In vitro simulation of the environment in the upper gastrointestinal lumen after drug administration in the fed state using the TIM-1 system and comparison with luminal data in adults

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ABSTRACT

We evaluated the environment in TIM-1 luminal compartments using paracetamol and danazol solutions and suspensions and the fed state configuration. Data were compared with recently published data in healthy adults. TIM-1 Experiments were performed with a 3-fold downscale. Volumes of secretions in gastric and duodenal compartments adequately reflected the luminal data in adults up to 3h post drug dosing. pH values in duodenal and jejunal compartments adequately reflected average pH values in adults. In gastric compartment pH values where initially higher than average values in adults and reached baseline levels earlier than in adults. The environment in the TIM-1 gastric compartment and jejunal compartment adequately reflected the average total paracetamol and danazol amounts per volume of contents in the adult stomach and upper small intestine, respectively. Total bile acids concentrations in the micellar phase of contents in duodenal and jejunal compartments overestimated micellar concentrations in the upper small intestine of adults. Adjustments in gastric emptying / acid secretion rates and bile acids identities in the duodenal and jejunal compartments, and application of dynamic bile acids secretion rates are expected to further improve the relevance of luminal conditions in TIM-1 compartments with those in adults.

KEYWORDS

1. INTRODUCTION

TIM-1 is a multi-compartmental dynamic model developed in the early 1990s as a physiologically relevant digestion model in food sciences (Minekus et al., 1995; Minekus et al., 2005). The TIM-1 system mimics the upper and middle gastrointestinal (GI) tract, comprising four serial compartments simulating the stomach, duodenum, jejunum and ileum. It also simulates gastric, biliary and pancreatic secretions. Herewith, lipid digestion products are removed for absorption via filtration from the jejunum and ileum compartments (Reis et al., 2008; Helbig et al., 2013). The TIM-1 system is also used in the pharmaceutical field for evaluating the impact of formulation and/or dosing conditions on oral drug absorption, after administration of immediate release products both in the fasted state (Barker et al., 2014; Dickinson et al., 2012; Souliman et al., 2007; Souliman et al., 2006; Verwei et al., 2016) and in the fed state (Blanquet et al., 2004; Lloyd et al., 2020; Souliman et al., 2006; Verwei et al., 2016). In all relevant studies, the water (fasted state) and the texture and composition of the meal (fed state) recommended by regulatory agencies to be used in association with the administration of the dose units (EMA, 2010; FDA, 2019), is simulated.

The majority of reported TIM-1 studies using drug products focus on API bioaccessibility. The number of published comparisons between luminal conditions in TIM-1 versus in vivo studies is limited, both, for the fed state (Brouwers et al., 2011; Hens et al., 2014; Van Den Abeele et al., 2017) and the fasted state (Hens et al., 2014). For the fed state, especially, investigations to date have involved comparisons with luminal data collected after co-administration of liquid meals with the drug products (Brouwers et al., 2011; Hens et al., 2014; Van Den Abeele et al., 2017).

The present investigation had two objectives:

1. To evaluate the conditions in TIM-1 gastric, duodenal and jejunal compartments using solutions and suspensions of two highly permeable drugs, paracetamol and danazol, in the gastric compartment and the fed state configuration by employing a solid, high fat meal that simulates texture and composition of the meal recommended by regulatory agencies (EMA, 2010; FDA, 2019).

2. To compare TIM-1 data with those collected recently in young healthy adults, after administration of similar paracetamol and danazol solutions and suspensions into the antrum, i.e. under conditions simulating the situation after intake of the meal recommended by regulatory agencies and after disintegration of immediate release tablets and capsules (Pentafragka et al., 2020b; Pentafragka et al., 2020a).

Typically, immediate release dose units ingested under the specific fed state conditions could empty from stomach only after their disintegration, a process which can be substantially delayed (Kelly et al., 2003; Rubbens et al., 2019). It should be clarified that both in the present in vitro study and in the recently published human study paracetamol and danazol were dosed as solutions and suspensions in the gastric compartment / antrum of the stomach to represent situations after non-substantially delayed intragastric disintegration of immediate release products, i.e., no drug products were used.

2. MATERIALS AND METHODS

2.1. Materials

Paracetamol for human use was from Lianyungang Kangle Pharmaceutical Co., Ltd, China (micronized, Batch No. CW-1704010M). Danazol for human use was from Coral Drugs Pvt. Limited, India (micronized, Batch No. 5201-B-17009).

Pancreatin from porcine pancreas, pepsin from porcine gastric mucosa, lipase from *Rhizopus oryzae*, amylase from *Bacillus sp.*, and trypsin were from Sigma-Aldrich, Gillingham, UK. Porcine bile was from Triskelion (Vion, Apeldoorn, Netherlands). Phenol red (phenolsulfophthalein sodium salt) was from Sigma-Aldrich Chemie GmbH. All other chemicals were of analytical grade from Sigma Aldrich Chemie GmbH or from E. Merck (Germany).

Gastric electrolyte solution (GES) was prepared by dissolving 8 g/L sodium chloride, 1.7 g/L potassium chloride and 0.16 g/L calcium chloride di-hydrate in water. HPMC 0.4% and bile 0.04% gastric solution was prepared by dissolving 0.4 g/L bile extract in water, subsequently adding 4.0 g/L HPMC and stirring the solution overnight. Gastric enzymes solution contained 3 mL of 0.1 M sodium acetate buffer pH 5.0, 6000 FIP (Fédération Internationale Pharmaceutique Unit) units/cup for 300mL of lipase from Rhizopus oryzae as an alternative to gastric lipase, 1,440,000 units/cup for 300mL of pepsin, 14,000 units/cup for 300mL of amylase and 299 mL GES. The required amount/cup was estimated using the following equation:

$$\text{Amount(mg/cup)} = \frac{\text{Necessary units } (units/cup)}{\text{Activity } (units/mg)} \qquad (1)$$

Small intestinal electrolyte solution (SIES) was prepared by dissolving 7 g/L sodium chloride, 0.35 g/L potassium chloride and 0.1 g/L calcium chloride di-hydrate in water and adjusting the pH to 7.0 with 1 M sodium hydroxide. 17.5 g of USP Pancreatin was dispersed in 237mL MilliQ water and after centrifugation (12.500 g, 4°C, 20 min) the supernatant was used for the experiment. The bile solution consisted of 100% v/v filtered porcine bile.

The high-calorie, high-fat meal employed in TIM-1 experiments was based on the standard meal composition (FDA, 2019) and consisted of toasted white bread with margarine, milk, cooked bacon, eggs, and potatoes (165 kcal/100 g), i.e. the only difference from the meal employed in the clinical study was the presence of sunflower oil instead of margarine; liquid and fat fractions were roughly similar.

2.2 Experiments with TIM-1

A schematic representation of the TIM-1 system is given in Figure 1. The system consists of four serial compartments simulating the stomach, duodenum, jejunum and ileum. Each compartment consists of two identical connected units each with a glass jacket and flexible silicone walls inside. Mixing of the contents and temperature control is achieved by circulating water around the flexible walls. Peristaltic valve pumps connect the compartments and control the longitudinal transit of contents. The volume of contents is controlled by level sensors. A pre-set pH curve for each compartment is monitored with pH probes. pH is regulated with secretion of 1M hydrochloric acid (gastric compartment) or 1M sodium bicarbonate (intestinal compartments). Solubilized drug and digestion products are continuously removed through lipid filters (Fresenius, polysulfone based Plasmaflux P1/dry filters) applied to the jejunal and ileal compartments.

Prior to the performance of each experiment the secretions fluids (e.g., gastric juice with enzymes, electrolytes, porcine bile and pancreatic juice) were freshly prepared, the pH electrodes were calibrated, and filter units were installed. Forty-five milli-litres of tap water, 70 g GES, and 10 g gastric start residue (enzymes, HPMC 0.4% and bile 0.04% solution) were mixed and added to the gastric compartment. In the experiments performed at Pfizer, instead of 45mL water, 15mL aqueous solution of 0.2 % phenol red and 30 mL water were added in the gastric compartment. The duodenal compartment was filled with 60 g of a duodenal start residue consisting of 22.5 g SIES, 22.5 g pancreatin solution, 45 g bile solution and 2 mg trypsin in 1 mL SIES. The jejunum compartment was filled with a mixture of 35 g SIES, 35 g pancreatin solution and 70 g bile solution (jejunal start residue). The ileum compartment was filled with 140 g SIES.

Due to the fact that total gastric intake at drug administration time in TIM-1 studies is 300 g and in studies in adults is ~782 mL [volume of gastric contents approx. similar to meal volume, 532 mL, water with the drugs 250mL (Pentafragka et al. 2020a)] the doses of paracetamol and danazol were scaled down to 30% of the doses administered in the recent clinical study (Pentafragka et al., 2020a).

No modifications with regards to secretions or transit processes were made and the default fed state configuration was applied.

Meal mastication was simulated with a food processor (Bellmann et al., 2016) and mixed with artificial salivary fluid containing electrolytes and alpha-amylase. Prior to the start of the TIM-1 experiment, the warm meal (37 °C) was added to the gastric compartment followed by administering the drug. The total volume of contents in the gastric compartment at t=0 was 300 mL, i.e. up to 34% of the total volume of gastric contents in the clinical study within half

hour (approx. 870 mL: 500 mL of meal, 250 ml of water with the drug, approx. 30 mL resting gastric volume, and approx. 90-120 mL salivary and gastric secretion and (Pentafragka et al., 2020a).

Each experiment was performed by using two drug solutions and two drug suspensions, as in the recent clinical study (Pentafragka et al. 2020). In experiments with drug solutions, paracetamol solution was prepared by dissolving 150 mg in 12 mL tap water, and 8 mL was used to rinse the beaker. Danazol solution was prepared by dissolving 30 mg in 6 mL sunflower oil. In experiments with drug suspensions, 150 mg of paracetamol was suspended in 6 mL of water with an additional 6 mL of water as rinse. And 30 mg of danazol were suspended in 6 mL water with 8 mL of water used as rinse. Drug solutions and suspensions were prepared a few minutes prior to the start of each experiment.

Samples from the gastric, duodenal, and jejunal compartments were collected at specific time intervals, as shown in Figure 2. To simulate the housekeeper wave, at 180 min, remaining contents of the gastric compartment were manually collected, the gastric compartment was rinsed with 30 mL of the duodenal start residue, which was subsequently mixed with the gastric residue. The pH of the mixture was adjusted to the pH of the duodenal compartment with sodium bicarbonate, and the mixture was transferred into to the duodenum compartment. Therefore, the last sampling from the gastric compartment was obtained at 180 min.

Experiments were performed at Pfizer, UK, and at The TIM Company, The Netherlands. At both sites, paracetamol solution and suspension experiments were performed in duplicate i.e., four experiments in total per site. Danazol experiments were performed at The TIM Company site only.

Three mL-samples were collected from the gastric, duodenum and jejunum compartments. The pH, phenol red concentration and total paracetamol amount per volume were measured in each sample. In two experiments 15mL-samples were collected from the duodenal compartment and in the other two experiments 15mL-samples were collected from the jejunal compartment. These samples were treated as follows: 200 μl of each sample were transferred in two vials for assaying phenol red concentration and the total paracetamol amount per volume. The remaining volume, after adding a cocktail of lipase/protease inhibitors (50 mM diisopropylfluorophosphate, 50 mM diethyl(p-nitrophenyl) phosphate, 50 mM acetophenone, and 250 mM phenylboronic acid at 2% v/v), was divided into two sub-samples. The first was stored at -20°C for measuring individual bile acids concentrations in the contents of duodenal and jejunal compartments. The second was immediately centrifuged (11000g, 37°C, 10 min) and, subsequently, ultracentrifuged (410174 g, 37°C, 2 h) to obtain the micellar phase (as in the clinical study, (Pentafragka et al., 2020a)) of contents in the duodenal and jejunal compartments, to measure in which individual bile acids concentrations.

At The TIM Company, phenol red was not included in the meal; also bile acids were not measured. One mL-samples, were collected, pH was measured and the total paracetamol amount per volume and total danazol amount per volume was analysed.

2.3 Sample Analysis

Phenol red, paracetamol and danazol were assayed as described recently in human aspirates (Pentafragka et al., 2020a). Phenol red in the contents of jejunal compartment was not assayed as it is removed from this compartment through the filters and, therefore, cannot be used as water-flux indicator in this compartment.

Bile acid content was quantified in the porcine bile, in the duodenal and the jejunal samples, and in the micellar phase of the samples collected from the duodenal and jejunal compartments. Glycochenodeoxycholic acid (GCDC), taurocholic acid (TC), taurochenodeoxycholic acid (TCDC), glycodeoxycholic acid (GDC), glycocholic acid (GC), ursodeoxycholic acid (UDC), glycohyodeoxycholic acid (GHDC) and glycohyocholic acid (GHC) were individually quantified as described previously (Vertzoni et al., 2008) and total bile acid concentrations were calculated.

2.4. Data presentation and treatment

In all figures TIM-1 data are presented with lines in comparison with data collected in the recent clinical study (Pentafragka et al. 2020a; Pentafragka et al. 2020b). Human data are presented as boxplots showing the median value, the 10th, 25th, 75th, and 90th percentiles and the individual outlying data points. The number of individual data points used for the construction of a box is indicated on top of the box. Within each box, horizontal dotted lines indicate mean values.

Data from both the duodenal- and jejunal compartments of TIM-1 are discussed versus the same data from human aspirates collected from the upper small intestine near the ligament of Treitz (Pentafragka et al. 2020a).

For both TIM-1 and human data, time zero (t=0) designates the time of drug administration, i.e. immediately after bringing the meal in the gastric compartment (TIM-1 data) and 30 min after initiation of meal consumption (adult data).

3. RESULTS AND DISCUSSION

3.1 Water-flux in TIM-1 gastric and duodenal compartments

In the gastric compartment, phenol red concentrations slightly decrease over time and the intra-laboratory variability was low. This decrease can be explained by secretion influx in parallel to gastric emptying, resulting in an overall dilution of the phenol red concentration in the gastric compartment. (Figure 3A).

In the duodenal compartment, data up to 2h adequately reflect the adult data (Figure 3B) in the upper small intestine, indicating that the net result of water-flux, input from stomach and output to the jejunum is adequately simulated in TIM-1 system. After 2h TIM-1 data slightly underestimate adult data (Figure 3B), most likely due to faster gastric emptying in TIM-1 (180 min), compared with data in adults (Pentafragka et al. 2020b and references therein).

3.2 Drug Disposition in TIM-1 compartments

At Pfizer, paracetamol average recovery (% of intake) was 96% for the solution experiments and 95% in the suspension experiments.
At The TIM Company, paracetamol average recovery (% of intake) was 98% for the solution experiments and 107% for the suspension experiments. Danazol average recovery was 84% for the solution experiments and 65% for the suspension experiments, indicating a loss of API during the experiment, handling of samples and/or the TIM sample analysis.

3.2.1 Disposition of paracetamol in TIM-1 compartments

Total paracetamol amounts per volume of contents in the gastric compartment declined continuously over time with low intra- and inter-laboratory variability (Figures 4A and 4B). Data of the first timepoint after the administration of paracetamol solution and suspension were similar, as in the recent luminal study in adults (Pentafragka et al. 2020a). Total paracetamol amounts per volume of contents decreased faster than phenol red concentrations (Figures 4A and 4B vs. Figure 3A), in line with data in adults (Pentafragka et al. 2020a).

For the solution, TIM-1 data were in line with data in adults. For the suspension, TIM-1 data over-estimated data in adults at times between 75 and 135 minutes, which may be, at least partly, related to differences in the kinetics of the decline; apparent zero-order for the TIM-1 data (Figures 4A and 4B) and apparent first-order of the adult data (Pentafragka et al., 2020a). In adults, the first sample after dosing of the solution or the suspension showed high variability (Figures 4A and 4B). Average values were slightly higher than theoretically expected, i.e. higher than about 656 µg/mL (Pentafragka et al. 2020a). This observation may reflect inhomogeneous distribution of paracetamol early after administration. In contrast, the average value early after initiation of TIM-1 experiments was similar with the theoretically expected, i.e. 150mg / 300 mL = 500 µg/mL, suggesting a homogenous distribution.

Total amounts per volume of contents in the duodenal compartment (Figure 4C and 4D) and in the jejunal compartment (Figure 4E and 4F) peaked at 60-90 min and at 90-120 min, respectively. In adults, total amounts per volume in the upper small intestine had been estimated to peak earlier than 30 min (Pentafragka et al., 2020a). The discrepancy is attributed to the increased initial concentration of paracetamol in the antrum in vivo (see Fig 4A and 4B) while in TIM-1, the homogenously distributed paracetamol follows the pre-programmed gastric

emptying curve, according to zero-order emptying rate kinetics. The increase and later decrease of paracetamol in the jejunum compartment might also be attributed to the absence of a filtration membrane in the TIM-1 duodenal compartment. In line with these arguments, data collected from the jejunum compartment of TIM-1, where active filtration occurs, show smaller differences from data collected in the luminal study in adults (Figure 4C vs 4E, and 4D vs 4F), i.e. average peak total amounts per volume of contents in the duodenal compartment were 297.06 µg/mL (n=4) at 60 min and 293.85 µg/mL (n=4) at 90 min for the solution and the suspension, respectively, whereas the corresponding values in the jejunal compartment were 206.69 µg/mL (n=3) at 90 min and 148.91 µg/mL (n=4) at 120 min respectively.

Data suggest that application of a filtration membrane in the duodenal compartment of TIM-1, and, perhaps, a differently designed gastric compartment (to better simulate the presence of drug in the gastric compartment over time after initiation of the experiment) would further improve predictability of luminal concentrations of rapidly absorbed drugs in adults. Both suggestions could be immediately applicable, given the existence of the Advanced Gastric Compartment (TIM*agc*) and the tiny-TIM system (Bellmann et al., 2016; Verwei et al., 2016). A preference for the tiny-TIM system when dealing with immediate release products has already been indicated for experiments performed by using both the fasted state and the fed state configurations (Verwei et al., 2016).

3.2.2 Disposition of danazol in TIM-1 compartments

Danazol amounts per volume of contents in the gastric compartment peaked at 135 min for the sunflower oil solution and at 180 min for the suspension (Figures 5A and 5B). Values were in line with data in adults, however, no peaks were apparent in the antrum of adults in the study performed by Pentafragka et al (2020a) (Figures 5A and 5B). Because danazol was added

dissolved in sunflower oil after meal administration, it is likely that this oil layer emptied early due to the semi-supine position of study subjects. In contrast, the TIM-1 pylorus is located at the bottom of the gastric compartment, and therefore the floating danazol – sunflower oil mixture empties later. Consequently, peak values were higher than 100 μg/mL, the nominal total amount per volume under perfect mixing conditions and in absence of secretions (30 mg dose / 300 mL initial volume in gastric compartment). These observations are in line with earlier observations after administration of *liquid* meals in adults according to which gravity has a major effect on the intragastric distribution and relatively little effect on total stomach emptying of oil (Horowitz et al. 1993). The peak was not apparent in the recent luminal study performed with *homogenized solid* meal, these observations may relate to body posture, since semi supine position was used in the in vivo study, which is of special importance for the gastric emptying of non-homogenously mixed contents, such as the administrated sunflower oil (Imai et al 2013).

Total danazol amounts per volume in the duodenal and jejunal compartments from the sunflower oil solution (Figures 4C and 4E) had low intra-laboratory variability. In adults, data variability was higher and no peaks were observed in the upper small intestine. They peaked after the application of the housekeeper wave in the gastric compartment (Figures 5C and 5E). Total amounts per volume in the duodenal and jejunal compartments after the suspension (Figures 5D and 5F) had low intra-laboratory variability. Values were in line with those in the upper intestinal lumen of adults which had also low inter-subject variability (Figures 5D and 5F).

3.3 pH in TIM-1 compartments

In the gastric compartment, pH values were initially higher than those in the antrum of healthy adults and reached baseline levels earlier than in healthy adults (Figure 6A, (Pentafragka et al., 2020b); (Dressman et al., 1990; Koziolek et al., 2015; Rubbens et al., 2019).

Faster return to baseline levels is in line with the smaller meal volume employed, the more homogenously mixed gastric content and "upright" positioning of the TIM-1 gastric compartment compared with the in vivo conditions. Slight adjustment of acid secretion rates in the gastric compartment might also be considered for a closer simulation of luminal conditions in adults. In addition, the faster return to baseline levels might be related to the faster meal gastric emptying rate in TIM-1 ($t_{1/2}$=80 min) compared to the $t_{1/2}$ of 105-120 min observed in vivo (Koziolek et al., 2014).

pH values in the duodenal compartment are generally in line with median pH values in the upper intestinal lumen of healthy adults (Figure 6B, (Pentafragka et al., 2020b). pH values in the jejunal compartment slightly overestimate median pH values data but they remained within the 90th percentile of luminal values at all time points (Figure 6C, (Pentafragka et al., 2020b).

3.4 Bile acids in TIM-1 duodenal and jejunal compartments

Total bile acids in the TIM-1 porcine (bladder) bile were measured to be 26.4 ± 1.8 mM. Bile is continuously secreted in the duodenal compartment at a rate of 0.5 mL/min. In the jejunal compartment an electrolyte solution containing 10% of the bile solution is continuously secreted at a rate of 2.8 mL/min. Half of the bile acid composition in porcine bile (33% GCDC, 7% TCDC and 9% TC) are similar with half of bile acids in the contents of upper small intestine in humans (Figure 7). The second half of bile acids in porcine bile (35% GHDC and 16% GHC) is

composed of different bile acids from the second half of bile acids in the contents of the human upper small intestine (13% GDC, 28% GC and 7% UDC) (Figure 7). However, the ratio of taurine to glycine conjugates is similar in porcine bile and in the contents of upper small intestine of adults. Bile acids identity and percentages in porcine bile used in TIM-1 are in line with previous findings (Effinger et al., 2021).

Bile acids concentrations in the duodenal compartment (Figure 8A) and in the jejunal compartment (Figure 8B) were similar to that in the upper small intestine of adults (Pentafragka et al., 2020b) during the first and second hour, after the beginning of the experiment. After that, bile acids concentrations increased in TIM-1 whereas they decrease in adults (Figures 8A and 8B). This may be a consequence of the constant secretion in TIM-1 even at times when fasted state conditions are apparent in the gastric compartment, i.e. at times when the pH in gastric compartment has reached baseline levels.

Bile acids concentrations in the micellar phase of the duodenal and jejunal compartments in TIM-1 followed a similar pattern with concentrations in total contents; unlike with data in the upper small intestine of adults, they increased with time at time-points longer than 1h and 2h, in the duodenal and the jejunal compartment, respectively (Figure 8A and Figure 8B, bold dotted lines vs. dotted lines).

Concentrations in total contents and in the micellar phase of contents were similar (Figure 8A and Figure 8B, continuous vs. dashed lines). In adults, average concentrations in the micellar phase were lower than average concentrations in total contents (Figure 8A and Figure 8B, lined vs. empty boxes). As a result, average bile acids concentrations in the micellar phase of TIM-1 in the duodenal and the jejunal compartment were higher as compared to the micellar phase found in the upper small intestine of adults after 30 minutes (Figure 8A and 8B, dashed lines vs. empty boxes). This could be attributed, at least partly, to suboptimal simulation of bile acid

secretion rates and to the higher hydrophilicity of the glycohyodeoxycholic acid and hyocholic acid in the porcine bile as compared to glycodeoxycholic and ursodeoxycholic acid in humans (Roda *et al*., 1990). These arguments are in line with the apparent agreement of total amounts per volume of contents in the duodenal and jejunal compartment of TIM-1 and data in upper small intestine of adults for lipophilic danazol (Figures 5C-5E), despite the low total recovery in the TIM-1 experiments, especially with the suspension (Section 3.2).

4. CONCLUDING REMARKS

The present study aimed to compare TIM-1 data with those collected from young healthy adults, after administration of paracetamol and danazol solutions and suspensions during fed state conditions. TIM-1 is a computer controlled dynamic gastrointestinal model, set to simulate one average individual in a highly reproducible way. This is achieved by using controlled mixing, transit, secretions, pH profiles and concentrations of digestive fluids. If we consider the TIM-1 as a virtual twin for the average clinical study volunteer, it is important to recognize that the highly controlled and reproducible gastrointestinal environment which is simulated will describe a narrower physiological design space than that which is represented by a clinical study cohort of human volunteers. Realistically, intra- and inter-individual variability cannot be simulated in their totality with aspects such as variations in body mass, anatomy and the complexity of inter-digestive motility patterns outside the scope of an in vitro gastrointestinal simulator. In addition to these individually derived sources of variability, clinical data are also influenced by further factors linked to the experimental protocol such as control of body posture and movement during the study, method of drug administration and the luminal sampling procedure employed. It was with this context that the current study sought to assess the relevance of fed state conditions in the TIM-1 and to evaluate the general relevance of the in vitro fed-state conditions in terms of physiological simulation and bio-performance of solutions and suspensions that result after the disintegration of products of drugs with different solubility profiles.

The results for drug disposition suggest that the conditions employed to simulate the average fed state in the TIM-1 capture a relevant region of the physiological design space expected in a typical controlled clinical study. It is important to note, that downscaling the dose of single unit solid dosage forms may be problematic and, in case of certain modified release products, not

feasible. For the reasons outlined above, the full range of physiological variation is not fully described but could be addressed using re-parameterized experimental settings. In broad terms, differences between drug disposition in the TIM-1 and the reported clinical study dataset could be attributable to gastric mixing hydrodynamics and relatively small differences in gastric emptying times. The gastric content of TIM-1 is more homogeneously mixed as compared to the human stomach. In vivo, this results in regional pH differences (section 3.3), phase separation (section 3.2.2) and differences in luminal compound concentrations (sections 3.2.1 and 3.2.2). Nevertheless, paracetamol total amount per volume and danazol total amount per volume in both the gastric compartment and the jejunal compartment were generally in line with those in the gastric contents and contents of the upper small intestine of adults, respectively. It should be noted that the TIM-1 system used in the current study was fitted with a horizontal gastric compartment rather than the more anatomically accurate advanced gastric compartment described by Barker et al (Barker et al, 2014).

In addition, the small intestinal samples in TIM-1 were collected from duodenal and jejunal compartments, both simulating average conditions of each small intestinal segment, whereas in vivo sampling was collected over a region of approximately 10 cm around the ligament of Treitz. The results show that the TIM-1 jejunum data are more in line with the in vivo data obtained by sampling near to the ligament of Treitz. The absence of a filtration membrane in the duodenal compartment will be a significant factor in this respect. However, this may have limited relevance for profiling the performance of most formulations as transit across the short anatomical length of the duodenum is rapid with the small intestinal transit time to the mid-jejunum around 20-30mins [Lennernäs, 2014]. For compounds with a very rapid absorption profile then the tiny-TIM system which utilises a single intestinal compartment and filtration unit could be considered.

The constant secretion of bile in the TIM-1 will also be a significant factor although it should be noted that the overall concentrations of bile acids in both the duodenal and jejunal compartments were similar to those observed in the upper small intestine of adults during the first two hours of the experiment [Pentafragka, 2020b]. In terms of measured bile acids, approximately half of the used porcine bladder bile composition compared well with around half of the bile acid composition measured humans. The significance of variations in the remaining pool of bile acids for solubilization of poorly-soluble drugs was not the subject of the current study but a comparison of the colloidal species formed in the TIM-1 to human intestinal fluid aspirates may be useful additional future experiment to consider in this respect.

Finally, the current set of data is a unique comparison between luminal samples of TIM-1 and human aspirates under fed state conditions. Assuming that downscale of the dose is applied, adjustment of gastric emptying / acid secretion rates in gastric compartment that could be addressed by using of TIM advanced gastric compartment, and application of dynamic bile acids are expected to further contribute to the relevance of luminal conditions in TIM-1 compartments with those in adults. This data set provides an opportunity to establish alternative conditions to capture physiologically relevant variability which for some compounds may be particularly important in understanding significant factors for oral drug product performance.

ACKNOWLEDGMENTS

FIGURE CAPTIONS

Figure 1: Schematic representation of the TIM-1 system. (A. gastric compartment; B. pyloric sphincter; C. duodenal compartment; D. peristaltic valve; E. jejunal compartment; F. peristaltic valve; G. ileal compartment; H. ileocecal valve; I. gastric secretion; J. duodenal secretion; K. bicarbonate secretion; L. pre-filter; M. filtration system; N. filtrate with bio-accessible fraction; O. hollow fiber system (cross section); P. pH electrodes; Q. level sensors; R. temperature sensors; S. pressure sensor) (Minekus, 2015).

Figure 2: Schematic representation of the TIM-1 sampling protocol from the contents of the gastric, duodenal and jejunal compartments.

Figure 3: Phenol red total amount per volume of contents in the gastric compartment (A) and duodenal compartment (B) of TIM-1 system (continuous lines, individual data, n=4) vs. data in antral contents (A - box plots) and contents of the upper small intestine of adults (B - box plots); number of individual data points for the construction of each box indicated on top of a box. Data from healthy adults have been extracted from Pentafragka et al (2020a).

Figure 4: Paracetamol total amount per volume of contents in the gastric compartment (A, B), duodenal compartment (C, D), and jejunal compartment (E, F) of TIM-1 system, after administration of aqueous solution (left panel) and aqueous suspension (right panel) in the TIM-1 gastric compartment [individual data from Pfizer (continuous lines) and from The TIM company (dashed lines)] vs. data in antral contents (A, B - box plots) and contents of the upper small intestine of adults (C, D, E, F - box plots); number of individual data points for the construction of each box indicated on top of a box. Data from healthy adults have been extracted from Pentafragka et al (2020a).

Figure 5: Danazol total amounts per volume of contents in the gastric compartment (A, B), duodenal compartment (C, D), and jejunal compartment (E, F) of TIM-1 system, after administration of sunflower oil solution (left panel) and aqueous suspension (right panel) in the TIM-1 gastric compartment [individual data from The TIM Company (continuous lines)] vs. data in antral contents (A, B - box plots) and contents of the upper small intestine of adults (C, D, E, F - box plots); number of individual data points for the construction of each box indicated on top of a box. One outlier in the upper small intestinal human data [2087.5 µg/mL at 30 min] is not shown in the graph (B, C, E, F) to improve visibility. Data from healthy adults have been extracted from Pentafragka et al (2020a).

Figure 6: pH of contents in the gastric compartment (A), duodenal compartment (B) and jejunal compartment (C) of the TIM-1 system (continuous lines, individual data, n=8) vs. pH in antral contents (A - box plots) and contents of the upper small intestine of adults (B - box plots); number of individual data points for the construction of each box indicated on top of a box. Data from healthy adults have been extracted from Pentafragka et al (2020b).

Figure 7: Individual bile acids quantified in porcine bile used in TIM-1 system (A) vs. individual bile acids in the human upper small intestine (B). Data from healthy adults have been extracted from Pentafragka et al (2020b). Bile acids that are present in TIM-1 but not in the upper small intestine of adults and vice versa have no colored background. GCDC: glycohenodeoxycholic acid, TCDC: taurochenodeoxycholic acid, TC: taurocholic acid, GDC: glycocholic acid, UDC: ursodeoxycholic acid, GC: glycochlic acid, GHDC: glycohyodeoxycholic acid, GHC: glycohyocholic acid.

Figure 8: Individual data (n=2) of total bile acids (sum of individual bile acids) in the duodenal compartment (A) and jejunal compartment (B) of TIM-1 system [total amounts per volume

(bold dotted lines); concentrations in the micellar phase (dotted lines)] vs. total bile acids in contents of the upper small intestine of adults [total amounts per volume (lined boxplots); concentrations in the micellar phase (empty boxplots)]; number of individual data points for the construction of each box indicated on top of a box. Data from healthy adults have been extracted from Pentafragka et al (2020b).
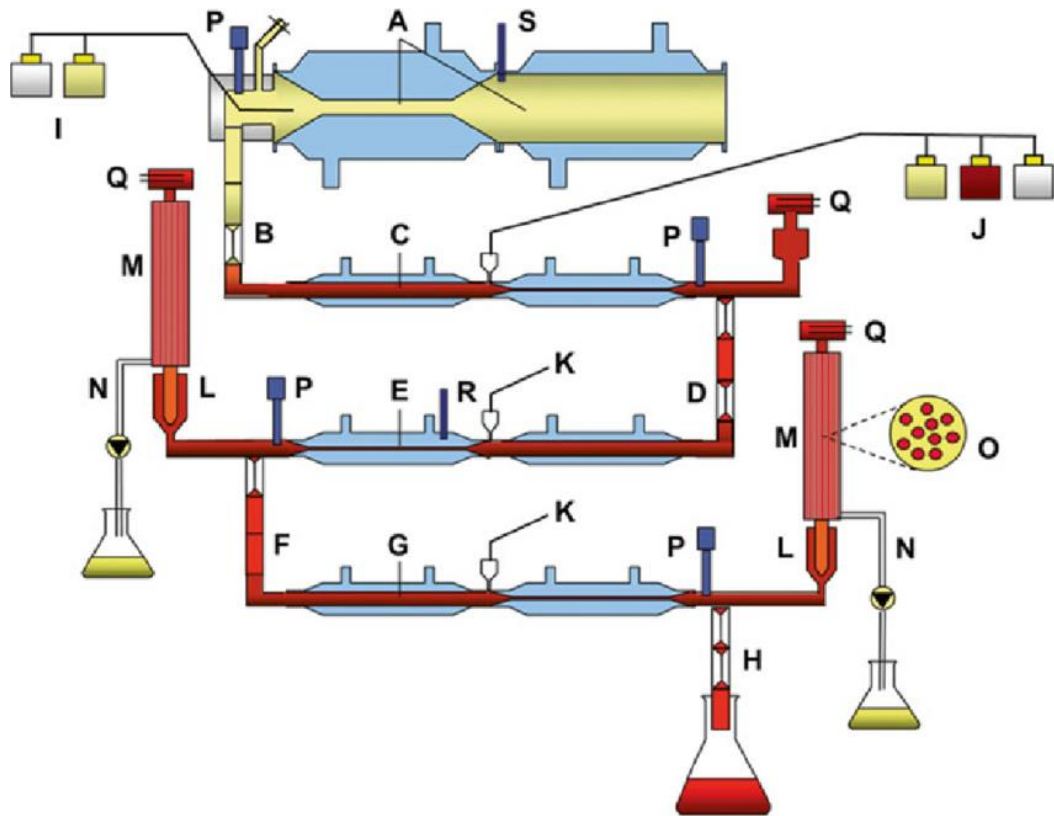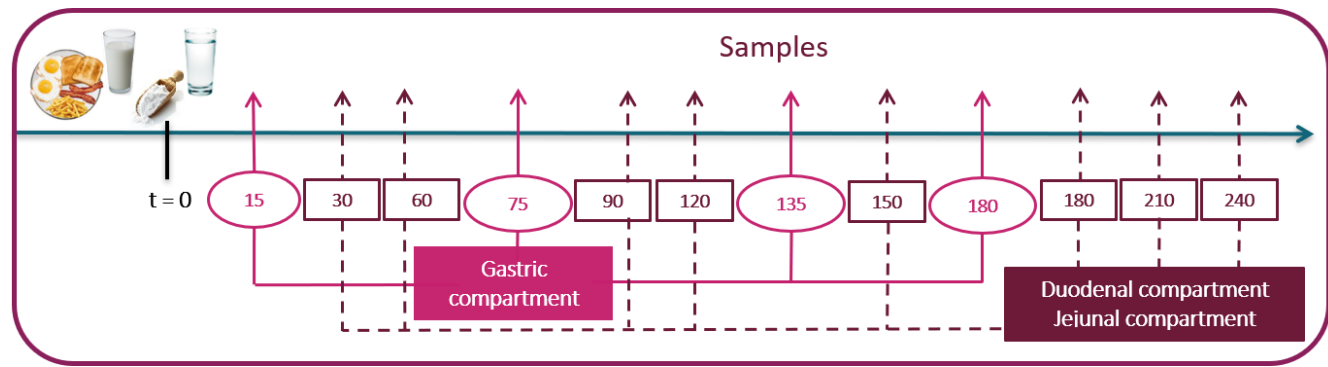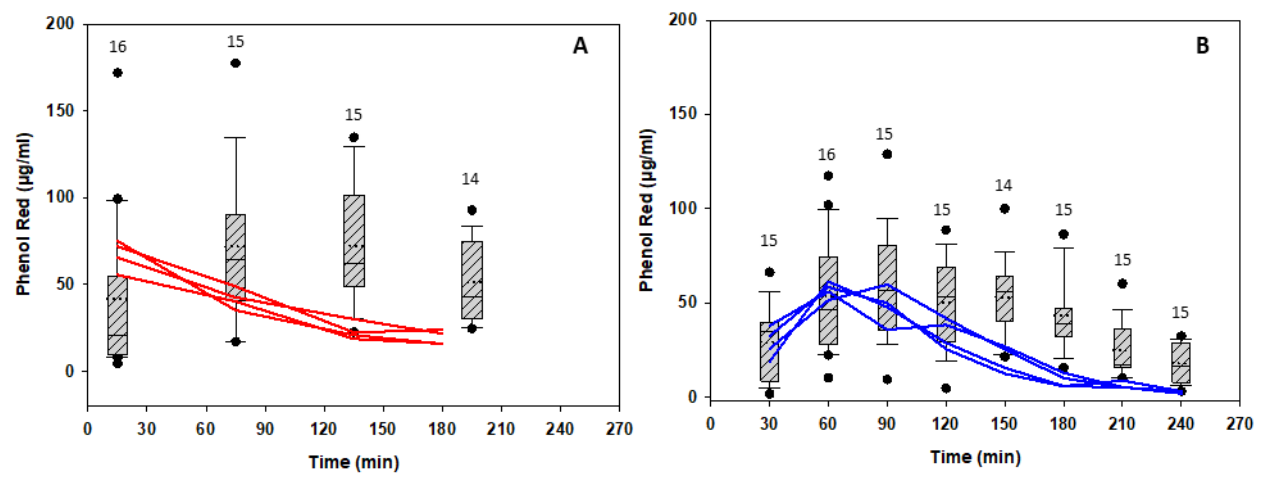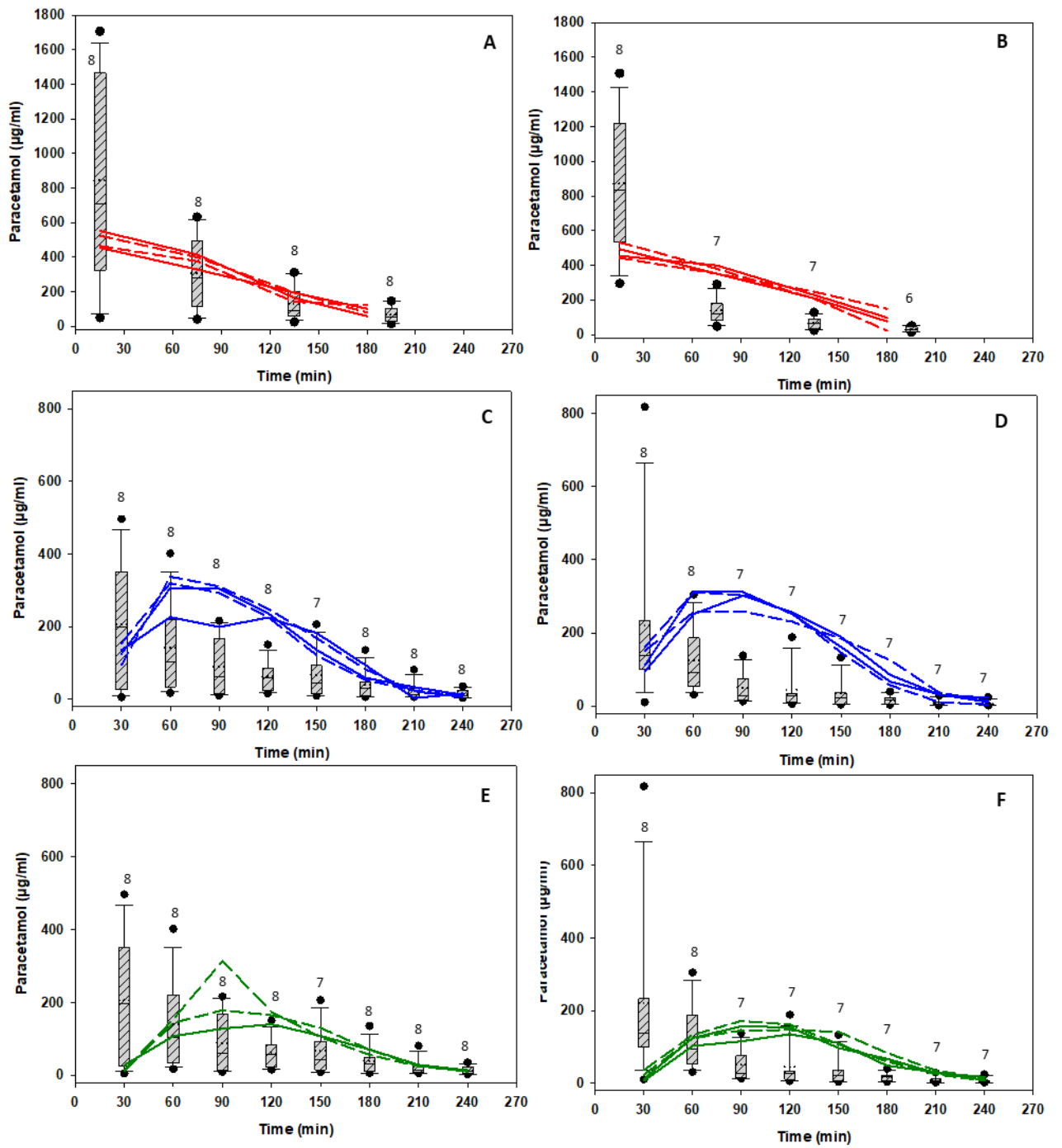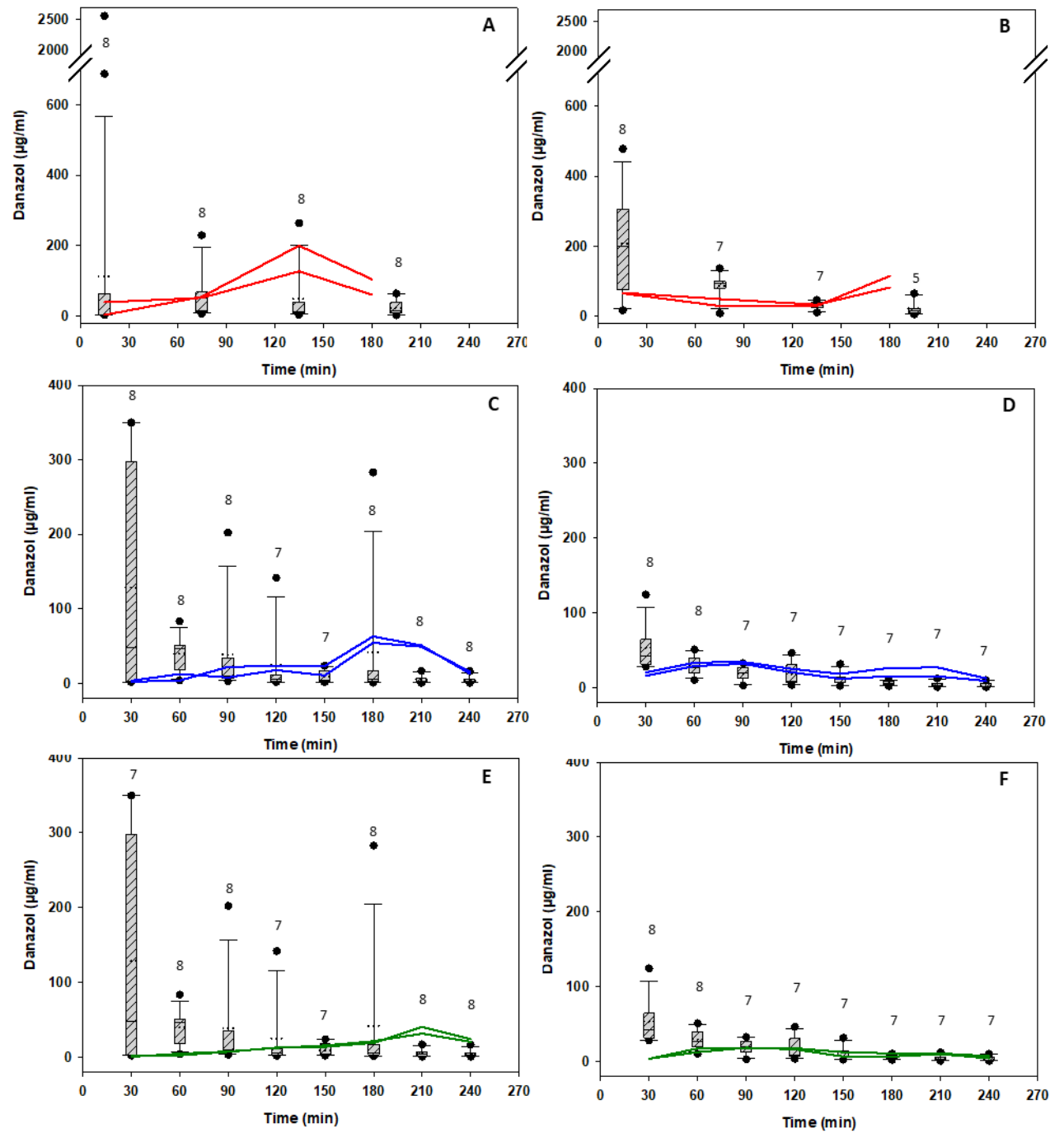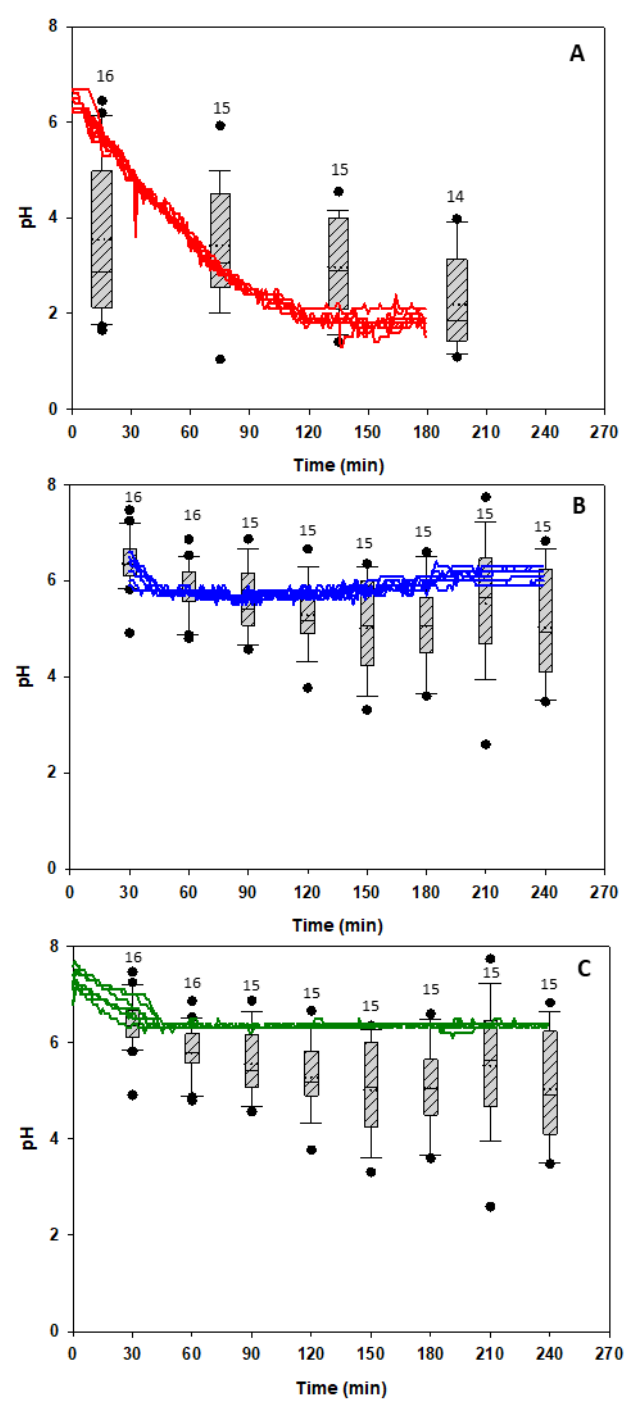
Figure 1

Figure 2
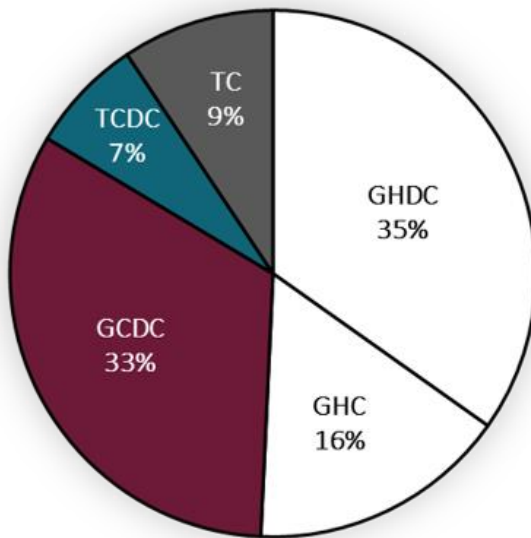
Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

A. TIM-1
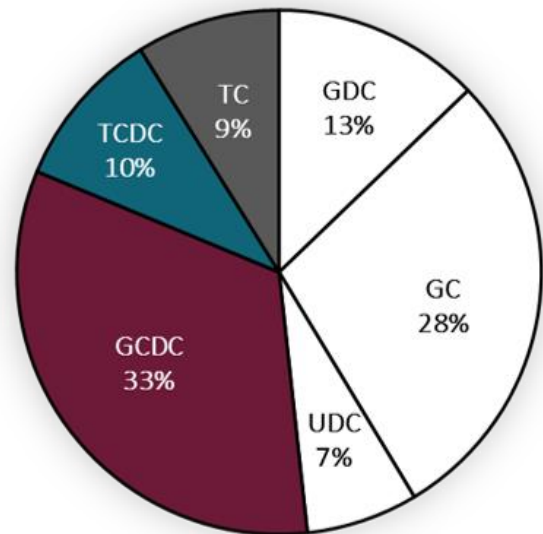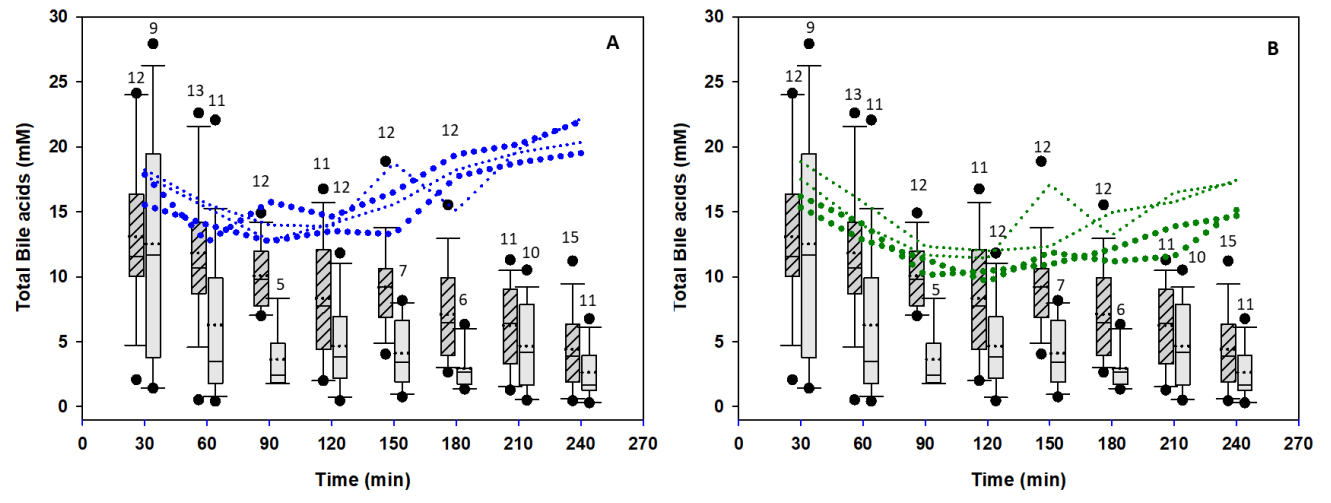**B. Human upper small intestine**

Figure 8

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