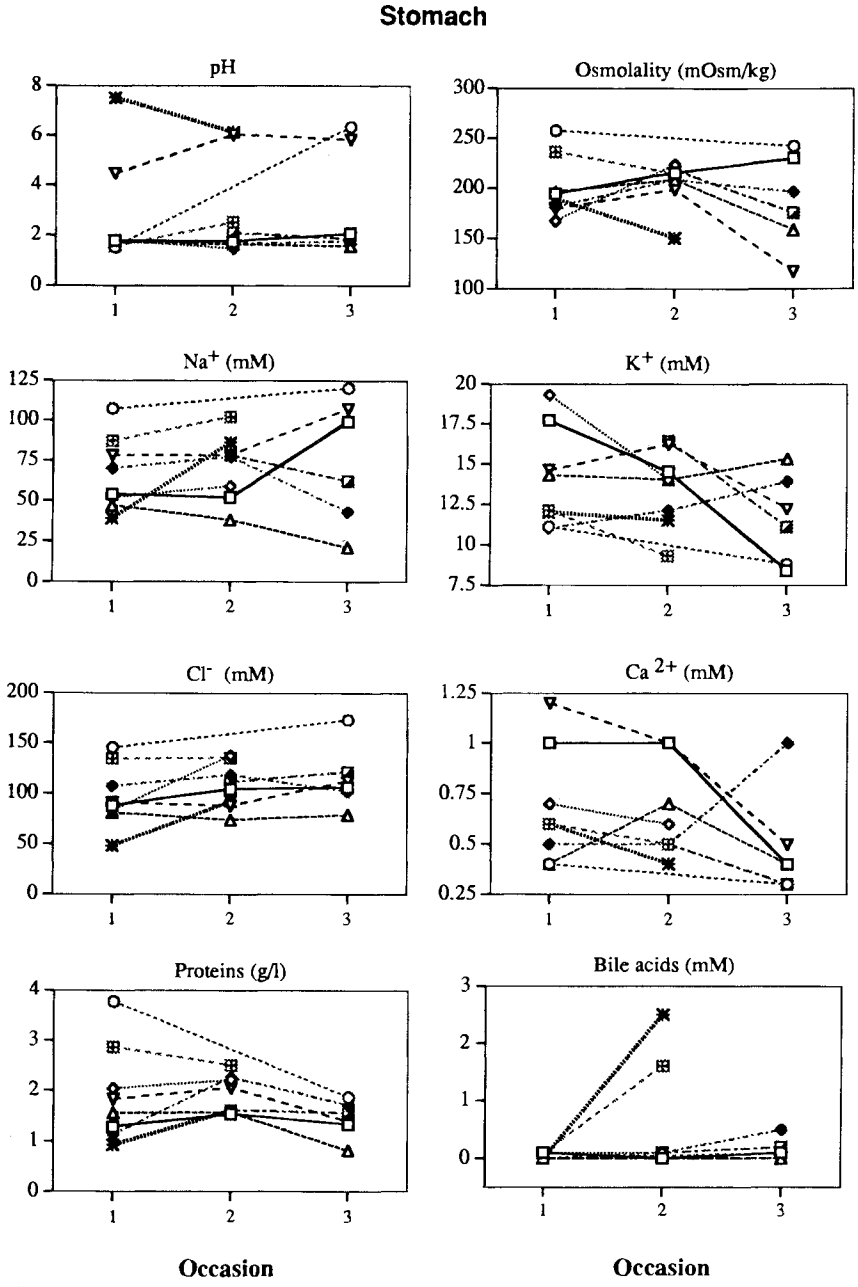

Fig. 2. Day to day variations in the content of the gastric fluids from 9 healthy human subjects in the fasted state. Each subject is represented by the same plot symbol in all the graphs.

after ingestion of food will more or less reflect the composition of the food itself (14).

Although not a dominating component of the gastrointestinal fluids, calcium ions have been shown to form low-solubility chelates with tetracycline derivatives resulting in an impaired intestinal absorption (15). Most of the intraluminal calcium is of dietary origin, since the amount of calcium secreted into the duodenum by the digestive glands is negligible compared to the calcium content of even low calcium meals (14,16). We found an average concentration of calcium during the fasted state of about 0.6 and 0.5 mM in the stomach and proximal jejunum, respectively,which agrees with a reported duodenal value of 0.75 mM (17).

The osmolality measured in the gastric and jejunal fluids was in the same range as that reported by others (12). Chloride and sodium ions contributed most to the osmolality, with chloride as the dominating ion in the stomach and sodium in the proximal jejunum.

Bile acids are known to affect the dissolution rate of different drugs by affecting their solubility, and/or by wetting of the drug powder which will increase the surface area available for dissolution (4). Bakatselou and coworkers have reported that the major contribution to the increased dissolution rate of steroids at fasting bile salt concentrations was always wetting (4). However, at higher concentrations of the bile salts the dissolution enhancement was primarily related to solubilisation (4). Bile

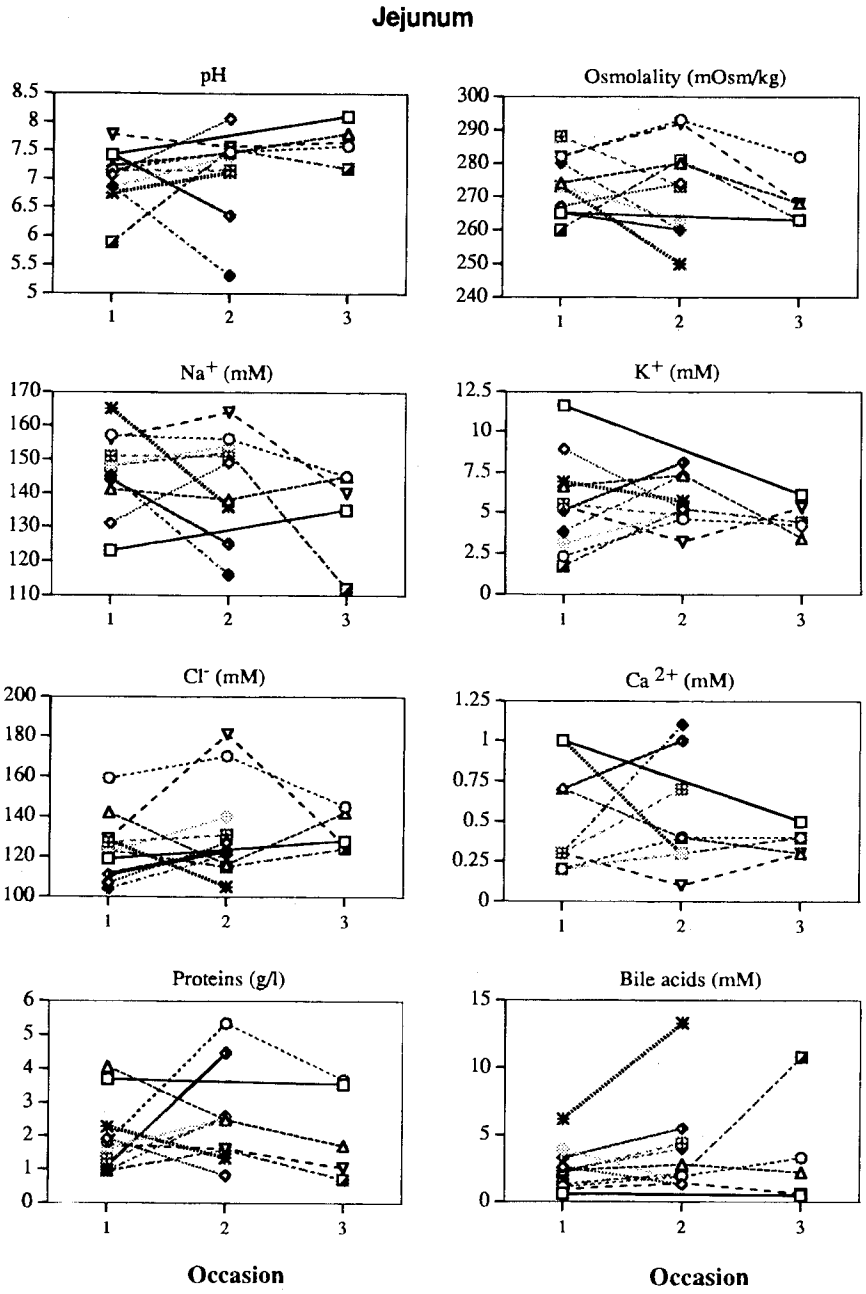

Fig. 3. Day to day variations in the content of the jejunal fluids from 11 healthy human subjects in the fasted state. Each subject is represented by the same plot symbol in all the graphs.

acids can increase the absorption by altering the barrier function by affecting the mucus layer, the cell membrane and/or the tight junctions (18,19). Furthermore, it is known that bile acids are able to associate with certain drugs, which has been suggested to be the mechanism underlying the low dose dependent and variable absorption of the β-blocker pafenolol observed *in vivo* (20). In the upper part of jejunum, the mean concentration of total bile acid was 2.9 mM, which agrees with the results of other studies (21). Although most of the individuals in the present study had consistent concentrations of bile acids over time, two subjects (1 male, 1 female) had increased bile acid concentration on one occasion (Fig. 3). Such a variation could contribute to variability of absorption by increased dissolution rate and/or complex binding, as discussed above.

In the present study, we also examined the potential gender differences in the chemical composition of the gastrointestinal fluids. For instance, gastric acid secretion has been reported to be lower in women than in men (8), but no significant gender difference in gastric pH during the fasted state has been found (7). This agrees with the results in the present study. Furthermore, we could not detect any significant gender differences regarding osmolality and the gastrointestinal concentrations of sodium, potassium, chloride, calcium, total bile acids and proteins (Table II).

Table II. Gender Differences in the Concentrations of Different Constituents of Gastric and Jejunal Fluids During the Fasted State

		Ventricle					Jejunum				
		Males (n = 19)		Females (n = 17)			Males (n = 22)		Females (n = 15)		
		Average	±S.D.	Average	±S.D.	p	Average	±S.D.	Average	±S.D.	p
Ionic Strength		0.100	0.027	0.101	0.023	NS	0.140	0.011	0.137	0.017	NS
pH		2.3	1.53	3.5	2.26	NS	7.2	0.56	7.1	0.68	NS
Osmolality	mOsm/kg	195	41.0	186	30.4	NS	270	11.0	271	19.1	NS
Na+	mM	66	31.8	71	26.5	NS	142	11.8	142	15.8	NS
K+	mM	13	2.9	14	3.0	NS	6	2.3	5	1.7	NS
Cl−	mM	102	29.5	102	26.0	NS	128	17.5	122	21.2	NS
Ca2+	mM	0.6	0.21	0.7	0.24	NS	0.5	0.30	0.4	0.31	NS
Bile Acids	mM	0.2	0.41	0.3	0.62	NS	2.6	2.25	3.2	3.79	NS
Proteins	g/l	1.7	0.74	1.9	0.74	NS	2.3	1.30	1.7	1.03	NS

Note: NS: Not Significant ($p > 0.05$); n: number of samples.

In conclusion, the present study demonstrates the complex nature of the fluids available for drug dissolution in the stomach and the upper small intestine in humans, which affects the *in vivo* drug dissolution and the transport of drug molecules across the intestinal mucosa. The chemical characteristics of the gastrointestinal fluids not only varied between individuals, but also showed a pronounced day to day variation in the same individual, which might be crucial for the overall rate and extent of drug absorption. The settings of *in vitro* dissolution standards must, therefore, be performed on the basis of the knowledge of the conditions existing *in vivo*. Our study also clearly demonstrates that no gender differences exist in the studied physicochemical properties in the human stomach and proximal jejunum, and consequently we suggest that gender differences do not have to be taken into account when designing a more physiological *in vitro* dissolution medium for the fasted state.

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