# Characterization of Human Duodenal Fluids in Fasted and Fed State Conditions

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**ABSTRACT:** This work provides an elaborate characterization of human intestinal fluids (HIF) collected in fasted- and fed-state conditions. HIF from 20 healthy volunteers (10 M/F) were aspirated by intubation near the ligament of Treitz in a time-dependent manner (10-min intervals) and characterized for pH, bile salts, phospholipids, cholesterol, triacylglycerides (TAG), diacylglycerides (DAG), monoacylglycerides (MAG), free fatty acids (FFA), pancreatic lipase, phospholipase A2, and nonspecific esterase activity. For almost all parameters, a food-induced effect was observed. Results were characterized by a high variability, as illustrated by the broad ranges observed for each parameter: pH (fasted: 3.4–8.3; fed: 4.7–7.1), bile salts (fasted: 0.03–36.18 mM; fed: 0.74–86.14 mM), phospholipids (fasted: 0.01–6.33 mM; fed: 0.16–14.39 mM), cholesterol (fasted: 0–0.48 mM; fed: 0–3.29 mM), TAG (fed: 0–6.76 mg/mL), DAG (fed: 0–3.64 mg/mL), MAG (fasted: 0–1.09 mg/mL; fed: 0–11.36 mg/mL), FFA (fasted: 0–3.86 mg/mL; fed: 0.53–15.0 mg/mL), pancreatic lipase (fasted: 26–86 μg/mL; fed: 146–415 μg/mL), phospholipase A2 (fasted: 3–6 ng/mL; fed: 4.3–27.7 ng/mL), and nonspecific esterase activity (fasted: 270–4900 U/mL; fed: 430–4655 U/mL). This comprehensive overview may serve as reference data for physiologically based pharmacokinetic modeling and the optimization of biorelevant simulated intestinal fluids for the use in *in vitro* dissolution, solubility, and permeability profiling. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

**Keywords:** intestinal drug absorption; intestinal secretion; gastrointestinal; oral drug delivery; food effects; lipids; pH; phospholipids

## INTRODUCTION

Variability in human drug bioavailability is often attributed to variations in hepatic first-pass extraction,[1] body distribution, and kidney excretion. For those orally administered drugs, variability may be further increased by gastrointestinal factors,[1] including gastrointestinal motility, gastric emptying,[2] and human intestinal fluid (HIF) composition.[3] HIF composition is especially important for its effect on the dissolution and permeation of lipophilic drugs with a limited aqueous solubility, that is, BCS (biopharmaceutical classification system) class II and IV compounds.[4] Predicting the oral absorption potential of this type of drugs requires *in vitro* tools for dissolution, solubility, and permeability, and physiologically based pharmacokinetic simulation models that account for HIF composition and its related variability.

The importance of bile salts, pH, phospholipids, lipid degradation products, cholesterol, and enzymatic secretions for absorption is generally accepted. The pH is known to affect drug ionization behavior.[5,6] The surfactant properties of bile salts[7] and phospholipids[8,9] contribute to the solubilization of lipophilic compounds. After intake and enzymatic digestion of a meal, lipolytic hydrolysis products [triacylglycerides (TAG), diacylglycerides (DAG), monoacylglycerides (MAG), and free fatty acids (FFA)] form colloidal structures and are a part of mixed micelles, which impact the solubility/permeability of lipophilic compounds.[10–12] Cholesterol improves bilayer stability[13] and is incorporated in mixed micelles[14]; more recently, cholesterol has been shown to affect solubility of several BCS II class drugs.[15]

As HIF is composed of several constituents, it is prone to a large variability. In addition to intersubject differences, day-to-day fluctuations may also lead to intrasubject variability. Moreover, the dynamic nature of the intraluminal environment, for instance, mediated by meal intake and biliary/pancreatic secretions, further enhances complexity. The pronounced variability obviously complicates the definition of simulated intestinal fluids (SIF).[3] Additionally, several limitations of the currently available literature data on HIF composition hinder a clear consensus on the simulation of HIF composition and its related variability. Data are scattered throughout numerous publications,[3,16–21] all focusing on different factors and utilizing different sampling protocols. Fasted- and fed-state conditions are often collected on separate test days, making it impossible to discriminate between the net effect of food and the intrinsic day-to-day variability in human volunteers. Moreover, the time-dependent evolution in HIF composition is usually neglected by using only pooled samples.

This study aims to provide a comprehensive and relevant characterization of the composition of duodenal fluids and its variability. Therefore, pH, individual bile salts, phospholipids, cholesterol, lipid degradation products (TAG, DAG, MAG, and FFA), and enzymatic secretions (pancreatic lipase, phospholipase $A_2$, and nonspecific esterase) were analyzed in consecutively collected fasted- and fed-state, time-dependent aspirates from 20 healthy volunteers. To judge the use of pooled aspirates as representative samples, volunteer pools were also created and analyzed. The data enabled insight into intersubject and

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time-dependent intrasubject variability and may contribute to the use of pooled HIF samples and their derived SIF composition for physiologically based absorption profiling.

## MATERIALS AND METHODS

### Materials

Taurochenodeoxycholic acid (TCDC), taurodeoxycholic acid (TDC), glycoursodeoxycholic acid (GUDC), glycochenodeoxycholic acid (GCDC), glycodeoxycholic acid (GDC), glycocholic acid (GC), chenodeoxycholic acid (CDC), deoxycholic acid (DC), lithocholic acid (LC), and cholic acid (C) were purchased from Sigma–Aldrich (St. Louis, Missouri). Tauroursodeoxycholic acid (TUDC), ursodeoxycholic acid (UDC), and taurocholic acid were acquired from Calbiochem (Darmstadt, Germany). Deuterated cholic acid (cholic-2,2,4,4-$d_4$) was purchased from CDN Isotopes (Quebec, Canada). NaCl, glacial acetic acid, chloroform, methanol, hexane, and pyridine were obtained from Merck (Darmstadt, Germany). The silylating agent bis(trimethylsilyl)trifluoroacetomide (BSTFA) was obtained from Pierce (Rockford, Illinois). The lipids DL-α-stearin, DL-1,2-dipalmitin, glyceryltripalmitin, and palmitic acid as well as the general lipase inhibitor Orlistat were obtained from Sigma–Aldrich. Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, UK). Ensure Plus (Abbott Laboratories B.V., Zwolle, The Netherlands) was used to simulate a standard meal. One portion of 200 mL has an energy content of 300 kcal, of which lipids, carbohydrates, and proteins constitute 29%, 54%, and 17%, respectively

### Sampling of HIF

The sampling of the intestinal fluids was performed at the University Hospitals Leuven and was approved by the Committee of Medical Ethics (ML7918). Twenty healthy Caucasian volunteers were enrolled in the study after giving written informed consent. The volunteers were selected as in a typical bioavailability/bioequivalence study. Ten men and 10 women, aged between 18 and 31 years and with a BMI between 19 and 25 kg/m$^2$, participated in the study. None of the volunteers had a history of gastroenterological diseases and medication was omitted for 2 days before participation in the study. After an overnight fast, one double-lumen catheter (Salem Sump Tube 14 Ch, external diameter 4.7 mm; Sherwood Medical, Petit Rechain, Belgium) was introduced in the duodenum (D2–D3) via the nose or mouth and the position was checked fluoroscopically (Fig. 1a). This double-lumen catheter enabled the collection of intestinal fluids by means of a syringe without creating under pressure in the gastrointestinal tract.

After an overnight fast of at least 12 h (no food and only water), the volunteers were given 250 mL of water before initiating the sampling of fasted-state intestinal fluids. Following the fasted state, 400 mL of Ensure Plus was ingested to simulate a standard meal; this condition is referred to as the fed state. A glass of 250 mL water was consumed 20 min after the intake of the liquid meal (Fig. 1b). After the ingestion of water (both for fasted and fed state), intestinal fluids were sampled every 10 min for a period of 90 min. In total, 18 time-dependent samples were obtained per healthy volunteer; sample volumes did not exceed 10 mL per time point. Intestinal samples were collected in test tubes containing the lipase inhibitor Orlistat dissolved in ethanol to arrest further lipolysis *in vitro*. A stock

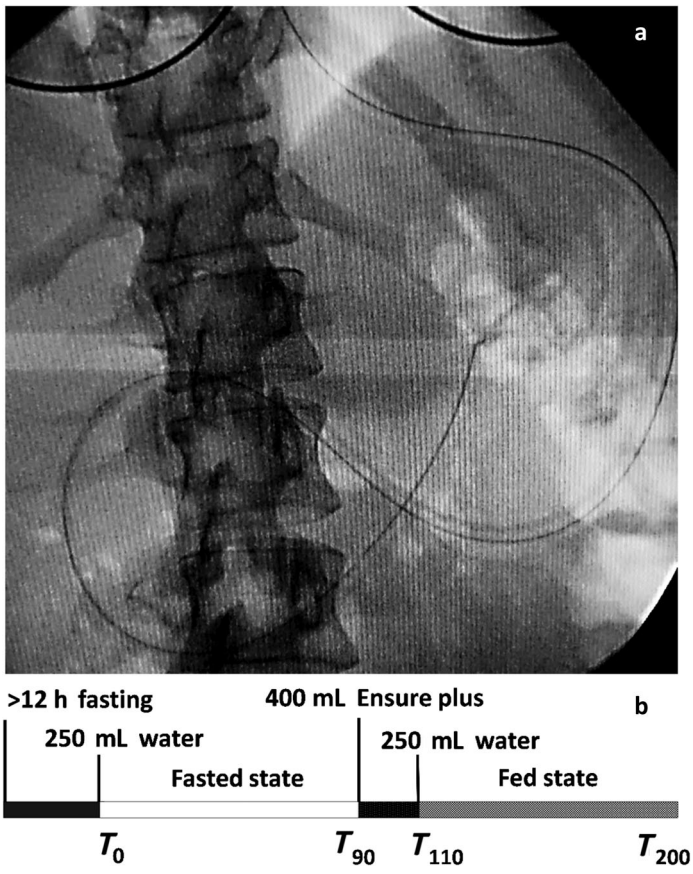


**Figure 1.** (a) A double-lumen catheter is positioned near the ligament of treitz. (b) Sampling protocol over time. Sampling was performed every 10 min for a period of 1.5 h in both fasted and fed state. T0 and T110 were not included. Between T90 and T110 was a resting period.

solution of 1 mM Orlistat in ethanol was used to bring the final concentration of Orlistat in the duodenal sample to 1 μM. This protocol allowed minor dilution of the collected samples as well as negligible addition of ethanol (0.1%, v/v). The applied concentration of this lipase inhibitor is 100-fold the IC50 (11 nM), as determined previously (Unilever R&D, Vlaardingen, data not published).

### Handling of Samples

All aspirated fluids were kept on ice in closed test tubes till the end of sampling, followed by a 15-min centrifugation at 1500*g* and 4°C. All samples were stored at −20°C until further analysis. Aliquots (<1 mL) of each sample were stored for characterization. The remainder of the collected fractions was pooled for each volunteer in fasted and fed state (further referred to as volunteer pools). Pools were created immediately after the collection was complete. For a selected set of samples, separate aliquots without Orlistat were collected in order to measure esterase activity; additionally, protease inhibitors were added to aliquots of another set of samples used for lipase quantification.

### Analysis of Samples

#### *pH*

The pH of all samples (see *Sampling of HIF* section) was measured immediately upon collection (Hamilton Slimtrode); the

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