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Research Paper

A Clinical Single-Pass Perfusion Investigation of the Dynamic *in Vivo* Secretory Response to a Dietary Meal in Human Proximal Small Intestine

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Purpose. To investigate the gastrointestinal secretory and enzymatic responses to a liquid meal during *in vivo* perfusion of the proximal human jejunum.
Methods. Human intestinal fluid was collected from the proximal jejunum by single-pass *in vivo* perfusion (Loc-I-Gut). The fluid was quantitatively collected at 10-min intervals during 90 min while perfusing a nutritional drink at 2 mL/min. Quantification of lipids in the fluid leaving the segment was performed by using novel chromatographic methods.
Results. The overall bile acid concentration varied between 0.5 and 8.6 mM with a peak level 40 min after the start of the liquid meal perfusion. The total concentration of phospholipids was between 0.1 and 3.9 mM and there was a rapid degradation of phosphatidylcholine to lysophosphatidylcholine. The tri-, di-, monoglycerides and free fatty acid levels increased sharply in the beginning and reached steady-state levels between 7 and 9.5 mM.
Conclusions. There is a rapid secretion of bile in response to food. Most of the dietary lipids are found in the form of their degradation products *in vivo* in human jejunum. This novel *in vivo* characterization, based on direct and high-recovery sampling of intestinal fluids, forms a basis for further development of improved *in vitro* drug dissolution test media.

KEY WORDS: bile acids; drug absorption; drug dissolution; intestinal fluid; neutral lipids; phospholipids.

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ABBREVIATIONS: AUC, area under the concentration time curve; BCS, biopharmaceutical classification system; C, the concentration of the component found in the analyses; C_{in}, the concentration of the lipid components in the NuTRIflex entering the intestinal segment; C_{max}, maximum perfusate concentration; C_{out}, the mean steady-state concentration of the nutritional lipids analyzed in the sample of the perfusate leaving the intestinal segment; $C_{out\text{-}comp}$, the concentration of each NuTRIflex component in the intestinal fluid leaving the segment after compensation for dilution in the intestinal segment has been made; $C_{t,\,in\ vivo}$, the concentration of each component upon dilution in the small intestine in the time interval t; ELS, evaporative light scattering; HIF, human intestinal fluid; HPLC, high-performance liquid chromatography; L, the length of the perfused jejunal segment (10cm); *LOQ*, limit of quantification; *PEG*, polyethylene glycol; PEG_{in}, concentration of ^{14}C-PEG 4000 (dpm/mL) entering the segment; PEG_{out}, concentration of ^{14}C-PEG 4000 (dpm/ml) leaving the segment; *NWF*, net water flux; SEM, standard error of the mean; T_{max}, the time at which the maximum perfusate concentration occurred; Q_{in}, the flow rate (mL/min) of the perfusion solution entering the segment; $Q_{out,t}$, the flow rate of the perfusate leaving the segment during the time interval t.

INTRODUCTION

Bioavailability is a primary pharmacokinetic variable in determining the potential successful development of a drug. Several clinically useful drugs and candidate drugs are highly hydrophobic and poorly water soluble. In the widely used biopharmaceutics drug classification system (BCS), highly hydrophobic compounds fall into low solubility, high/low permeability classes II and IV (1). Nowadays, strategies for the pharmaceutical formulation of hydrophobic and poorly soluble compounds are crucial for the successful pharmaceutical development of a drug. These strategies include traditional formulation approaches such as formulating different salts and attempting different particle sizes and more recent approaches like nanotechnology and the production of advanced lipid formulations. However, lipid formulation development strategies remain largely empirical, due in part to the lack of simple *in vitro* tests that are predictive of the complex processes that occur *in vivo* with a lipid based dosage form along the gastrointestinal tract (2–5). It is also well known that the absorption and plasma exposure of a low solubility hydrophobic drug may be significantly increased in the presence of a fatty meal, especially when the drug is given in a relatively high dose in relation to its low solubility. The bioavailability increased after administration in fed state and/or in a lipid-based formulation for drugs such as halofantrine (6), griseofulvin (7), and danazol (8) and is

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considered to be explained by increased solubility in the more lipidic environment present in the lumen.

Dietary lipids stimulate the secretion of bile acids, which results in the formation of different colloidal phases within the intestinal lumen. Class II and IV drugs may be unevenly distributed between these intraluminal lipid phases, and the composition and functional properties of these lipid phases are affected by secretion, digestion, and absorption. Futhermore, the relative intraluminal concentrations of bile salts, biliary lipids, and lipid digestion products change continuously in the postprandial phase. Furthermore, the relative proportions of the high lipid load emulsion droplets and multilamellar vesicles decrease relative to the end-stage mixed micelle (hydrodynamic radii ~20 nm) and unilamellar vesicles (hydrodynamic radii ~40–60 nm) will be affected significantly over time in the fed state (9–11). Accordingly, *in vivo* dissolution and absorption of class II and IV drugs is subjected to changes not only as a function of the fed/fasted cycle, but also dependent on the intraluminal *in vivo* kinetics of lipid digestion and absorption.

Previously, complex *in vitro* dissolution media for both the fasted and fed states have been developed and compared with corresponding *in vivo* dissolution data (12–16). These physiologically based *in vitro* dissolution media are simplified and mainly composed of buffers to which lipids, such as bile acids and phosphatidylcholine have been added. Not surprisingly, it has been shown that the *in vitro* dissolution rate of class II and IV drugs in these physiologically static *in vitro* media does not always correlate with the dissolution rate in aspirated gastrointestinal fluids from humans or with *in vivo* dissolution within the small intestine (17,18). In addition, the complex physiological based *in vitro* media simulating the fed state are static compared to *in vivo* conditions, where the composition of fluids in the small intestine changes with the progress of intraluminal enzymatic digestion and absorption. Recently, several reports on *in vitro* dissolution investigations based on relatively simple models of lipid digestion have been published (2–5). These models incorporate the kinetics of lipid digestion and the coincidental formation of various colloidal lipid digestion products (micelles, emulsions, vesicles, etc.) with the solubilization profile of coadministered drugs (11,19–22). Even if these *in vitro* lipid digestion studies have substantially broadened our understanding of the dissolution mechanisms of low solubility drugs, we consider that accurate comparisons with the complex *in vivo* situation in humans have not yet been fully comprehended. Apparently, there is a need for in-depth investigations of both the composition and degradation kinetics of components in gastrointestinal fluid collected in humans in various states of fasted and fed conditions.

The aim of the human study reported here was to make online *in vivo* determinations of the dynamic intestinal secretory response over time when a liquid meal was introduced in the proximal small intestine. The results will form the basis for further development of dissolution media based on dynamic *in vivo* gastrointestinal physiology in humans. The intestinal fluid used was quantitatively collected from the proximal jejunum by use of the Loc-I-Gut technique. Degradation of lipids was inhibited with a lipase inhibitor, and lipid characterization was carried out using a new analytical method that incorporates high performance liquid chromatography (HPLC) with an evaporative light scattering (ELS) detector.

MATERIALS AND METHODS

Study Design and Subjects

The single-pass jejunal perfusion studies were performed at the Clinical Research Department, University Hospital, Uppsala, Sweden, and were approved by the Ethics Committee of the Medical Faculty of Uppsala University. Six healthy volunteers (four men and two women), aged 24 to 40 years and weighing 66–86 kg (males) and 50–70 kg (females), gave informed consent for their participation in this study.

Clinical Method

Human intestinal fluid (HIF) was collected quantitatively using the Loc-I-Gut method described by Knutson *et al.* (23) and Lennernäs *et al.* (24). In brief, the perfusion tube is a 175-cm long (5.3-mm external diameter) multichannel polyvinyl tube with two inflatable balloons, and a tungsten weight at the tip (Fig. 1). The subjects had fasted overnight before the experiment. The tube was introduced through the subjects' mouths after applying of local anesthesia to the upper throat with a lidocaine spray. Once the perfusion tube had been positioned in the proximal part of the jejunum, with the positioning being checked by fluoroscopy, the distal balloon was inflated with approximately 26–30 mL of air. Another tube was positioned in the stomach to drain gastric juice to avoid nausea during the intestinal perfusion experiment. After rinsing the jejunal segment with isotonic saline (37°C), the perfusion solution was pumped into the jejunal segment at a flow rate of 2.0 mL/min for 90 min by using the

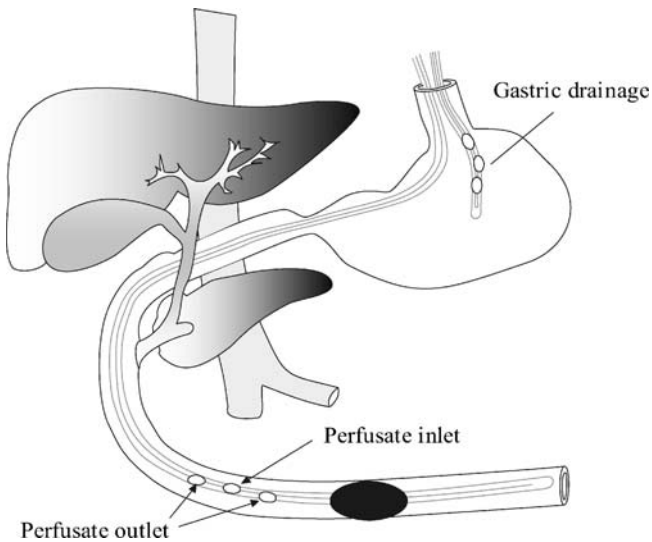

Fig. 1. Schematic drawing of the Loc-I-Gut® tube. The perfusate inlet and outlet holes were positioned in the jejunum. In the present *in vivo* perfusion study, only the distal balloon was inflated to prevent fluid from continuing further down the gastrointestinal tract. A gastric drainage was used to avoid nausea.

Table I. The Composition and Amount of the Major Nutrient Groups Contained in a Liquid Meal Exposed to a Segment of the Human Proximal Jejunum During a Single-Pass *in Vivo* Perfusion

Nutrient	Composition (g/L)	Amount given (g)
Nitrogen	5	0.8
Amino acids	32	6
Glucose	64	12
Lipids	40	7
Energy	3200 kJ	576 kJ

single-pass approach. The perfusion solution chosen to simulate fed conditions was a well-defined nutritional drink used for parenteral administration to patients with mild to moderate catabolism (NuTRIflex®, Braun, Table I). The total dose of each of the nutrients was 0.8 g nitrogen, 5.8 g amino acids, 11.5 g glucose, and 7.2 g lipids. ^{14}C-labeled polyethylene glycol (^{14}C-PEG 4000) (2.5 μCi/L, Amersham Pharmacia Biotech, Little Chalfont, England, UK) was included in the perfusion solution as a volume marker, which is not absorbed from the intestinal lumen. The experiment started as the NuTRIflex was introduced through the Loc-I-Gut tube. The perfusate leaving the jejunal segment, containing both endogenous lipids and lipids from the NuTRIflex, was quantitatively collected on ice at 10-min intervals. The HIF was pooled and stored at −70°C prior to performing the analyses.

Chemicals

Orlistat was purchased from Apin Chemicals, UK. All the bile acids for the analyses came from Sigma-Aldrich, Germany. The bile acids included in the analyses were cholic acid, glycocholic acid, taurocholic acid, deoxycholic acid, glycodeoxycholic acid, taurodeoxycholic acid, chenodeoxycholic acid, glycochenodeoxycholic acid, taurochenodeoxycholic acid, and lithocholic acid. Phosphatidylinositol was ordered from ICN Biomedicals, Germany, and all other phospholipids from Avanti Polar Lipids, USA. The phospholipids used for the analyses were phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, diphosphatidylglycerol, phosphatidic acid, and sphingomyelin. For the neutral lipid analyses, cholesterol, cholesteryloleate, dipalmitin, and tripalmitin were purchased from Sigma-Aldrich, Germany; monoolein from Larodan AB, Sweden; and palmitic acid from Aldrich Chemie, Germany.

Analytical Method

The nutritional drink NuTRIflex and the different time fractions of fed HIF were characterized to determine their lipid content (i.e., by measuring the bile acids, phospholipids, and neutral lipids including free fatty acids). The enzyme inhibitor orlistat was added (1 mg/mL) to the HIF prior to performing the analyses to inhibit the enzymatic degradation of lipids. Orlistat binds covalently to and inhibits gastric and pancreatic lipase, but it does not inhibit phospholipase A2 (25–27). To purify the samples of intestinal fluids, 500 mg C18 columns and 100 mg silica (Si) columns (Isolute International Sorbent Technology, UK) were used. The lipids were divided into three different groups: bile acids, phospholipids, and neutral lipids including free fatty acids (Fig. 2), each of which was analyzed with HPLC using an ELS detector (Persson *et al.*, in preparation). Each analysis was

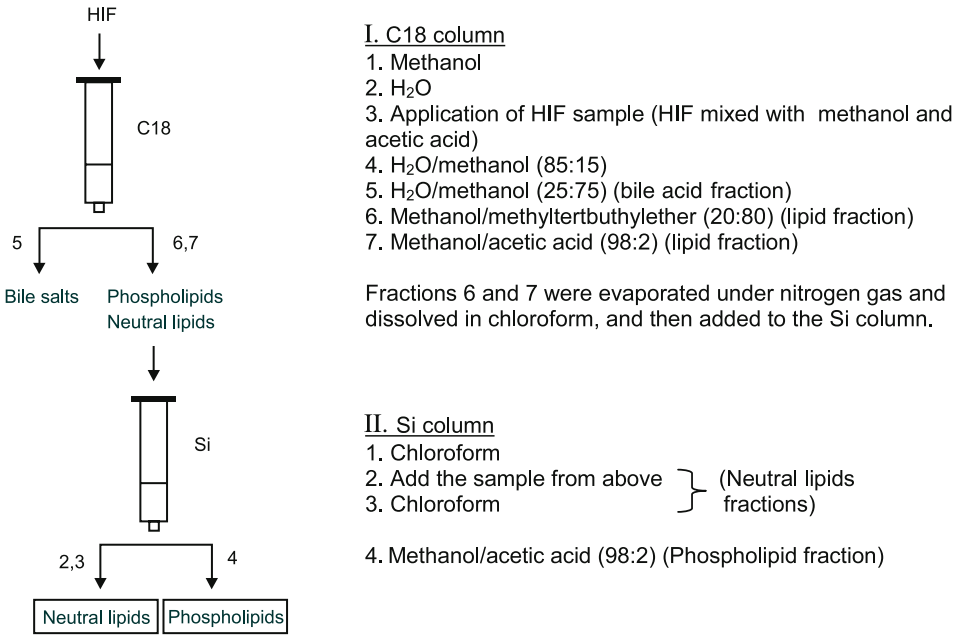

Fig. 2. Schematic illustration of the novel solid-phase extraction method for human intestinal fluids. Bile acids were separated from phospholipids and neutral lipids by use of a C18 column. A Si column was subsequently used for separation of phospholipids and neutral lipids. Each fraction was analyzed for their species with HPLC and ELS detection.

performed in triplicate; the results presented are the mean concentrations. In brief, the bile acids were separated on a Zorbax C18 Extend column (150 × 4.6 mm, 3.5 μm) (Agilent Technologies, USA). The flow rate was set to 1 mL/min, and 25 μL of the samples was injected directly into the HPLC. A binary gradient was used to separate the bile acids. The solvents were as follows: A) methanol/buffer (ammonium acetate 15 mM, 0.2% triethylamine, 0.5% formic acid, pH 3.15) 60:40 (v/v) and B) methanol/buffer 95:5 (v/v). Phospholipids were separated on a YMC-Pack Diol column (250 × 2.1 mm, 5 μm) (YMC Inc, USA). The flow rate was set to 0.25 mL/min, and 10 μL of the samples was injected. A binary gradient was used to separate the phospholipids. The two solvents were as follows: A) hexane/2-propanol/acetic acid/triethylamine 82:18:0.5:0.014 (v/v/v/v) and B) 2-propanol/H_2O/acetic acid/triethylamine 85:15:0.5:0.014 (v/v/v/v). Neutral lipids and free fatty acids were separated using an Apex II Diol column (150 × 4.6 mm, 5 μm) (Jones chromatography, USA). The flow rate was set to 0.8 mL/min, and 10 μL of the samples was injected. A binary gradient was used to separate the neutral lipids. The two solvents were as follows: A) hexane/acetic acid 99:1 (v/v) and B) isohexane/2-propanol/acetic acid 84:15:1 (v/v/v). The limit of quantification (LOQ), as determined from the lowest standard concentration with a coefficient of variation of less than 20% (n = 3), for the lipids found in the intestinal fluid was between 0.02 and 0.1 mM, which was well below the actual values.

Data Analyses

Perfusate Data

The net water flux (*NWF*, in milliliters per hour per centimeter) in the isolated jejunal segment was calculated according to Eq. (1):

$$NWF = \left(1 - \frac{PEG_{out}}{PEG_{in}}\right) \cdot \frac{Q_{in}}{L} \quad (1)$$

in which PEG_{in} and PEG_{out} are the concentrations of ^{14}C-*PEG* 4000 (dpm/mL) entering and leaving the segment, respectively. Q_{in} (mL/min) is the flow rate of the perfusion solution entering the segment, and L is the length of the perfused jejunal segment (10 cm). The calculation was made from steady-state concentrations of *PEG* 4000 in the outlet jejunal perfusate.

Each individual value of the area under the perfusate concentration–time curve (AUC) was calculated for all of the substances measured in the outlet intestinal perfusate. The calculations were performed using the linear and logarithmic trapezoidal rules for ascending and descending perfusate concentrations, respectively, in the time interval 0 to 90 min. In addition, the maximum perfusate concentration (C_{max}) and the time at which it occurred (T_{max}) were obtained by visual inspection.

When the perfusate (NuTRIflex) reached the intestinal lumen it was immediately diluted according to the well-mixed hydrodynamics (28). The concentrations of the lipidic components in the NuTRIflex solution upon entry in the jejunum were therefore calculated for each time interval under the assumption that dilution took place immediately upon entering the small intestine and that no degradation or absorption occurred. The calculation was performed according to Eq. (2):

$$C_{t,\,in\,vivo} = \frac{C_{in}}{Q_{out,t}/Q_{in}} \quad (2)$$

where $C_{t,\,in\,vivo}$ is the concentration of each component upon dilution in the small intestine in the time interval t, C_{in} is the concentration of the component in the NuTRIflex and $Q_{out,t}$ is the flow rate of the perfusate leaving the segment during the time interval t. $C_{t,in\,vivo}$ was calculated for comparison with the analytically determined concentrations in the outflow samples containing both endogenous and NuTRIflex lipids. This revealed if there was any absorption, secretion, formation, or degradation of the lipids. The concentration of the NuTRIflex components in the outflow samples was diluted in comparison to the concentration in the inlet perfusate. To be able to compare these two, the compensated concen-

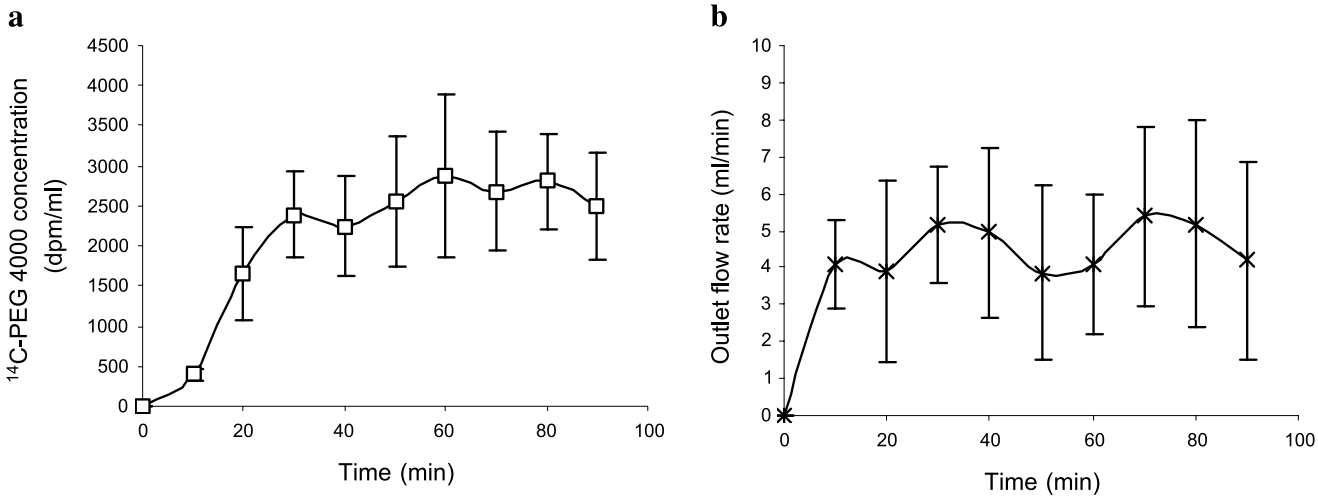

Fig. 3. a) The concentration-time profile of the nonabsorbable volume marker ^{14}C-PEG 4000 in the jejunal fluid leaving the perfused jejunal segment (mean ± SD) and b) the flow rate (mL/min) of the jejunal fluid leaving the perfused jejunal segment (mean ± SD).

Table II. Concentrations of Lipid Components in the Perfusate Entering and Leaving the Small Intestine

	Perfusate inlet C_{in} [a]	Perfusate outlet C_{out} [b]	Perfusate outlet $C_{out\text{-}comp}$ [c]
Triglycerides (mM)	15 ± 3	1 ± 0.5	3
Free fatty acids (mM)	14 ± 0.2	12 ± 3	30
Cholesterol (mM)	0.4 ± 0.02	0.3 ± 0.2	0.8
Phosphatidylcholine (mM)	2 ± 0.3	0.5 ± 0.07	1.3

Perfusate outlet gives the concentration in the analyzed samples and the mean outlet concentration corrected for the dilution in the intestine [according to Eq. (3)]. The values given for perfusate inlet and outlet are mean ± SD.

[a] The measured concentration of the lipids in the NuTRIflex administered as a perfusate through the Loc-I-Gut tube.

[b] The determined mean concentration of the lipid components in the NuTRIflex in the outlet samples.

[c] The calculated mean concentration in the outlet perfusate corrected for dilution according to Eq. (3).

trations of each NuTRIflex component leaving the intestinal segment ($C_{out\text{-}comp}$) were calculated as follows [Eq. (3)]:

$$C_{out\text{-}comp} = C_{out} \cdot \frac{Q_{out}}{Q_{in}} \quad (3)$$

in which C_{out} is the mean concentration analyzed in the perfusate leaving the intestinal segment at steady state and Q_{out} is the mean flow rate out of the segment. The calculated values of $C_{out\text{-}comp}$ were compared to the analytically determined concentrations in the inlet perfusate (containing only NuTRIflex lipids).

Throughout the paper all data are expressed as mean values ± standard deviation (mean ± SD) unless stated otherwise.

RESULTS

The perfusion tube was successfully positioned in the proximal jejunum for all six of the volunteers. The time required to position the tube and to start the perfusion was about 60 min. The perfusion experiment was carried out for another 90 min in all subjects without any discomfort. The recovery of the nonabsorbable volume marker ^{14}C-PEG 4000 in the intestinal perfusate leaving the jejunal segment was complete (101.5 ± 4.8%). A steady-state perfusate concentration of ^{14}C-PEG 4000 leaving the segment was obtained from 50 to 90 min after start of the perfusion (Fig. 3). The net water secretion and the flow rate of the fluid leaving the segment were 6.2 ± 1.7 mL/h · cm and 4.5 ± 1.1 mL/min, respectively.

The concentrations of dietary lipids in the nutritional drink (NuTRIflex) that was given as perfusate, entering and leaving the intestinal segment were determined and are shown in Table II. It is evident when comparing C_{in} and $C_{out\text{-}comp}$ that the concentration of triglycerides and phosphatidylcholine was lower in the outlet perfusate than in the perfused nutritional drink, whereas the concentration of free fatty acids and cholesterol had increased. The calculated *in vivo* concentrations ($C_{t,in\ vivo}$) of the NuTRIflex components are displayed in Figs. 4 and 5.

A lag phase was observed in all the outlet perfusate concentration–time profiles (Figs. 4 and 5). This is probably due to the time required for the perfusate to complete the length of the Loc-I-Gut tube and reach the intestine. If we consider the length and the inner diameter of the tube, the time needed for the perfusate to reach the intestine would be approximately 5–10 min, thus the time zero in Figs. 4 and 5 represents the start of the perfusion and not the actual time when the nutritional drink reached the intestine, and the concentrations of the perfusate leaving the intestine at 10 min represents the fasted state.

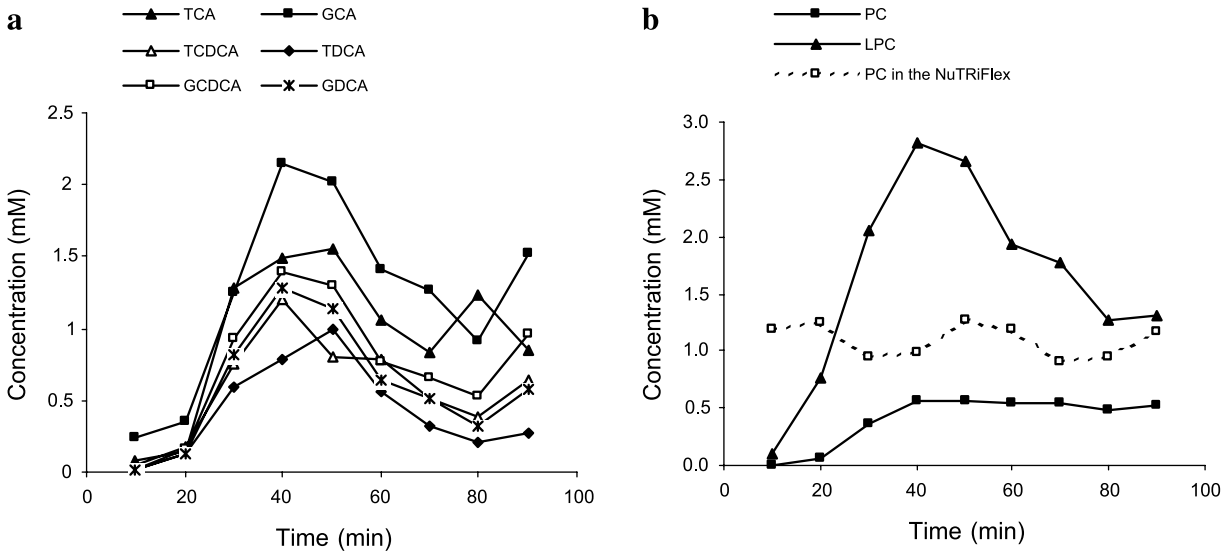

Fig. 4. Mean concentrations (mM) of a) bile acids and b) phospholipids from intestinal secretions and NuTRIflex in intestinal fluids 10–90 min after the start of perfusion of a nutritional drink. The theoretical *in vivo* concentrations ($C_{t,\ in\ vivo}$) of the NuTRIflex components calculated according to Eq. (2) are included in the figure as dotted lines. TCA, taurocholic acid; GCA, glycocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; PC, phosphatidylcholine; LPC, lysophoshatidylcholine.

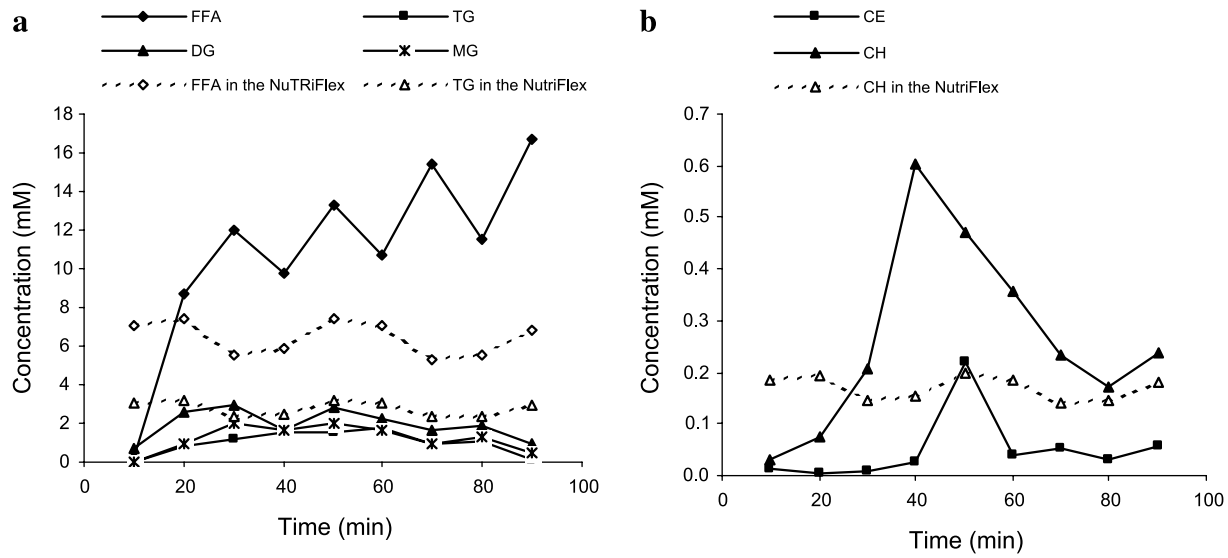

Fig. 5. Mean concentrations (mM) of a) neutral lipids including free fatty acids and b) cholesterols from endogenous secretions and NuTRIflex in intestinal fluids 10–90 min after start of the perfusion with a nutritional drink ($n = 6$). The theoretical *in vivo* concentrations ($C_{t,in\ vivo}$) of the NuTRIflex components calculated according to Eq. (2) are included in the figures as dotted lines. FFA, free fatty acids; TG, triglycerides; DG, diglycerides; MG, monoglycerides, CE, cholesterol ester; CH, cholesterol.

The concentration time profiles of six different bile acids in the small intestine during digestion are shown in Fig. 4a. Since the NuTRIflex did not contain any bile acids, the concentration in the outlet perfusate represents only endogenous secreted bile acids. Twenty minutes after the start of the perfusion, there was a release of bile acids from the gall bladder into the proximal small intestine and a maximum concentration (T_{max}) in the outlet perfusate was reached for all the bile acids after about 40 min (Table III). Interestingly, after 80 min the perfusate outlet concentration of all the bile acids increased again (Fig. 4a). The total bile acid concentration varied between 0.5 and 8.6 mM during the perfusion. Glyco- and taurocholic acid were the predominant bile acids with AUCs of 103.7 ± 18.3 and 80.9 ± 29.2 mM min, respectively, which corresponded to 28 and 22% of the total bile acid contents in the small intestinal lumen (Table III). Kinetic data for all the bile acids in the outlet perfusate are shown in Table III. Interestingly, one of the subjects had to be excluded as no bile acids were secreted.

Table III. The Local Kinetic Variables of Six Bile Acids, Two Phospholipids, and Six Neutral Lipids Including Free Fatty Acids in the Analyzed Samples of the Perfusate Leaving the Human Proximal Jejunum in the *in Vivo* Postprandial Condition, Expressed as the Mean ± SEM

Substance	AUC (mM · min)	C_{max} (mM)	T_{max} (min)
Bile acids			
Taurocholic acid	81 ± 29	2.4 ± 0.7	43 ± 3
Glycocholic acid	104 ± 18	3.2 ± 0.6	43 ± 3
Taurochenodeoxycholic acid	50 ± 6	1.5 ± 0.1	40 ± 0
Taurodeoxycholic acid	38 ± 14	1.5 ± 0.6	43 ± 3
Glycochenodeoxycholic acid	63 ± 10	2.1 ± 0.2	43 ± 3
Glycodeoxycholic acid	52 ± 13	1.9 ± 0.5	43 ± 12
Phospholipids			
Phosphatidylcholine	34 ± 9	1.0 ± 0.3 SS[a]	50 ± 6
Lysophosphatidylcholine	140 ± 16	3.4 ± 0.7	43 ± 4
Neutral lipids			
Cholesterol ester	4 ± 3	0.2 ± 0.2	47 ± 8
Free fatty acids	900 ± 111	14.5 ± 1.2 SS	38 ± 5
Triglycerides	90 ± 55	2.3 ± 1.1 SS	35 ± 7
Cholesterol	23 ± 6	0.7 ± 0.2	47 ± 3
Diglycerides	169 ± 20	3.3 ± 0.6 SS	22 ± 3
Monoglycerides	107 ± 16	2.1 ± 0.3 SS	30 ± 0

[a] Steady state.

Figure 4b shows the luminal concentration–time profiles of phosphatidylcholine and lysophosphatidylcholine, which were the only phospholipids found. Only trace amounts of phosphatidylserine were found in the outlet perfusate. The concentration of lysophosphatidylcholine increased after 10 min and reached a C_{max} of 3.4 ± 0.7 mM after 40 min (Table III). The concentration of phosphatidylcholine increased after 20 min and reached a steady-state concentration of 0.5 mM after 40 min. The total concentration of phospholipids was between 0.1 and 3.9 mM during the perfusion. This concentration is a mixture of endogenous secreted phospholipids and phospholipids in the NuTRIflex.

The neutral lipids including free fatty acids found in the intestinal fluids 0–90 min after the start of perfusion with a nutritional drink (NuTRIflex) are shown in Fig. 5. The concentrations displayed for free fatty acids and cholesterol are a mixture of endogenous secreted and NuTRIflex. The NuTRIflex contributed with free fatty acids, triglycerides, and cholesterol to the overall concentration in the outflow samples. The total concentrations of tri-, di-, and monoglycerides and free fatty acids were between 0.8 and 9.5 mM during the perfusion. The concentration–time profiles for these lipid species showed that the intestinal concentration was low in the beginning of the perfusion (below the concentration in the NuTRIflex, 15 and 14 mM for triglycerides and free fatty acids, respectively) but started to

increase after 10 min and reached a steady-state level after approximately 30 min (Table III). The concentrations of cholesterol and cholesterol ester were between 0.04 and 0.70 mM during the perfusion and T_{max} was reached after 40 and 50 min, respectively (Table III). The concentration of cholesterol was higher than that of cholesterol ester and also than the calculated *in vivo* concentration in the perfusate (Fig. 5b).

DISCUSSION

In the clinical study being presented here we investigated the dynamic intestinal and biliary secretion in response to the luminal presence of a liquid meal. This is the first step in our strategy to develop more *in vivo* like *in vitro* dissolution methods for low solubility compounds (belonging to the BCS classes II and IV) to simulate the dynamic physiology in the small intestine. By using this *in vivo* single-pass jejunal perfusion approach, we were able to examine the net changes of relevant lipid components over time as a consequence of secretion, enzymatic degradation, and absorption. These are novel data, as earlier clinical data were obtained with an open perfusion technique with a significantly lower level of recovery (29). The current single-balloon technique has a high level of recovery (101.5 ± 4.8%) and enables continuous sampling to take place. In addition, the analyses were performed using novel and improved assay methods where separate lipid components were quantified with high precision (Persson *et al.*, in preparation).

The water transport and the flow rate of the fluid leaving the segment in this study were three and two times higher, respectively, than determined in earlier human intubation studies with drug solutions (11). This is in accordance with earlier reports where the dilution in the intestine had been approximated to be about two to three times (30). Hence, the luminal concentration of the lipid components and drugs *in vivo* in the fed state are expected to be diluted, compared with those in the fasted state. Knowing that there is a certain degree of dilution in the fed state is valuable when trying to predict the local concentration–time profile in the intestinal tract, because it has an impact on the carrier-mediated transport/metabolism and potential drug–drug interactions and specific food–drug interactions at the intestinal level.

It was previously shown that at least 10 g of fat is required to stimulate release of bile and that 25 g will induce the maximum contraction of the gall bladder *in vivo* in humans (31). Normally, the gall bladder empties to approximately 75% after food intake (32). The lipid content in a standard breakfast (FDA Office of Generic Drugs) is 27 g. The total fat content in the perfusate entering the jejunal segment in the present study was 7.2 g, which is below the amount needed to trigger an extensive contraction of the gall bladder. Despite this, we observed a significant increase in the concentration of bile acids, cholesterol, and phospholipids (all of which are components of human bile) 20 min after the start of the perfusion (Fig. 4a). This indicates that there was a rapid *in vivo* response to the liquid meal, leading to a fast release of bile from the gall bladder into the sampling segment in the proximal small intestine. Using the length and the inner diameter of the perfusion tube, we approximated the time for the perfusate to reach the intestine to be 5–10 min, which means that the real response *in vivo* would have been about 10 min. A second increase in the concentrations was seen after 80 min (Fig. 4), which suggests a second contraction of the gall bladder. These new *in vivo* data clearly indicate that a lower amount of lipids in the intestinal lumen might be sufficient to stimulate an *in vivo* response. It was also an important observation that one subject did not secrete any bile acids at all. This lack of response in fed state should be better described because it certainly affects the interindivdual variability in absorption for poorly dissolved drugs.

It is important for the kinetics of the dissolution enhancement to better characterize the *in vivo* relevant concentration time profile of each bile acid found in the human intestine. In this investigation, we determined the concentration of six different bile acids during the perfusion. The two dominant bile acids were glyco- and taurocholic acid, which agreed with previous results from studies in fasted state HIF (33). The luminal C_{max} values for these two bile acids were 1.5 and 2 mM, respectively. The overall concentration of bile acids was in accordance with earlier data where the concentration was approximately 9 mM after intake of food (4,34). In a study by Yao *et al.* (35), the maximum concentration of total bile acids was about 80 mM, and it was reached 20 min after administration of a liquid meal (33.6 g fat, 123 mg cholesterol, 3.7 g protein, and 13.6 g carbohydrates) to normal subjects through a nasogastric tube. The concentration–time profile was similar, but without the second increase in concentration, which suggests that the gall bladder had already been completely emptied at the first exposure to the liquid meal (35). The nutritional drink administered in our study contained a lower total amount of fat and proteins, which might explain the slower, lower, and incomplete release of bile acids from the gall bladder. The concentration profile of bile acids differs significantly in the aspirated intestinal fluids and from those applied in the reported artificial dissolution media. In the aspirated fluids, the concentrations of bile acids are low at the beginning of the perfusion and an increase is observed when bile acids are secreted in the postprandial state (22,36). In contrast, in *in vitro* lipolysis models, the concentration of bile acids has its maximum value early on and then decreases with time. This might affect the compositions of lipids in the colloidal phases formed. As a consequence of our observation, we suggest that bile acids should be added to *in vitro* digestion and dissolution models so that the kinetics better reflect the complex *in vivo* processes. Since glyco- and taurocholic acid are present in almost the same concentration in human intestinal fluid, either one could be used as a model bile acid in artificial intestinal media as long as the chosen acid is equally potent as a dissolution-enhancing agent.

The only phospholipids found in the small intestinal fluid in humans were phosphatidylcholine and lysophosphatidylcholine (Fig. 4b). Both of these have been shown to be able to increase the solubility of poorly soluble drugs, but no comparative studies seem to have been published that reveal a difference in the solubilization capacity (7,37). The concentration of phosphatidylcholine in the intestinal fluid increases 10 min after start of perfusion and reaches a steady-state concentration in the outlet at 0.5 mM after 40 min (Fig. 4b).

The concentration–time profile for lysophosphatidylcholine during the perfusion is similar to that of the bile acids (Fig. 4a). Thus, it seems that phosphatidylcholine is rapidly degraded to lysophosphatidylcholine and fatty acids. Lysophosphatidylcholine also seems to be absorbed very quickly because the concentration at 20 min, when the perfusate should have reached the intestine, is lower than the concentration of phosphatidylcholine in the inlet perfusate (Fig. 4b). Earlier studies have shown that lysophosphatidylcholine seems to be present at low concentrations in human bile and in high concentrations in the contents of the duodenal region of the small intestinal lumen (38,39). Borgström has reported that following a test meal free of phospholipids, most of the phospholipids in intestinal chyme were in the form of lysophosphatidylcholine (39). The total concentration of phospholipids in the intestinal fluid agreed with that found in earlier reports (40). In clinical studies where a meal with a higher fat load was given, the concentration of phospholipids was higher, probably owing to increased secretion from the gall bladder (35). Thus, the primary phospholipid in human intestinal fluid is lysophosphatidylcholine, rather than phosphatidylcholine, which is included in artificial fluids. Further studies are needed to examine whether there is any difference in the solubilization capacity of low solubility drugs with different chemical structure between these two phospholipids.

The ratio of bile acids to phospholipids obtained in this study was 6:1 at the start of the perfusion and 1:1–2.7:1 after contraction of the gall bladder (Fig. 6). This is in agreement with the results obtained by Schersten, where the ratio measured from the release of bile acids and phosphatidylcholine from the gall bladder varied between 5:1 in the beginning when the experiment commenced and then decreased to 2:1 by interruption of enterohepatic circulation and duodenal refeeding of bile acids during interrupted enterohepatic circulation (41,42). The ratio of bile acids to phospholipids in the fed state was also similar to artificial intestinal fluids (13,17).

The lipase inhibitor, orlistat, was added to the HIF in the sample tube to inhibit the degradation of triglycerides by gastric and pancreatic lipases. In earlier studies of the physicochemical characteristics of lipid digestion, the inhibition of degradation was most commonly achieved by heating the intestinal fluids to 70°C (34,40,43–48). However, heating intestinal fluid to this temperature may affect the stability of the lipid composition. The concentrations of intact triglycerides in this study were surprisingly low in the intestinal fluid compared to the concentration in the NuTRIflex. This might, as for phosphatidylcholine, be due to rapid hydrolyses of the triglycerides. A low concentration of triglycerides at the start of the perfusion has also been observed in earlier human studies and an *in vitro* lipolysis study with simulated intestinal fluids has also reported rapid degradation of triglycerides (35,36). The increase in the concentration of free fatty acids early in the perfusion correlates very well with the formation of lysophosphatidylcholine and mono- and diglycerides (Figs. 4b and 5a) and is, therefore, believed to be the result of the degradation of both phosphatidylcholine and triglycerides. The concentration of triglycerides, diglycerides, monoglycerides, and free fatty acids reached a steady-state level after approximately 30 min (Fig. 5a). The ratio of these for the steady-state level was 1:1:1:6. This is in agreement with other studies where the ratios were approximately 1:1:1:5–10 (45). The importance of incorporating lipolytic products (free fatty acids and monoglycerides) into bile salt micelles for solubilization has been shown with probucol and danazol in *in vitro* lipolysis models (5) and in studies with human intestinal fluid (49). The static artificial media in use today lack the addition of these dietary lipids. The human *in vivo* study reported here shows that there is a rapid degradation of triglycerides in the intestinal lumen, resulting in diglycerides, monoglycerides, and free fatty acids, and that inclusion of monoglycerides and free fatty acids in artificial media should be approximately in the ratio 1:6.

The concentration–time profile of cholesterol and cholesterol ester (Fig. 5b) correlates with that for other bile

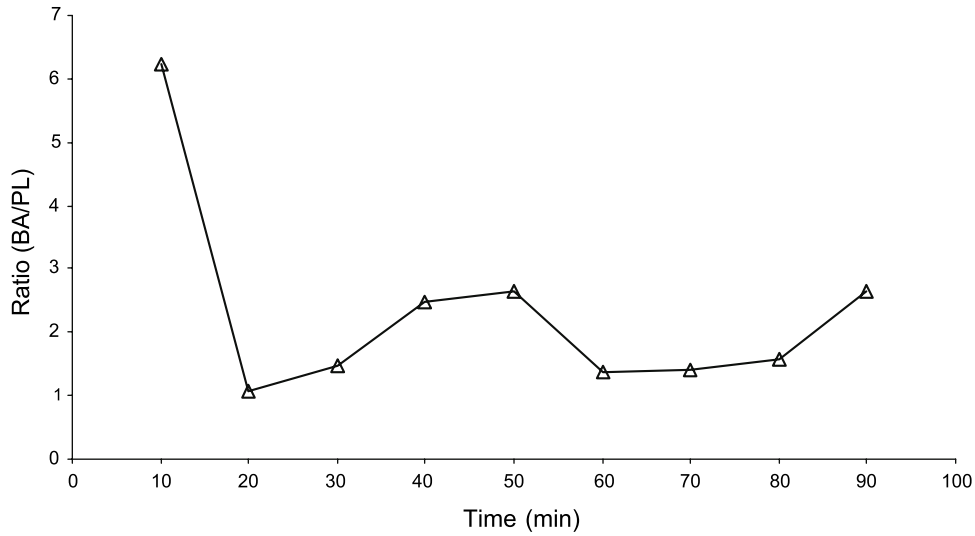

Fig. 6. Ratio of bile acids to phospholipids in intestinal fluids 10–90 min after start of the perfusion with a nutritional drink ($n = 5$). The ratio increases upon secretion of bile and follows the same profile as the bile acids.

components. The degradation of cholesterol was not as extensive as the degradation of triglycerides and phosphatidylcholine, as the concentrations of cholesterol esters were low throughout the whole *in vivo* jejunal perfusion. The concentration of cholesterol was higher than the theoretical concentration derived from NuTRIflex. This also indicates a release of bile from the gallbladder. Cholesterol is incorporated into mixed micelles in the small intestine, but its importance for solubilization of poorly soluble drugs have not been studied. It is evident from this study that inclusion of cholesterols in artificial media should be in the form of cholesterol rather than the degradation product, as the concentration of cholesterol was much higher throughout the perfusion.

CONCLUSION

Gall bladder contraction can be stimulated by a lower amount of dietary fat than had earlier been believed to be the case, but with a lower output. The composition of fluids in the small intestine changes over time with the progress of lipolysis and bile secretion. Most of the lipids are found primarily in the form of their degradation products when sampled *in vivo* in human jejunum. The dominant phospholipid is lysophosphatidylcholine rather than phosphatidylcholine. This degradation product should, therefore, be included in artificial media together with the addition of tauro- or glycocholic acid in a ratio of 1:2. The demonstrated importance of lipolytic products suggests that monoglycerides and free fatty acids in a ratio of 1:6 should be incorporated into an artificial media. These *in vivo* findings based on direct *in vivo* intestinal sampling are relevant for reevaluation and the development of novel physiological based *in vitro* drug-dissolution test media. Further studies using the same methodology are needed to determine the effect of different lipid content in the test meal on the output of bile.

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REFERENCES

1. G. L. Amidon, H. Lennernas, V. P. Shah, and J. R. Crison. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res.* **12**(3):413–420 (1995).
2. A. M. Kaukonen, B. J. Boyd, C. J. H. Porter, and W. N. Charman. Drug solubilization behaviour during *in vitro* digestion of simple triglyceride lipid solution formulations. *Pharm. Res.* **21**(2):245–253 (2004).
3. A. M. Kaukonen, B. J. Boyd, W. N. Charman, and C. J. H. Porter. Drug solubilization behaviour during *in vitro* digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. *Pharm. Res.* **21**:254–260 (2004).
4. K. J. MacGregor, J. K. Embleton, J. E. Lacy, A. P. Perry, L. J. Solomon, H. Seager, and C. W. Pouton. Influence of lipolysis on drug absorption from the gastrointestinal tract. *Adv. Drug Deliv. Rev.* **25**:33–46 (1997).
5. N. H. Zangenberg, A. Müllerz, H. Gjelstrup Kristensen, and L. Hovgaard. A dynamic *in vitro* lipolysis model II: evaluation of the model. *Eur. J. Pharm. Sci.* **14**:237–244 (2001).
6. S. M. Caliph, W. N. Charman, and C. J. Porter. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and the assessment of mass balance in lymph-cannulated and non-cannulated rats. *J. Pharm. Sci.* **89**:1073–1084 (2000).
7. T. R. Bates and P. J. Carrigan. Apparent absorption kinetics of micronized griseofulvin after its oral administration on single and multiple-dose regiments to rats as a corn oil-in-water emulsion and aqueous suspension. *J. Pharm. Sci.* **64**:1475–1481 (1975).
8. W. N. Charman, M. C. Rogge, A. W. Boddy, and B. M. Berger. Effect of food and a monoglyceride emulsion formulation on danazol bioavailability. *J. Clin. Pharmacol.* **33**:381–386 (1993).
9. J. S. Patton and M. C. Carey. Watching fat digestion. *Science* **204**:145–148 (1979).
10. J. S. Patton, R. D. Vetter, M. Hamosh, B. Borgström, M. B. Lindström, and M. C. Carey. The light microscopy of triglyceride digestion. *Food Microstruct.* **4**:29–41 (1985).
11. O. Hernell, J. E. Staggers, and M. C. Carey. Physical–chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analyses and aggregation states of luminal lipids during duodenal fat digestion in healthy adult beings. *Biochemistry* **29**:2041–2056 (1990).
12. L. J. Naylor, V. Bakatselou, N. Rodríguez-Hornedo, N. D. Weiner, and J. B. Dressman. Dissolution of steroids in bile salt solutions is modified by the presence of lecithin. *Eur. J. Pharm. Biopharm.* **41**(6):346–353 (1995).
13. E. Galia, E. Nicolaides, D. Hörter, R. Löbenberg, C. Reppas, and J. B. Dressman. Evaluation of various dissolution media for predicting *in vivo* performance of class I and II drugs. *Pharm. Res.* **15**(5):698–705 (1998).
14. J. B. Dressman, G. L. Amidon, C. Reppas, and V. P. Shah. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharm. Res.* **15**(1):11–22 (1998).
15. J. B. Dressman and C. Reppas. *In vitro–in vivo* correlations for lipophilic, water-soluble drugs. *Eur. J. Pharm. Sci.* **11**(2) S73–S80 (2000).
16. B. L. Pedersen, H. Brondsted, H. Lennernäs, F. N. Christensen, A. Müllertz, and H. G. Kristensen. Dissolution of hydrocortisone in human and simulated intestinal fluids. *Pharm. Res.* **17**(2):183–189 (2000).
17. B. L. Pedersen, H. A. Müllertz, H. Brondsted, and H. G. Kristensen. A comparison of the solubility of danazol in human and simulated gastrointestinal fluids. *Pharm. Res.* **17**(7):891–894 (2000).
18. L. Bönlökke, L. Hovgaard, H. G. Kristensen, L. Knutson, A. Lindahl, and H. Lennernäs. A comparison between direct determination of *in vivo* dissolution and the deconvolution technique in humans. *Eur. J. Pharm. Sci.* **8**:19–27 (1999).
19. J. E. Staggers, O. Hernell, R. J. Stafford, and M. C. Carey. Physical–chemical behaviour of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behaviour and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. *Biochemistry* **29**:2028–2040 (1990).
20. L. Sek, C. J. H. Porter, and W. N. Charman. Characterisation and quantification of medium chain and long chain triglycerides and their *in vitro* digestion products by HPTLC coupled with *in situ* densitometric analysis. *J. Pharm. Biomed. Anal.* **25**:651–661 (2001).
21. L. Sek, C. J. H. Porter, A. M. Kaukonen, and W. N. Charman. Evaluation of the *in vitro* digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *J. Pharm. Pharmacol.* **54**:29–41 (2002).
22. N. H. Zangenberg, A. Mullerz, H. G. Kristensen, and L. Hovgaard. A dynamic *in vitro* lipolysis model: I. Controlling the rate of lipolysis by continuous addition of calcium. *Eur. J. Pharm. Sci.* **14**:115–122 (2001).
23. L. Knutson, B. Odlind, and R. Hallgren. A new technique for segmental jejunal perfusion in man. *Am. J. Gastroenterol.* **84**:1278–1284 (1989).
24. H. Lennernäs, Ö. Ahrenstedt, R. Hällgren, L. Knutson, M. Ryde, and L. K. Paalzow. Regional jejunal perfusion, a new *in vivo* approach to study oral drug absorption in man. *Pharm. Res.* **9**:1243–1251 (1992).

25. P. Hadvary, H. Lengsfeld, and H. Wolfer. Inhibition of pancreatic lipase *in vitro* by the covalent inhibitor tetrahydrolipstatin. *Biochem. J.* **256**(2):357–361 (1988).
26. P. Hadvary, W. Sidler, W. Meister, W. Vetter, and H. Wolfer. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active serine of pancreatic lipase. *J. Biol. Chem.* **266**(4):2021–2027 (1991).
27. Y. Shi and P. Burn. Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. *Nat. Rev.* **3**:695–710 (2004).
28. H. Lennernäs, I. D. Lee, U. Fagerholm, and G. L. Amidon. A residence-time distribution analysis of the hydrodynamics within the intestine in man during a regional single-pass perfusion with Loc-I-Gut: *in-vivo* permeability estimation. *J. Pharm. Pharmacol.* **49**(7):682–686 (1997).
29. H. Lennernäs. Human intestinal permeability. *J. Pharm. Sci.* **87**(4):403–410 (1998).
30. J. S. Fordtran and T. W. Locklear. Ionic constituents and osmolality of gastric and small intestinal fluids after eating. *Am. J. Dig. Dis.* **11**:503–521 (1966).
31. F. Froehlich, J. J. Gonvers, and M. Fried. Role of nutrient fat and cholecystokinin in regulation of gallbladder emptying in man. *Dig. Dis. Sci.* **40**(3):529–533 (1995).
32. E. A. Shaffer. Review article: control of gall-bladder motor function. *Aliment. Pharmacol. Ther.* **14**:2–8 (2000).
33. E. S. Kostewicz, A. S. Carlsson, G. Hanisch, K. Krumkühler, R. G. Nilsson, L. Löfgren, and B. Abrahamsson. Comparison of dog and human intestinal fluid and its impact on solubility estimations. *Eur. J. Pharm. Sci.* **17**(1):S1–S148 (2002).
34. M. C. Mansbach, R. S. Cohen, and P. B. Leff. Isolation and properties of the mixed lipid micelles present in intestinal content during fat digestion in man. *J. Clin. Invest.* **56**:781–791 (1975).
35. L. Yao, J. E. Heubi, D. D. Buckley, H. Fierra, K. D. R. Setchell, N. A. Granholm, P. Tso, D. Y. Hui, and L. A. Woollett. Separation of micelles and vesicles with luminal aspirates from healthy humans: solubilization of cholesterol after a meal. *J. Lipid Res.* **43**:654–660 (2002).
36. J. O. Christensen, K. Schultz, B. Mollgaard, H. G. Kristensen, and A. Müllertz. Solubilisation of poorly soluble drugs during *in vitro* lipolysis of medium- and longchain triacylglycerols. *Eur. J. Pharm. Sci.* **23**:287–296 (2004).
37. I. W. Kellaway and L. Saunders. The solubilization of some steroids by phosphatidyl choline and lysophosphatidyl choline. *Biochim. Biophys. Acta* **144**:145–148 (1967).
38. H. L. Spitzer, E. C. Kyriakides, and J. A. Balint. Biliary phospholipids in various species. *Nature* **17**:204–288 (1964).
39. B. Borgström. Studies of the phospholipids of human bile and small intestinal content. *Acta Chem. Scand.* **11**:749 (1957).
40. H. P. Porter, D. R. Saunders, G. T. Tytgat, O. Brunser, and C. E. Rubin. Fat absorption in bile fistula man. A morphological and biochemical study. *Gastroenterology* **60**(6):1008–1019 (1971).
41. T. Schersten. Formation of lithogenic bile in man. *Digestion* **9**(6):540–553 (1973).
42. T. Schersten. Bile acids as a determining factor for synthesis and excretion of human bile lecithin. *Helv. Med. Acta* **37**(2):161–168 (1973).
43. A. F. Hofmann and B. Borgström. Physico-chemical state of lipids in intestinal content during their digestion and absorption. *Fed. Proc.* **21**:43–50 (1962).
44. A. F. Hofmann and B. Borgström. The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. *J. Clin. Invest.* **43**:247–257 (1964).
45. C. Ricour and J. Rey. Study of the oil and micellar phases during digestion in the normal child. *Rev. Eur. Étud. Clin. Biol.* **15**:287–293 (1970).
46. G. R. Thompson, J. Barrowman, L. Gutierrez, and R. H. Dowling. Action of neomycin on the intraluminal phase of lipid absorption. *J. Clin. Invest.* **50**:319–323 (1971).
47. T. A. Miettinen and M. Siurala. Bile salts, sterols, sterol esters, glycerides and fatty acids in micellar and oil phases of intestinal contents during fat digestion in man. *Z. Klin. Chem. Klin. Biochem.* **9**:47–52 (1971).
48. A. F. Hofmann and A. Roda. Physicochemical properties of bile acids and their relationship to biological properties: an overview of the problem. *J. Lipid Res.* **25**:1477–1489 (1984).
49. E. M. Persson, A. Gustafsson, A. S. Carlsson, R. G. Nilsson, L. Knutson, P. Forsell, G. Hanisch, H. Lennernäs, and B. Abrahamsson. The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. *Pharm. Res.* **22**(12):2141–2151 (2005).