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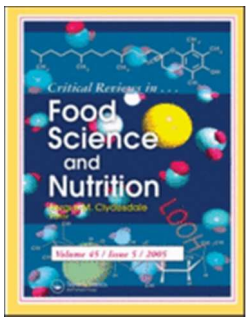

Can dynamic in vitro digestion systems mimic the physiological reality?

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Can dynamic *in vitro* digestion systems mimic the physiological reality?

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Abstract

During the last decade, there has been a growing interest in understanding the fate of food during digestion in the gastrointestinal tract in order to strengthen the possible effects of food on human health. Ideally, food digestion should be studied *in vivo* on humans but this is not always ethically and financially possible. Therefore simple static *in vitro* digestion models mimicking the gastrointestinal tract have been proposed as alternatives to *in vivo* experiments but these models are quite basic and hardly recreate the complexity of the digestive tract. In contrast, dynamic models that allow pH regulation, flow of the food and injection in real time of digestive enzymes in the different compartments of the gastrointestinal tract are more promising to accurately mimic the digestive process. Most of the systems developed so far have been compared for their performances to *in vivo* data obtained on animals and/or humans. The objective of this article is to review the dynamic digestion systems available and their validation towards *in vivo* data. This is the result of a cooperative international effort made by some of the scientists involved in Infogest, an international network on food digestion

Keywords: dynamic *in vitro* digestion, food, stomach, small intestine, colon

Introduction

Digestion is a complex process that will provide nutrients to the body and release molecules in the gastrointestinal tract that can have a beneficial or a deleterious effect on human health. Therefore, understanding the fate of food in the digestive tract is a way to increase our knowledge on the effect of food on health. When entering in the gastrointestinal tract food will be disintegrated in the different compartments (mouth, stomach, small and large intestine), macronutrients will be hydrolyzed and micronutrients will be absorbed.

Investigating food digestion using *in vivo* models (animals or humans) is rather difficult, expensive and sometimes ethically questionable. For this reason, several *in vitro* models have been developed. Most of the numerous protocols described in the literature are static ones and consist in placing the food in a series of bioreactors where the physicochemical and enzymatic environment of each digestive compartment is recreated. However, digestion is a dynamic process and therefore these models exhibit strong limitations: there is no flow of the food between the different compartments and the pH, digestive enzymes and bile concentrations are kept constant. For these reasons, dynamic systems have been designed and protocols are available for simulating food digestion.

Dynamic systems are either monocompartmental (simulate one compartment of the gastrointestinal tract) or multicompartmental (several compartments). The different systems available have been described recently (Guerra et al., 2012) and a general description of the different systems investigated is presented in Table 1. In this review, we particularly would like to focus on their ability to simulate the physiological reality and recreate what happens in the gastrointestinal tract of animals or humans. This is the contribution of scientists involved in the international Infogest network (www.cost-infogest.eu) that aims at understanding the fate of food in the gastrointestinal tract.

Mono-compartmental systems

The Dynamic Gastric Model (DGM)

Origins of the system

The Dynamic Gastric Model was developed at the Institute of Food Research (Norwich, UK) to address the need for a model that could simulate both the biochemical and mechanical processes occurring during human gastric digestion in a physiologically relevant manner. The DGM was initially developed to further food research and to enable the study of parameters such as nutrient bioaccessibility, effect of food structure on nutrient delivery, nutrient interactions, and survival and delivery of functional foods. However, for obvious reasons the DGM has also increasingly been used by the pharmaceutical industry as an *in vitro* tool to study the effect of food matrices on the disintegration and dissolution of drug formulations and the delivery profile of drugs to the duodenum. This success is in part due to its ability to realistically process any complex food matrix for direct comparison with the results of *in vivo*/clinical studies. The design of the DGM is based on extensive research into gastric digestion and the physiology of the human stomach, both biochemical and mechanical (Wickham et al., 2012).

Short description of the system

The gastric digestion of food involves secretions from the gastric mucosa and a change in peristaltic contractions. Within the DGM, acid and enzyme solutions are added through a perforated loop situated at the top of the fundus and allowing a flow of secretion down the wall of the stomach. The flow rates of secretions are controlled dynamically and the rate of acid addition slows in response to the drop in pH as detected by the pH electrode positioned in the fundus. The DGM simulates the fundus and the antrum of the stomach. Within the fundus/main body, the food bolus is subjected to rhythmic squeezing brought about by cyclical pressurization of the 37°C water jacket surrounding it. The DGM antrum consists of a barrel and a piston, which move within a water jacket. While the piston draws portions of food bolus through an inlet valve from the fundus into the antrum, it is the upward and downward movement of the barrel during processing which exerts shear stresses on the antral contents. This is due to a flexible annulus mounted within the top part of the barrel through which food (and formulations) passes during every stroke, thereby simulating the rhythmic peristaltic contractions of the human stomach. While the speed of movement has been calibrated to provide physiological shear forces (Vardakou et al., 2011b), the actual volume of food bolus processed within the antrum at any one time, as well as duration of processing are tailored to the specific meal used (volume, composition, calorific content). At pre-defined intervals, the inlet valve closes and the outlet valve opens, allowing the processed chyme to be ejected from the DGM. Gastric sieving is simulated within the DGM through the use of a "dead volume," i.e. a defined space between barrel and piston whose volume is maintained during ejection thereby allowing large, dense particles to remain in the antrum and undergo repeated processing cycles. At the end of a simulated digestion, any material remaining in this dead volume is ejected to simulate the phase III contraction (housekeeper wave) which fully empties the human stomach at the end of gastric digestion. Following ejection from the DGM, samples can be subjected to further digestion using a static duodenal model. To this end, the pH of the samples is elevated and a physiological mix of bile salts with lecithin and cholesterol and pancreatic enzymes, is added to simulate conditions found within the duodenum.

Validation of the system towards in vivo data animal and/or human

The grinding forces of the DGM and a Dissolution Apparatus USP-II operated at two rotational speeds (50 and 100 rpm) were measured using the breakdown of agar gel beads of various fracture strengths in high and low-viscosity meals and compared to in vivo data collected on human volunteers (Vardakou et al., 2011a). For this experiment, the DGM was designed to replicate the real-time changes in pH, enzyme addition, shearing, mixing, and retention time of an adult human stomach. The model can be fed 'meals' ranging from a glass of water to high fat meals (i.e. the FDA high fat American breakfast) and deliver samples from its 'antrum' in the same processed form and at the same rate as seen in vivo. The data used to program the DGM were derived from echo-planar imaging studies (Marciani et al., 2009; Marciani et al., 2001b) and from published references detailing physiological ranges for the rate of production of gastric secretions (Geigy, 1981). All beads tested in the DGM broke after a certain amount of gastric processing. The results expressed as MBT obtained for the beads at the four strengths administered in low (LV LBG) and high viscosity Locust Beam Gum (HV LBG) meals are represented in Figure 1b in order to facilitate a direct visual comparison with the in vivo data, Figure 1a. Increasing the viscosity of the meal reduced the survival time of the harder beads. The interrelationship between the in vitro data obtained with the DGM and those observed in vivo (Marciani et al., 2001a) is clearly visible from the graphs depicted in Figure 2. Even though the R2 in Eqs. 5 and 6 are not very high, there is a clear correlation between the in vitro (DGM) and the in vivo data. One-way ANOVA analysis showed that no statistical difference exists

between any of the data collected from the DGM and those found in human. This indicates that the forces produced during the DGM processing are within the range of forces exerted by the human gastric compartment in vivo. Furthermore, the DGM showed to be able to discriminate between the two meals, similarly to the finding of (Marciani et al., 2001a). The different behavior observed for the beads in the LV and HV meals is of special interest particularly when considering the effect that it may have on dosage forms for which the drug release is greatly susceptible to the shear forces applied to its surface, as in the case of erodible matrixes.

Advantages and limitations of the system.

The DGM processes real food items and meals as eaten and simulates the physical mixing, transit and breakdown forces within the normal physiological range. The system adjusts for gastric residence time, acid and enzyme additions (quantity and rate) and physical processing depending on food matrix, allowing fed and fasted state comparisons and studies of the impact of different meals/food items on dosage form behavior. It provides samples of digested materials at any sampling time, within the total digestion period. However, the DGM only models the behavior of the gastric compartment, necessitating a method of simulating the oral phase (e.g. chew and spit) and the intestinal phase if the fate of nutrients and bioactives is to be investigated fully.

Human Gastric Simulator (HGS)

Origins of the system

The human gastric simulator (HGS) system was developed at the University of California, Davis to enable measurement of gastric food breakdown in a system with physiologically-relevant physical and chemical conditions to the stomach. This mono-compartmental system focus on gastric digestion. However, oral and/or small intestinal stages may be incorporated either before or after testing in the HGS, respectively. Two generations of this model have been developed (Guo et al., 2014; Kong and Singh, 2010; Phinney, 2013), both incorporating a flexible gastric vessel, continuous peristaltic contractions provided by rollers, controllable secretions (enzymes, pH), and gastric emptying.

Short description of the system

The HGS model (Table 1) consists of a flexible outer vessel to simulate the stomach. Although the vessel may be filled with several liters of material, the typical amount of material (e.g. the “meal” and secretions) is 0.9-1.0 L, which is the volume that can be ingested without resulting in increases in gastric luminal pressure (Ferrua and Singh, 2010). The rollers that simulate peristaltic contractions are controlled by a variable-speed motor, which can be used to change the contraction frequency. To simulate normal adult gastric digestion, a frequency of ~ 3 contractions/minute is used, according to previous *in vivo* studies in humans (Hocke et al., 2009; Marciani et al., 2001c). The gastric secretions are added through tubes entering the top of the vessel. The secretion rate and specific composition (pH, enzymes, salts, mucin) can be varied, depending on the goal of the study. Samples are emptied through a small tube in the distal portion of the vessel. A mesh with 1 mm openings is used to control the gastric emptying, such that only smaller particles (< 1 mm diameter) can exit the stomach. The

entire unit is kept inside of a temperature-controlled chamber maintained at 37°C using a small heater and fan (Guo et al., 2014; Kong and Singh, 2010; Phinney, 2013).

Validation of the system towards in vivo data animal and/or human

Data from the HGS model systems have been compared with previously published *in vivo* data, and a systematic validation of the model is currently ongoing in our laboratory. However, some comparisons can be made from the second generation HGS model with *in vivo* animal studies in the growing pig. For both studies, meals of white rice (medium grain, *Calrose* variety) were cooked following a standardized procedure (Bornhorst et al., 2013a; Bornhorst et al., 2013b). For the *in vivo* study, the growing pig (20.9 ± 0.2 kg) was used as a model for digestion in adult humans. Digestion was monitored for up to 8 h. For the *in vitro* study, the same meals of white rice were mixed with simulated saliva and fed into the second generation HGS model. Digestion was monitored for up to 3 h. Specific experimental details are given elsewhere (Bornhorst et al., 2013a; Bornhorst et al., 2013b; Bornhorst et al., 2014; Phinney, 2013).

Figure 3 shows the correlation between the gastric emptying rate of dry matter from the *in vitro* and *in vivo* experiments. The solid line represents a 1:1 correlation (e.g. gastric emptying rate *in vitro* is the same as the gastric emptying rate *in vivo*). It can be seen that the dry matter gastric emptying rate was similar between the *in vitro* and *in vivo* studies, especially at later digestion times. For example, after 3 h gastric digestion, the *in vivo* and *in vitro* systems both had 64% dry matter remaining. The intragastric pH distribution between the HGS and the *in vivo* study also showed similarities at certain locations. pH measurements were taken at ten intragastric locations (Bornhorst et al., 2014), and values from the location closest to the pylorus (or HGS emptying tube) were compared as well as values from the top of the fundus (or top of HGS gastric vessel) were compared over the 3 h gastric digestion period (Figure 4). The pH values varied significantly between location (e.g. pylorus vs. fundus), but the values were similar between the *in vitro* and *in vivo* systems. For example, after 60 min digestion, the pH in the fundus location was 6.9 ± 0.1 *in vivo* and 7.0 ± 0.1 *in vitro*. Although most values compared here were similar between the *in vitro* and *in vivo* system, some differences were observed. After 180 min digestion, the pH in the fundus location was 4.9 ± 1.3 *in vivo* compared to 6.2 ± 0.4 *in vitro*. These differences may be the result of varying gastric secretion rates *in vivo* that are difficult to predict and control *in vitro*.

Additionally, texture changes were monitored to quantify the white rice breakdown during gastric digestion in the proximal and distal stomach regions. Differences between the proximal and distal stomach regions can be observed from both *in vivo* and *in vitro* models, where rice grains from the proximal region have greater hardness compared to the distal region. This trend is observed at all digestion time points (20, 60, 120, and 180 min). At the shorter time points, the *in vivo* and *in vitro* systems have similar hardness values within each region. For example, after 60 min digestion in the distal region, the *in vivo* hardness was 26.2 ± 1.9 N compared to 28.2 ± 1.4 N in the HGS *in vitro* system. However, at longer digestion times, the hardness was lower in the *in vivo* model compared to the *in vitro* system in both the proximal and distal stomach regions. For example, after 180 min digestion in the proximal region, the hardness from the *in vivo* model was 23.7 ± 5.7 compared to 34.1 ± 5.6 in the HGS *in vitro* system. These promising results indicate that the HGS *in vitro* model has the capability of producing a similar gastric emptying rate, similar pH values at certain gastric locations, and similar trends in food breakdown in a white rice meal. However, a more complete validation, including additional measurements, longer digestion times, and varying meal types is necessary for the HGS *in vitro* model to be utilized in a wide variety of applications.

Advantages and limitations of the system.

The advantages of the HGS are that it can be used to study both the physical and chemical breakdown of food and other materials in the stomach with physiologically relevant parameters. The gastric secretion rate, pH, and gastric emptying can be controlled and varied as needed. The system can be used with larger meal volumes (up to 1 L), which may be important if greater amounts of sample are needed for analysis (e.g. physical property analysis). The limitations of this system are that the mixing and physical property changes of sample meals still needs to be validated with *in vivo* data. In addition, it does not account for the oral or small intestinal phases of digestion, although it may be coupled with other static or dynamic digestion model systems.

The artificial colon: ARCOL

Origins of the system

ARCOL (Artificial colon) is a one-stage fermentation model that reproduces the colonic environment of humans or animals. This model has been developed by the University of Auvergne (Clermont-Ferrand, France). It's the first one that has allowed the maintaining of anaerobiosis inside the fermentor by the sole metabolic activity of the microbiota and not by flushing with N_2 or CO_2, as usually done in other colonic *in vitro* models. Up to date, ARCOL has been used to reproduce the colon of humans (Blanquet-Diot et al., 2012; Cordonnier et al., 2015; Thevenot et al., 2015; Thevenot et al., 2013), pre-ruminant calves (Gerard-Champod et al., 2010) and pigs.

Short description of the system

ARCOL integrates the main parameters of *in vivo* fermentation in the large intestine, such as pH, temperature, anaerobiosis, supply of simulated ileal effluents, colonic residence time, presence of a complex, high-density, metabolically-active microbiota and passive absorption of water and microbial metabolites.

ARCOL is a 2-L bioreactor equipped with various ports and probes that is used in semi-continuous conditions. The fermentor is inoculated with fresh feces from healthy volunteers or animals, after suspension into phosphate buffer and filtration through a double layer of gauze. A culture medium, reproducing the composition of ileal effluents and containing various carbohydrate, protein, lipid, mineral and vitamin sources, is sequentially introduced into the bioreactor, while fermentation medium is sequentially withdrawn from the bioreactor. During fermentation, the fermentation medium and the atmospheric phase are continuously stirred. The pH and temperature are kept at a constant value by adding NaOH and heating with a water double-jacket. After initial sparging with O_2-free N_2 gas, the fermentative process allows the maintenance of anaerobic conditions in the bioreactor. A dialysis system using hollow fiber membranes (cut-off 30 kDa) maintains the appropriate electrolyte and metabolite concentrations and the operating volume.

Validation of the system towards in vivo data animal and/or human

ARCOL has been validated towards *in vivo* data in human, pig or calves regarding the composition of the colonic microbiota (main bacterial populations followed by qPCR or plating), its metabolic activity (production of major end products of fermentation, such as short chain fatty acids) and/or the composition of the nutritive medium used to feed the fermentor (Gerard-Champod et al., 2010; Thevenot et al., 2015). The relevance of the ARCOL model for probiotic studies was also shown as the survival of probiotic yeasts and their influence on SCFA production obtained *in vitro* corroborate the

available data in human adult volunteers (Blanquet-Diot et al., 2012; Cordonnier et al., 2015; Thevenot et al., 2015).

Advantages and limitations of the system

ARCOL reproduces the conditions that can be found in average in the human or animal colon but does not simulate the different biotic and abiotic conditions (e.g. pH, retention time, availability of substrates, microbiota) associated with the three parts of human or pig colon.

Multicompartmental systems

DIDGI®

Origins of the system

The DIDGI® system was built up at INRA in order to monitor the disintegration and the kinetics of hydrolysis of the food occurring during a simulated digestion. It focuses on the upper parts of the digestive tract, i.e. the stomach and the small intestine. To be physiologically realistic, the computer-controlled system reproduces the gastric and intestinal transit times, the kinetics of gastric and intestinal pH, the sequential addition of digestive secretions and the stirring of the stomach and small intestine contents.

Short description of the system

The DIDGI® system consists of two consecutive compartments simulating the stomach and the small intestine. Each compartment is surrounded by a glass jacket filled with water pumped using a temperature-controlled water bath. The system is equipped with temperature, pH and redox sensors and variable speed pumps to control the flow of meal, HCl, Na_2CO_3, bile, enzymes and the emptying of each compartment. Flow rates are regulated by specific computer-controlled peristaltic pumps. Anaerobic conditions can be simulated by purging air with nitrogen. A Teflon membrane with 2 mm holes is placed before the transfer pump between the gastric and the intestinal compartment to mimic the sieving effect of the pylorus in human, as described previously (Kong and Singh, 2008). The computer program was designed to accept parameters and data obtained from *in vivo* studies in animals or human volunteers, such as the quantity and duration of a meal, the pH curves for the stomach and small intestine, the secretion rates into the different compartments and the gastric and small intestine emptying rates. The system is controlled by software named StoRM® for Stomach regulation and monitoring (Guillemin et al., 2010). To control the transit time of the chyme in each compartment, a power exponential equation for gastric and intestinal delivery is used f = 2-(t) t / 1/2 b

where f represents the fraction of the chyme remaining in the stomach, t is the time of delivery, t½ is the half time of delivery and β is the coefficient describing the shape of the curve, as described previously (Elashoff et al., 1982).

Validation of the system towards in vivo animal data

Example – Digestion of infant formula

The DIDGI® system is a very recent one. Although several matrices (dairy, meat, fruits and vegetables, emulsions) have been submitted to digestion using the DIDGI® system, only data obtained on the digestion of infant formulas (Ménard et al. 2014), cheese (Adouard et al., 2016), bovine skim milk (Sanchez-Rivera et al., 2015) and human milk (de Oliveira et al., 2016a; de Oliveira et al., 2016b; Deglaire et al., 2016) have been published so far. In order to demonstrate that this system was physiologically-relevant, a comparison of the *in vitro* and *in vivo* digestion of an infant formula was performed. The *in vivo* trial was conducted on 18 piglets that were fed the infant formula for which the concentration in lipids and proteins was increased compared to a standard one, but the ratio lipids/proteins was kept constant. In parallel, *in vitro* gastro-intestinal digestion was performed on this enriched infant formula using the newly developed system and the extent of milk proteolysis was monitored and compared to the one obtained *in vivo*. All the details regarding the experimental conditions used for this validation have been described previously (Menard et al., 2014).

Volumes of the stomach content observed *in vitro* with the dynamic digestion system were compared to the ones observed *in vivo* in piglets. No significant differences were observed 30, 90 and 210 min after ingestion confirming that the parameters chosen for mimicking the gastric transit of infant formula *in vitro* were physiologically relevant. Evolution of caseins and β-lactoglobulin throughout *in vitro* and *in vivo* digestion, as determined by ELISA, was compared. Results showed that the kinetics of hydrolysis of both proteins during *in vitro* and *in vivo* digestion were similar. The proportion of immunoreactive caseins appeared not to be significantly different between both experiments for samples collected in the stomach as well as in the small intestine after 30, 90 and 210 min of digestion (Figure 5a). Similarly, the percentage of immunoreactive β-lactoglobulin showed no significant differences for samples collected *in vivo* and *in vitro* in the stomach after 30, 90 and 210 min (Figure 5b). However, the percentage of immunoreactive β-lactoglobulin in the small intestine was significantly higher *in vitro* than *in vivo*. The correlation coefficient, between *in vitro* and *in vivo* ELISA determination for caseins and β-lactoglobulin was 0.987 ($p<0.001$), proving a good agreement between *in vitro* and *in vivo* proteolysis during digestion.

Advantages and limitations of the system.

The main advantage of this system is that, since it is basic, it is quite robust and can handle real foods and full meals up to 200 g. The compartments are transparent allowing to see in real time the evolution of the food structure during digestion. In contrast, the mixing in the compartments consists only in basic stirring and, so far, absorption of nutrients in the small intestine is not simulated.

TIM

Origins of the TIM systems

In 1992 the authors initiated the development of *in vitro* gastrointestinal (GI) models at TNO. Realizing the limitations of static models, from the start we focussed on dynamic systems. Extensive literature data about anatomy and physiology of the GI tract were 'translated' to the TIM technology. The gastric and small-intestinal model (TIM-1) was described in details in 1995 (Minekus et al., 1995), and patented in the EU and USA. After that, the large-intestinal model (TIM-2) was developed (Minekus et al., 1999). Even today it is a continuous process of optimization, such as simulation of

infant GI conditions (Havenaar et al., 2013a) and development of the advanced gastric model 'TIMagc' (Bellmann et al., 2016). Over the years a broad variety of validation studies in nutrition research (section below) and pharmaceutical research (not part of this review) have been published.
Although the focus is on humans, the GI conditions and colon microbiota of pigs (Avantaggiato et al., 2007; Martinez et al., 2013) and dogs (Smeets-Peeters et al., 1999) can also be simulated in TIM.

Short description of the TIM systems

The TIM-1 system comprises compartments for the stomach, duodenum, jejunum and ileum, connected by peristaltic valves and linked with semi-permeable membrane units. In these compartments the successive dynamic conditions in the upper GI tract are simulated. Also a tiny-TIM system is available, comprising one compartment for the small intestine (Verwei et al., 2016). TIMagc simulates the specific conditions in the corpus and antrum part of the stomach, including peristaltic motility and pressure forces (Bellmann et al., 2016). The TIM-2 system simulates the dynamic conditions in the colon with a high density of metabolic active microbiota of human origin (Aguirre et al., 2015).
The settings in the computer software accurately and reproducibly control the TIM system, e.g. for temperature, peristaltic mixing, transit times, pH curves, and secretion of GI fluids (e.g. salivary and gastric juice, bile, pancreatic juice prepared according to SOPs). The settings and composition of secretion fluids can be adapted related to the type of drink and food, age, health status, and drug use. It can vary from rapid gastric emptying with low secretion after intake of water, up to slow gastric emptying with high initial gastric pH and high secretion of digestive fluids after intake of a high fat meal. The average dynamic GI conditions as well as the biological day-to-day and inter-individual variation can be simulated based on available physiological data. For example, the GI conditions of neonates, infants and toddlers in tiny-TIM and the consequences it has for oral drugs and digestion vs. adult conditions have been documented (Havenaar et al., 2013b).
Related to the research question, the TIM-2 systems can be inoculated with pooled or individual faecal samples (Aguirre et al., 2015; Aguirre et al., 2014b), from healthy volunteers, e.g. on different diets (Tabernero et al., 2011), from obese persons (Aguirre et al., 2014a), or patients with GI disorders (Rose et al., 2010). Phylogenetic analysis showed that the microbial density and composition in TIM-2 was rather similar to the human faecal microbiota (Kovatcheva-Datchary et al., 2009).

Validation and application in food and nutrition research

TIM is a pioneer *in vitro* digestion system and has been widely used during the last 2 decades. More than 100 papers have been published relating studies performed on all kinds of foods and micro/macronutrients. In the next paragraph, only the papers showing a comparison between *in vivo* and TIM data will be presented.
In a pioneer work, the gastric and ileal deliveries of the TIM model were shown to simulate accurately the pre-set curves for slow and fast deliveries of chime calculated from in vivo data obtained from studies with human volunteers (Figure 6).

Macronutrients. The digestion and fermentation of carbohydrates and dietary fibres in TIM-1 and TIM-2 (Venema et al., 2003; Venema et al., 2005), respectively, showed reliable results for the human situation. The human glycaemic response curve after carbohydrate intake can be predicted by combining TIM digestion studies with *in silico* modelling of the insulin response (Figure 7) (Bellmann et al., 2010).

The digestion of proteins and bioaccessibility of amino acids in TIM was compared with *in vivo* data (Schaafsma, 2005), showing a high predictive quality. Therefore, the TIM system is a suitable *in vitro* tool to determine the true ileal protein digestibility and amino acid bioaccessibility (Havenaar et al., 2016), e.g. to determine protein quality according to DIAAS.

Micronutrients. Human plasma concentrations after long-term intake of folate was accurately predicted using TIM in combination with *in silico* modelling (Verwei et al., 2006).
The stability and bioaccessibility of fat-soluble vitamins such as lycopenes and tocopherol (Déat et al., 2009) and fat-soluble phytochemicals (Ribnicky et al., 2014) were studied in TIM showing food matrix and food preparation effects consistent with *in vivo* data.
Different aspects of minerals and metals in TIM showed good correlation with human data such as the bioaccessibility of iron from various food products (Larsson et al., 1997) or the risks for young children of unintended lead intake via polluted soil (Van de Wiele et al., 2007).

Functional foods. Studies with functional foods vary from probiotics and prebiotics to anti-oxidants. The survival of probiotic bacteria during transit through TIM-1 was first validated in 1997 by Marteau et al. (Marteau et al., 1997) and during the years, many different bacterial strains were tested. Examples of anti-oxidants studies in TIM are about the bioconversion of phenolic acids (Gao et al., 2006) and fermentation of cereal fibre fractions by the colon microbiota (Anson et al., 2011b). The anti-inflammatory capacity measured in TIM samples using a macrophage assay (Anson et al., 2010) was confirmed in an *ex-vivo* human study (Anson et al., 2011a).

Advantages and limitations of the TIM system

Advantages of the TIM system are that (i) they simulate accurately the dynamic physiological GI conditions; (ii)) they can handle specific food ingredients and drugs as well as complete meals; (iii) they can simulate average GI conditions, biological variation, and disease conditions for different age groups;
(iv) therefore, they can be used for a broad scope of applications in the food and pharma research and are not limited to a specific application; (v) samples can be collected from the compartments during transit of the chyme for analysis, which results in detailed information about the fate of test products in the GI tract; (vi) due to the strict control over all settings and composition of secretion fluids the experiments are highly reproducible.
Limitations of the TIM systems are that (i) there is no feed-back on energy density of the food on the GI conditions; these parameters should be set in advance in the TIM-software; (ii) there is no intestinal mucosa, therefore absorption should be studied in combination with intestinal cell lines (Déat et al., 2009; Haraldsson et al., 2005) or tissues (Westerhout et al., 2014); (iii) in TIM the availability for absorption (bioaccessibility) is measured and not the bioavailability including metabolism and excretion; this can be overcome by combining TIM with *in silico* modelling (Naylor et al., 2006; Verwei et al., 2006).
In conclusion, the TIM system is a broadly validated, time- and cost-efficient, reliable *in vitro* tool to study the digestibility of foods, the bioaccessibility of nutrients, and the fate and efficacy of functional ingredients under simulated dynamic human adult and infant GI conditions.

Simulator or the Human Intestinal Microbial Ecosystem (SHIME®)

Origins of the system

The reactor setup was adapted from the original SHIME® model developed at Ghent University (Belgium), representing the gastrointestinal tract (GIT) of the adult human, as described by Molly *et al.* (Molly et al., 1993). During the years the system has been improved and nowadays, it is a computer-controlled device that can be used to simulate the gastrointestinal microbial ecology and physiology of healthy humans, babies, elderlies, some specific disease conditions (e.g. IBD, pathogen infection) and also pigs, dogs and cats (ProDigest, Belgium).

Short description of the system

The SHIME® consists of a succession of five reactors simulating the different parts of the gastrointestinal tract. The first two reactors are of the fill-and-draw principle to simulate different steps in food uptake and digestion, with peristaltic pumps adding a defined amount of SHIME nutritional medium (3x/day) and pepsin to the stomach and pancreatic enzymes with bile liquid in the small intestine. A specific software allows the subsequent simulation of the physiological conditions occurring in the duodenum, jejunum and ileum. The last three compartments are continuously stirred reactors with constant volume and pH control. Retention time and pH of the different vessels are chosen in order to resemble *in vivo* conditions in the different parts of the gastrointestinal tract. Upon inoculation with fecal microbiota, these reactors simulate the ascending, transverse and descending colon. Upon stabilization of the microbial community in the different regions of the colon, a representative microbial community is established in the three colon compartments, which differs both in composition and functionality in the different colon regions. Inoculum preparation, retention time, pH, temperature settings and reactor feed composition were previously described by Possemiers *et al*. (Possemiers et al., 2004). In order to investigate different compounds at the same time, a TWINSHIME® setup was developed by operating two systems in parallel at the same time. This makes the model an ideal system for direct comparison of two products or to perform placebo-controlled studies. More recently a TripleSHIME and a QuadSHIME model have been introduced to compare 3 or 4 conditions, respectively.

The most recent developments in relation to the SHIME technology consist in the automation of the process control (i.e. liquid transfer, pH, flushing), data acquisition and the development of an additional absorption unit that can be used to simulate the small intestinal absorption processes. This unit is connected directly in line with the main operation unit and operated with the same software. Using the so-called M-SHIME® it is possible to mimic the mucosal microbial colonization by incorporation of mucin-covered microcosms therefore maintaining *in vitro* unique features of an individual's microbiome in terms of its mucosal composition (Van den Abbeele et al., 2013a). Systems have been developed to simulate the specific physiological conditions occurring in babies and elderly, as well as pig, dog and cat. Moreover, by combining the SHIME® with the so-called HMI™ module (Marzorati et al., 2014), it is possible to simulate online the host-microbiota interaction occurring at the level of the gut wall (i.e. biofilm formation under a shear stress and concomitant presence of enterocytes to evaluate the impact of a treatment in terms of gut wall modulation)

Last but not least, specific protocols have been developed to simulate diseased conditions: inflammatory bowel disease, treatment with antibiotics, infection with *Clostridium difficile* (PathoGut™ model)

Validation of the system towards in vivo data animal and/or human

The SHIME model was initially developed in 1993 and was validated based on a comparison with *in vivo* human data regarding indicator bacterial groups, short-chain fatty acid production (SCFA), enzymatic activities, headspace gases and microbiota-associated characteristics (MACs) (Molly et al., 1994). Over the years, a large number of experiments (i.e more than 100 papers) has been performed in which SHIME results were compared with *in vivo* animal and human experiments. Below, we summarize some key findings.

- The application of a high-resolution phylogenetic microarray (i.e. HITChip) pointed out that a wide range of intestinal microbes of *in vivo* human samples can be maintained in the SHIME model and are colon region-specific, similar to *in vivo* data (Van den Abbeele et al., 2010). One critical remark of this study was that the shift from an *in vivo* to an *in vitro* environment resulted in an increased Bacteroidetes/Firmicutes ratio as also occurs in other *in vitro* models (Rajilic-Stojanovic et al., 2010). In this respect, Van den Abbeele *et al.* (Van den Abbeele et al., 2012) introduced a simulated intestinal surface in the SHIME (M-SHIME®). As a result, in contrast to conventional models, washout of relevant mucin-adhered microbes was avoided. This resulted in the fact that unique inter-individual differences among human subjects are preserved in this *in vitro* model (Van den Abbeele et al., 2013a). Since then, the M-SHIME has also been applied to e.g. investigate the differences between healthy individuals and IBD patients (Vermeiren et al., 2012; Vigsnaes et al., 2013).
- Multiple case studies have also demonstrated that specific enzymatic conversions can be accurately simulated. As an example, Possemiers et al. (Possemiers et al., 2006) elucidated the mechanism of the intestinal activation of phyto-estrogens and showed that a high inter-individual variability exists in the capacity of the intestinal bacteria to perform this activation. Selection of specific metabolic phenotype *in vivo* and use of a fecal sample from that donor, resulted in the establishment of a SHIME with the same metabolic phenotype (= SHIME allows to maintain *in vivo* functionality). Animal (Possemiers et al., 2008) and human trials (Bolca et al., 2007) confirmed these *in vitro* data.
- Sulfasalazine is a pro-drug historically used for the treatment of inflammatory diseases in the gut. Sulfasalazine is partially absorbed in the small intestine (approx. 30%). The residual part enters into the colon, where it is reduced by the metabolic activity of the gut microbiota to to sulfapyridine and 5-ASA. The pro-drug behaved similarly in vivo and in the SHIME (Molly et al., 1994) (Figure 7).
- A high similarity between *in vitro* and *in vivo* data was also found for the metabolism of prebiotics. When introducing the same human fecal sample in germfree rats (Van den Abbeele et al., 2011) and in the SHIME model (Van den Abbeele et al., 2013b), similar fermentation profiles by specific microbial groups were found to be enhanced by specific prebiotics (i.e. arabinoxylans and inulin). Another study with inulin (Van de Wiele et al., 2004) confirmed that the administration of inulin to the SHIME model led to a 2-times increase of butyrate and propionate production by the microbiota and induced specific quantitative (1 log unit) and qualitative changes in the bifidobacterial community. The effects of inulin administration in a clinical validation study confirmed the predictive power and scientific quality of the SHIME with highly similar effects on bifidobacteria and butyrate production.

In the probiotic field, a typical example of validation of SHIME results is a study related to cholesterol-lowering activity of *Lactobacillus reuteri*. Using the SHIME model, it was shown that this probiotic strain exerted a high specific bile salt hydrolase activity, which alters bile

salt circulation in the intestine and the body. This altered bile salt metabolism may then lead to a cholesterol-lowering effect. Validation of the effect of the probiotic on cholesterol levels in pigs, showed a significant decrease of total and LDL cholesterol (De Smet et al., 1998).

Advantages and limitations of the system.

The advantages correlated with the use of a SHIME technology platform for experimental purposes can be listed as follows: i) presence of two to four full GIT in the same system (i.e. TWINSHIME to QuadSHIME) to study the mechanism of action of products and ingredients; ii) possibility to work with volumes close to the *in vivo* ones; iii) possibility to culture the intestinal microbiota in the different colonic compartments for periods up to several months. This allows studies based on repeated daily dosing strategy to evaluate the adaptation of the activity and composition of the microbiota to a specific treatment; iv) the M-SHIME allows to accurately mimic the mucosal microbial colonization. Due to its close proximity to host epithelial cells, the mucosal microbiome is thought to have an intrinsically higher potency to modulate gut health, and by extension, human health; v) the modular setup, which characterizes the SHIME, makes possible to explore the inter-individual variability in microbiome behavior upon specific treatments; vi) finally, an important read-out from SHIME experiments consists of the evaluation of host-microbe interactions. Colon suspension can be brought in direct contact with host epithelial cells. This allows assessing to what extent changes in microbiome composition, microbial metabolites, signaling molecules or antigens have differential effects at the level of the host in terms of gut barrier permeability and parameters related to inflammation. As any other *in vitro* simulator, the SHIME suffers of the absence of a physiological environment. Moreover, water and metabolites absorption are not routinely simulated in the colonic compartment.

Engineered Stomach and small INtestinal - ESIN

Origins of the system

The Engineered Stomach and small INtestinal -ESIN- system is a new multi-compartmental dynamic *in vitro* model of the human stomach and small intestine (Guerra et al., 2012). This model has been developed by the University of Auvergne (Clermont-Ferrand, France) to overcome some limitations identified in the current *in vitro* multi-compartmental gastrointestinal models, even in the most complete like TIM and SHIME. Indeed, such models do not allow a close imitation of real food bolus entering the stomach, as they proceed with mixed food rather than with food particles of a realistic size. They also do not reproduce the differential gastric emptying of liquids and solids as observed during digestion in human. Then, ESIN presents an original architecture, especially for the gastric compartment that has been patented (Alric and Denis, 2009).

Short description of the system

ESIN is composed of six successive compartments: a meal reservoir allowing a progressive introduction of food particles with a realistic size into the gastric compartment, a salivary ampoule dedicated to a progressive mixing of food with saliva, the stomach and the three parts of the small intestine, the duodenum, jejunum and ileum. This model reproduces the main parameters of human

digestion: body temperature, temporal and longitudinal changes in pH, salivary, gastric, pancreatic and biliary secretions, transit times, chyme mixing and passive absorption of digestion products.
The most striking innovation of ESIN is the architecture of its gastric compartment that enables to reproduce the biphasic nature of gastric emptying observed *in vivo*. An indentation inside the gastric chamber allows the passage of small size particles (< 2 mm) and liquids in a second chamber. Large size particles (> 2 mm) stay in the main chamber to be further degraded. Two openings, each connected to a peristaltic pump allow the differential gastric emptying of "liquids" and "solids", respectively. These two pumps are programmed to follow specific profiles as observed in human: the "liquids" emptying follows an exponential "Elashoff" curve (Elashoff et al., 1982) without a lag phase period, while "solids" emptying fulfills a linear law after a 30 min lag phase (Siegel et al., 1988).

Validation of the system towards in vivo data animal and/or human

The model has been validated for pharmaceutical applications against *in vivo* data in human (Guerra et al., 2016). Two model drugs were studied: an immediate release form of paracetamol and a sustained release form of theophylline. Both *in vitro* and *in vivo*, the drugs were ingested with a glass of water. In ESIN, the amount of absorbed paracetamol and theophylline was measured in the dialysis samples while in human, saliva (paracetamol) or blood samples (theophylline) were collected (Souliman et al., 2007; Souliman et al., 2006). Paracetamol and theophylline tablets showed similar absorption profiles in ESIN and in healthy subjects (Figure 9). For theophylline, a level A *in vitro in vivo* correlation (IVIVC) was established with a slope of 1.097 and a correlation coefficient (r^2) of 0.989, showing the predictive value of the *in vitro* system. These results demonstrate the high level of efficacy of ESIN in mimicking the behavior of soluble drugs in the human gastrointestinal tract.

Advantages and limitations of the system

As ESIN is a new model, it has been validated up to now only for pharmaceutical applications during liquid digestion. Additional validation experiments are necessary to validate the model during digestion of solid foods and for nutritional or microbiological applications. In its current state, the model doesn't include resident microbiota, but the small intestinal compartments of the model has been designed to allow inoculation with human fecal sample and their maintaining under anaerobic conditions by flushing with nitrogen.

SIMulator Gastro-Intestinal: simgi®

Origin of the system

The simgi® (SIMulator of the GastroIntestinal tract) has been developed at the Institute of Food Science Research CIAL (CSIC-UAM, Madrid, Spain). It is a computer-controlled gastrointestinal *in vitro* model designed to simulate the physiological processes taking place during digestion in the stomach and small intestine, as well as to reproduce the colonic microbiota responsible for metabolic bioconversions in the large intestine.

Short description of the system

The simgi® comprises five interconnected compartments that simulate the stomach, small intestine and three stages of the large intestine that can operate jointly or independently. The gastric compartment consists of two cylindrical transparent and rigid methacrylate plastic modules covering a reservoir of flexible silicone walls where the gastric content is mixed by peristaltic movements. The peristalsis is achieved by changing the pressure of water that flows in the jacket between the plastic modules and the reservoir. The stomach compartment has different ports for input of experimental food components, gastric juice, and acid.

The small intestine consists in a double jacket glass reactor vessel stirred that receives the gastric content and mixes it with pancreatic juice and bile. The stages of the large intestine are simulated in three double jacket stirred glass reactors. The pH in the colonic units named ascending (AC), transverse (TC) and descending (DC) is controlled by addition of NaOH and HCl. When the digested content of the small intestine is transferred to the proximal colon compartment, the transit of colonic content between the AC, TC and DC compartments is simultaneously initiated at the same flow rate. The intestinal and colonic vessels contain ports for the transit of intestinal content, sampling, continuous flushing of nitrogen allowing a permanent anaerobic atmosphere and control of pH and temperature.

Flow rates, compartment volumes, pH, temperature and pressure are computer controlled through a programmable logic panel (Unitronics Vision 120TM) and the system stores the on-line monitored values such as volumes pumped, temperature, and pH during the whole experiment.

Validation of the system towards *in vivo* data

Milk whey proteins have been used as model proteins to follow the gastric digestion outcome (Miralles et al., 2015). Progress of protein degradation was followed by SDS-PAGE and band integration. Intact protein decline agreed with data reported in human subjects after whey proteins ingestion (Sullivan et al., 2014). This study incorporated a detailed peptide profile analysis. Comparison of the resistant protein sequences with those reported in duodenal effluents from mini-pigs fed milk (Barbe et al., 2014), that correspond to the end of the gastric digestion (Barbe, et al., 2014), showed a remarkably close pattern. From the identified sequences in the dynamic model, 73% were common with those reported in the porcine *in vivo* study.

The flexible-modulating characteristics of the system and the computer-control of physiological parameters open possibilities for variation of conditions that would allow the simulation in the simgi® as model of microbial dysbiosis associated to pathological conditions or due to unbalanced diets. Using this model, short fatty acids (SCFA) and ammonium formation under high energy diet (during microbiota stabilization period) followed by a low energy diet (during dietary intervention) have been compared. Shift from high to a low energy diet resulted in a two-fold decrease in the average content of total SCFA of the three colon compartments. Besides, a two-fold increase in the ammonium content in the distal colon compartments (TC and DC) and a remarkable six fold increase in the proximal colon compartment (AC) were accounted when changing from high to low energy diet (Barroso et al., 2015a). The SCFA and ammonium results were contrasted with *in vivo* data from obese subjects where a significant decrease of SCFA and increase of proteolytic products were observed when the individuals consumed high protein diets reduced in total carbohydrates (Russell et al., 2011).

The system allows the development of a stable and colon region specific microbial ecosystem that has been shown representative of the *in vivo* situation in terms of microbial composition and activity

(Barroso et al., 2015b). The evaluation of the polyphenol metabolic activity of the colonic microbiota of two volunteers using the simgi® has demonstrated that moderate red wine consumption produces a significant increase in 3,5-dihydroxybenzoic acid, 3-O-methylgallic acid, vanillic acid, protocatechuic acid and syringic acid (Cueva et al., 2015). This rise was consistent with previous data obtained in human feces in an intervention study using the same wine (Munoz-Gonzalez et al., 2013). However, it has to be noted that the microbiota metabolic activity observed was individual-dependent.

Advantages and limitations of the system

The advantage of the model is associated to its flexible modulating characteristics and the automated control of the working parameters that can be adjusted to physiological conditions parameters. The peristaltic mixing movements of the stomach are one of the distinctive technical features.

The microbiota stabilized in the simgi® has demonstrated to induce the phenotypical maturation of human monocyte-derived dendritic cells (Barroso et al., 2015a). However, a limitation is the lack of devices to evaluate the formation of microbial biofilms adhering to the colonic epithelium and the simulation of intestinal absorption to remove end products of microbial metabolism to prevent inhibition of the colon microbiota.

Conclusion and perspectives

The present paper reviews some of the main *in vitro* dynamic digestion systems currently available. It has however to be emphasized that all the systems presented in this review are not at the same stage of development. Indeed, systems like the TIM and the SHIME models have been developed more than 20 years ago and have been regularly improved during all these years. Other systems such as the ESIN, simgi® or DIDGI® have been developed more recently. Nevertheless, from these examples, it is clear that dynamic *in vitro* digestion systems, when programmed with physiologically-relevant parameters, can mimic the complexity of the digestive process. However, one can wonder whether when a system is validated for the digestion of a certain food it is relevant for other types of foods and it might be useful to validate those systems for, at least, families of foods with similar rheological properties (liquids, solids, gels, foams…). Food structure is not always taken into account in this system and food needs sometime to be submitted to drastic physical dispersion (ultra-turrax, blender etc) before being submitted to digestion in order to avoid blockage of the system tubes. Connection with a mastication simulator could be an added value for the digestion of solid foods. Other improvements could be envisaged to make these systems even more relevant. Absorption is over simplified but coupling of the dynamic digestion systems with cellular models (Caco-2, HT-29, IPEC-J2 or co-culture of Caco-2 and HT-29MTX) could allow to better simulate the epithelial transport (Déat et al., 2009). The absence of microbiota in the distal parts of the small intestine can appear as a limit. In the future, dynamic digestion systems will probably become compulsory for understanding the mechanisms of food digestion, especially because of the increased ethical and economic constraints of *in vivo* trials. They will also become key players in the field of drug delivery which will also require microsystems able to investigate the release of expensive pore molecules in small volumes. Some microfluidic devices have already been developed to perform protein digestion before identification by mass spectrometry (Jansson et al., 2012; Kecskemeti and Gaspar, 2017) and the devices developed could be interesting starting points for the development of new

"microdigestors". Interestingly, microfluidic has been used to study the digestion of one lipid droplet (Marze et al., 2014). Microsystems could allow to run digestion experiments in parallel, allowing to screen several compounds with high throughput.

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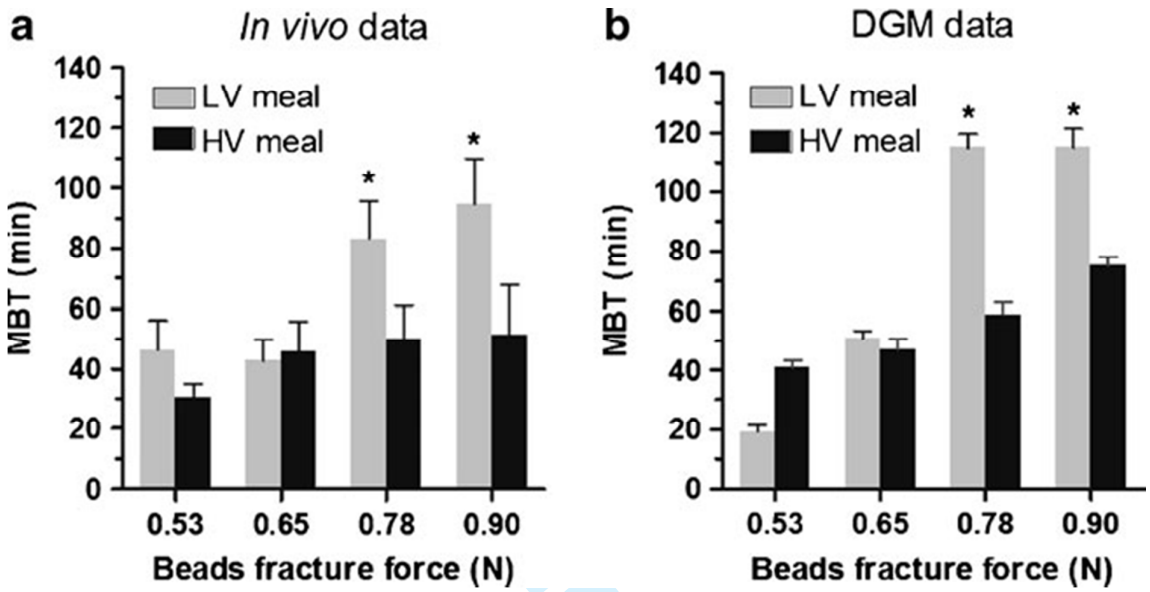

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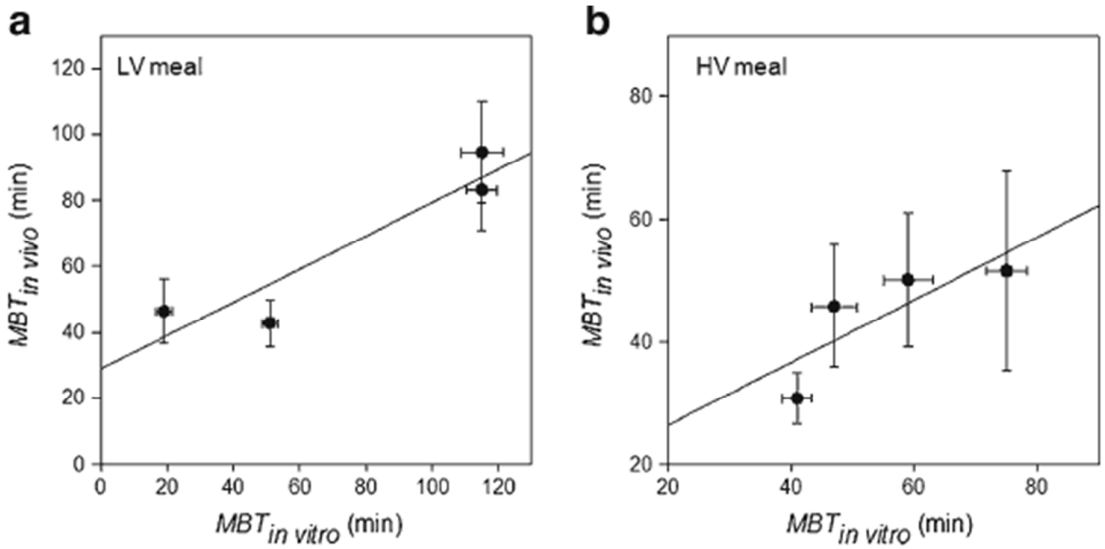

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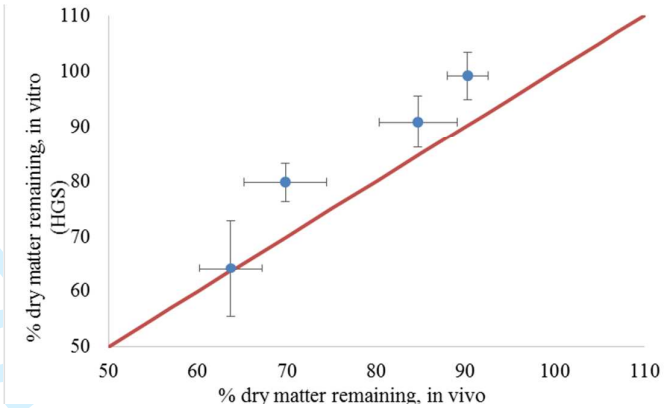

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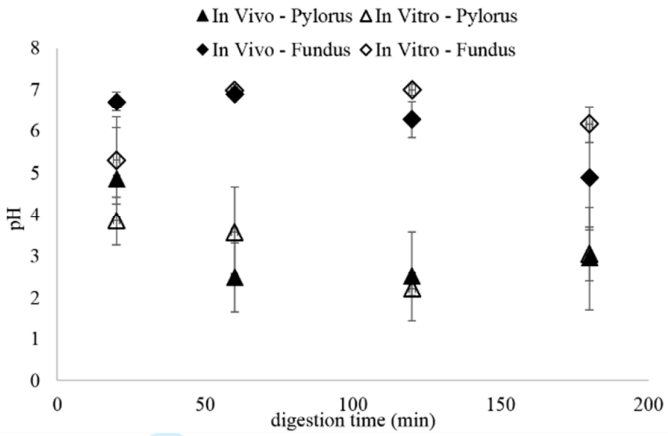

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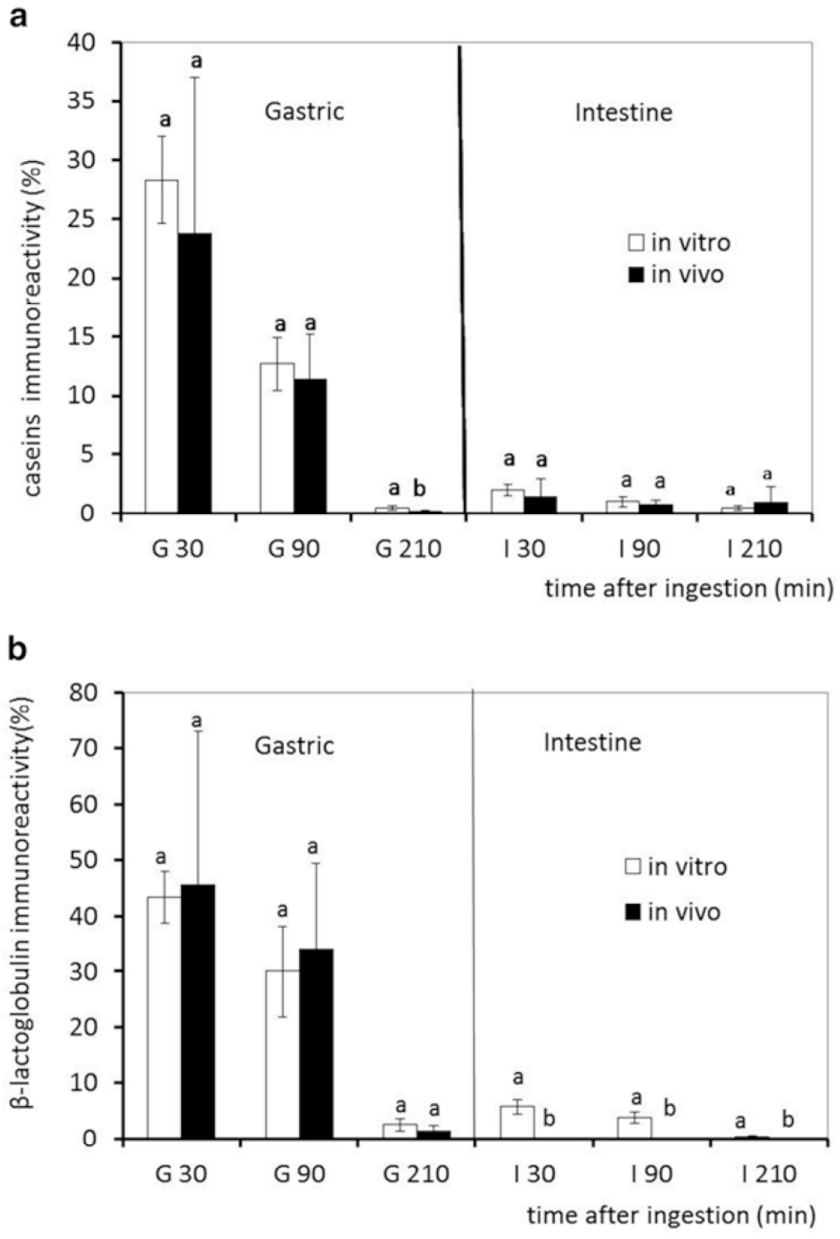
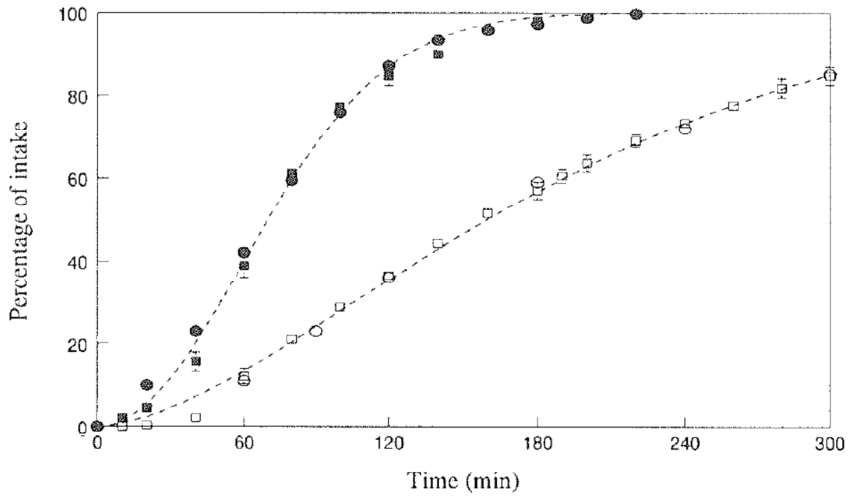

a
caseins immunoreactivity (%)
Gastric
Intestine
in vitro
in vivo
G 30
G 90
G 210
I 30
I 90
I 210
time after ingestion (min)
b
β-lactoglobulin immunoreactivity(%)
Gastric
Intestine
in vitro
in vivo
G 30
G 90
G 210
I 30
I 90
I 210
time after ingestion (min)

Figure 5



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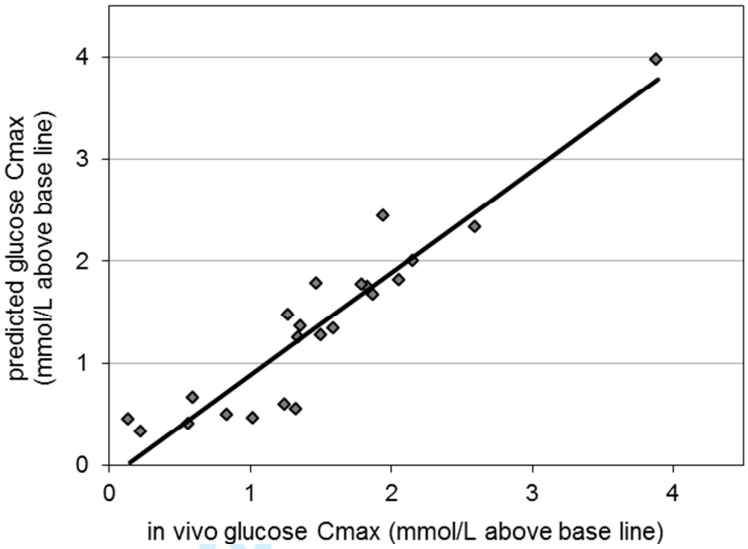

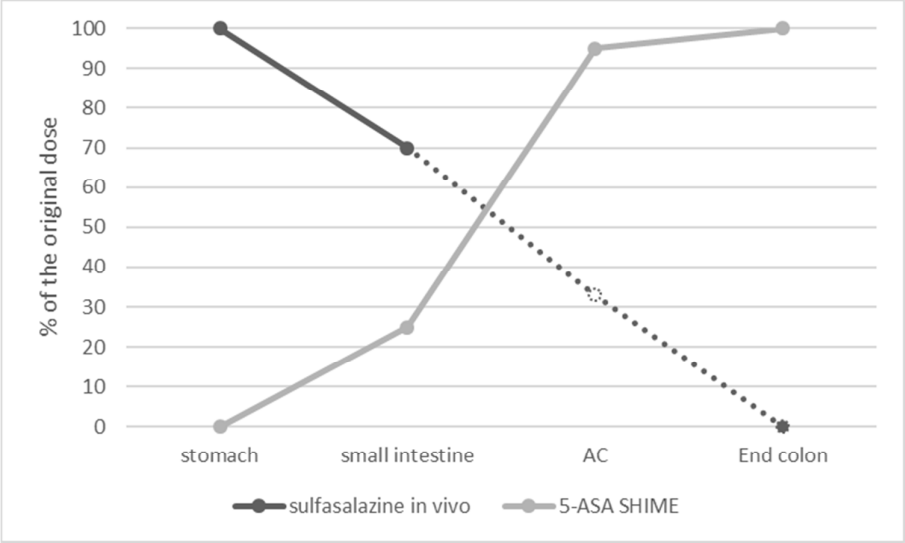

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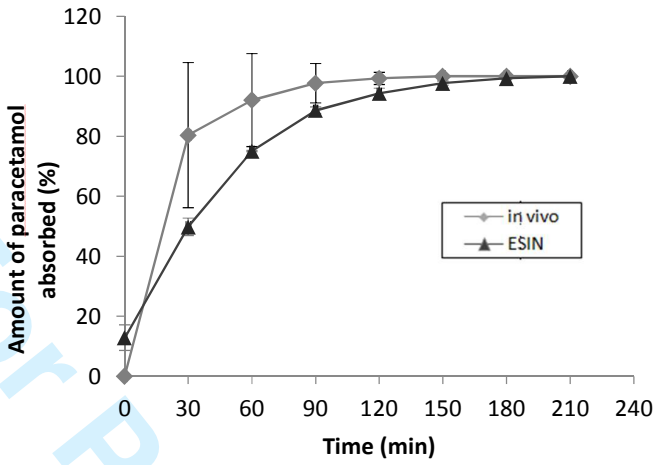
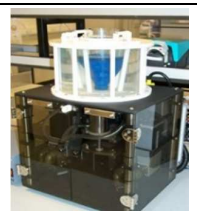
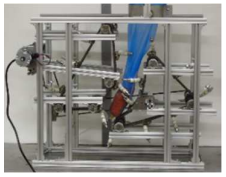
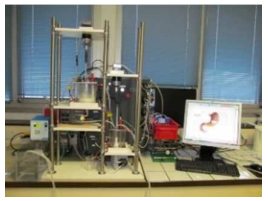


Figure 9

System	Body temp.	Control of gastric pH	Mixing in the stomach	Gastric emptying	Control of intestinal pH	Digestive secretions	Intestinal transit	Intestinal absorption	Intestinal microbiota	Photo
DGM	+	+	Contractions by water pressure with piston and barrel	+	N/A	Saliva Gastric fluid HCl	N/A	N/A	N/A	
HGS	+	+	Contractions by mechanical driving device	+	N/A	Saliva Gastric fluid HCl	N/A	N/A	N/A	
ARCOL	+	N/A	N/A	N/A	N/A	N/A	+ colonic retention time controlled by the inlet flow of nutritive medium, outlet flow of fermentation medium and volume inside the bioreactor	+ passive absorption of water and microbial metabolites with a dialysis system using hollow fibers	+	
DIDGI	+	+	Rotational stirring	+	+	Saliva Gastric fluid HCl Intestinal fluid Bile NaHCO3	+	-	-	

TIM-1	+	+ Meal specific curves	+ Contractions flexible wall by water pressure. Also for small intestinal compartments	+ Simulation of pyloric sphincter. Meal specific curves	+ Site specific for duodenum, Jejunum and ileum	+ Saliva Gastric fluid HCl Intestinal fluid Pancreatic juice Bile NaHCO3	+ Based on meal specific gastric emptying, intestinal transit and ileal-emptying curves, controlled with peristatic valve-pumps.	+ Jejunum and ileum: Dialysis for water soluble compounds. Filtration for lipid soluble compounds	+ TIM-2: Complex high density ($>10^{11}$ cfu/g) microbiota of human or animal origin	
tiny-TIM + AGC	+	+ Meal specific curves Incl. infant conditions	+ Contractions flexible walls for corpus, proximal and distal antrum. Also for small intestinal compartment	+ Simulation of pyloric sphincter. Meal specific curves	+ Conditions for duodenum of 'overall' small intestine	+ Saliva Gastric fluid HCl Intestinal fluid Pancreatic juice Bile NaHCO3	+ Based on meal specific gastric emptying. Optional: ileal emptying curves controlled with peristatic valve-pump.	+ Dialysis for water soluble compounds. Filtration for lipid soluble compounds	+ TIM-2	
SHIME	+	+	± Magnetic Stirring	+	+	+ Saliva Gastric fluid HCl Intestinal fluid Bile NaHCO3	+	+	+ Luminal and mucosal microbiota	

ESIN	+	+ HCl for the stomach and $NaHCO_3$ for the small intestine	+ 2 pistons inside cylinders with back and forth frequency fixed at 3 movements/min	+ Differential gastric emptying of solids and liquids with 2 separate pumps	+	Saliva Gastric fluid HCl Intestinal fluid Bile NaHCO3	+	+ Passive absorption of water and digestion products with dialysis fibers in the jejunum and ileum	-	
SIMGI	+	+	Contractions Water pressure	+	+	Saliva Gastric fluid HCl Intestinal fluid Bile NaHCO3	+	+	+	

Figure captions

Figure 1. Mean Breaking Time (MBT) of agar gel beads for four bead breakdown forces for both low-viscosity (LV—grey bars) and high-viscosity (HV—black bars) meals. n=9 for the LV and n=8 for the HV for each bead strength. Panel b in the DGM; n=5 for each bead strength. *p<0.05 vs. each of the two lower beads strengths for the LV meal.

Figure 2. Correlation of the in vitro MBT (DGM) and in vivo MBT for the beads at the different breaking forces. Panel a LV meal. Panel b HV meal. Straight lines are the regression lines. Bars represent SE

Figure 3. Comparison of gastric emptying of dry matter from an in vivo study with growing pigs (Bornhorst et al., 2013) and from the HGS in vitro system (Phinney, 2013). Values are averages (n = 3, in vitro; n = 6, in vivo) with error bars representing the standard deviation. The solid line represents a 1:1 correlation.

Figure 4. Comparison of intragastric pH between an in vivo study with growing pigs (Bornhorst et al., 2014) and from the HGS in vitro system (Phinney, 2013). Samples were taken at two intragastric locations: Pylorus, representing a location near the pyloric sphincter (or HGS emptying tube), and Fundus, representing a location at the top of the gastric fundus (or top of HGS gastric vessel). Values are averages (n = 3, in vitro; n = 6, in vivo) with error bars representing the standard deviation.

Figure 5. Comparative residual concentration of total casein (a) and β-lactoglobulin (b) determined by ELISA after in vitro (white) and in vivo (black) digestion.

Figure 6. Cumulative gastric and ileal delivery of a meal expressed as a percentage of total meal intake: *in vivo* (human n=7) gastric (●) and ileal (O) delivery of yoghurt and gastric (■) and ileal (□) delivery of blue dextran in the TIM-1 system simulating the gastrointestinal transit of yoghurt.

Figure 7. Prediction of glycaemic response in humans based on the digestion of carbohydrates and bioaccessibility of glucose and fructose in tiny-TIM in combination with *in silico* modelling of the insulin response: correlation (r=0.94) between predicted blood glucose Cmax and measured blood Cmax in humans for 22 different carbohydrate products.

Figure 8. Measurement of the concentration of the sulfasalazine in vivo (stomach, small intestine and end of the colon) and related 5-ASA production in vitro. Data on the concentration of sulfasalazine in the ascending colon in vivo are not available and no pro-drug was detected in the fecal samples. Adapted from Molly et al. 1994 (Molly, et al., 1994).

Figure 9. Paracetamol absorption in ESIN and in healthy human volunteers. Results are expressed as mean cumulative percentages ± standard deviations (n=3 in vitro and n=8 in vivo. * In vitro percentages statistically different from in vivo ones ($P < 0.05$))

Table 1. The dynamic digestion systems investigated and their main characteristics