EXTENDING IN VITRO DIGESTION MODELS TO SPECIFIC HUMAN POPULATIONS: PERSPECTIVES, PRACTICAL TOOLS AND BIO-RELEVANT INFORMATION

Carmit Shani Levi[a], Paula Alvito[bc], Ana Andrés[d], Ricardo Assunção[bc], Reyes Barberá[e], Stéphanie Blanquet-Diot[f], Claire Bourlieu[g], André Brodkorb[i], Antonio Cilla[e], Amélie Deglaire[h], Sylvain Denis[f], Didier Dupont[h], Ana Heredia[d], Sibel Karakaya[j], Concetta Valeria Lucia Giosafatto[k], Loredana Mariniello[k], Carla Martins[bc], Olivia Ménard[h], Sedef Nehir El[j], Gerd Elizabeth Vegarud[l], Ellen Ulleberg[l], and Uri Lesmes[a *]

[a]*Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa, Israel*

[b]*Food and Nutrition Department, National Institute of Health, Lisbon, Portugal*

[c]*CESAM, Centre for Environmental and Marine Studies, University of Aveiro, Portugal*

[d] *Institute of Food Engineering for Development, Universitat Politècnica de València, Valencia, Spain*

[e]*Faculty of Pharmacy. University of Valencia, Valencia, Spain*

[f]*Engineering and Development of Food and Drug, University of Auvergne, Clermont-Ferrand, France.*

[g] *IATE 1208, INRA, CIRAD, SupAgro, Montpellier, France*

[h]*STLO Agrocampus Ouest, INRA, Rennes, France*

[i] *Teagasc Food Research Centre, Cork, Ireland*

[j] *Food Engineering Department, Ege University, İzmir, Turkey*

[k]*Department of Chemical Sciences, University of Naples Federico II, Naples, Italy*

[l]*Norwegian university of Life Sciences, As, Norway*

* Corresponding Author: Assistant Prof. Uri Lesmes

Laboratory of Chemistry of Foods and Bioactives

Department of Biotechnology and Food Engineering,

Technion – Israel Institute of Technology, Haifa 32000, Israel

Tel. 972-77-8871869; Fax.972-4-8293399

Email: lesmesu@bfe.technion.ac.il

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Abstract

Background. *In vitro* digestion models show great promise in facilitating the rationale design of foods. This paper provides a look into the current state of the art and outlines possible future paths for developments of digestion models recreating the diverse physiological conditions of specific groups of the human population.

Scope and Approach. Based on a collective effort of experts, this paper outlines considerations and parameters needed for development of new *in vitro* digestion models, e.g. gastric pH, enzymatic activities, gastric emptying rate and more. These and other parameters are detrimental to the adequate development of *in vitro* models that enable deeper insight into matters of food luminal breakdown as well as nutrient and nutraceutical bioaccessibility. Subsequently, we present an overview of some new and emerging *in vitro* digestion models mirroring the gastro-intestinal conditions of infants, the elderly and patients of cystic fibrosis or gastric bypass surgery.

Key Findings and Conclusions. This paper calls for synchronization, harmonization and validation of potential developments in *in vitro* digestion models that would greatly facilitate manufacturing of foods tailored or even personalized, to a certain extent, to various strata of the human population.

Key words: Food digestion, *In vitro* digestion, gastric, infants, elderly, Gastro-Intestinal disorders

Abbreviations: CF-Cystic Fibrosis; EFFoST-European Federation of Food Science and Technology; GBP-Gastric Bypass; GI-Gastrointestinal; GIT-Gastrointestinal tract; IBD-inflammatory bowel disease; IVD- In vitro digestion; PTL:Pancreatic Triglyceride Lipase; SG: Sleeve Gastrectomy

1. Introduction

1.1 *In vitro* models for food research

In vitro digestion (IVD) modelling is a vivid field of research that shows great promise in facilitating the development of foods and oral formulations based on better understanding of their digestive fate in the stomach and small intestine in as well as downstream ramifications to the gut microbiome(Bornhorst, Gouseti, Wickham, & Bakalis, 2016; Guerra, et al., 2012; Hur, Lim, Decker, & McClements, 2011; Payne, Zihler, Chassard, & Lacroix, 2012). Although human or *in vivo* animal studies are still considered a "gold standard" for tackling issues of bioaccessibility, absorption, bioavailability, metabolism and excretion, IVD methods have the advantage of being more rapid, less labor intensive and having significantly less bioethical restrictions. In fact, various IVD models have been increasingly applied to assess the digestive fate and potential toxicity of ingested natural and engineered nano-materials(Lefebvre, et al., 2015). This has led to great variability in scientific efforts, including some contradicting studies, and stimulated the recent effort of the INFOGEST network of scientists to develop a consensus harmonized static *in vitro* digestion model based on physiologically relevant conditions gathered from humans(Minekus, et al., 2014). This harmonized protocol was validated in a wide inter-laboratory trial(Egger, et al., 2016) and is currently pending on-going efforts to correlate findings of protein digestibility with an *in vivo* trial in pigs and biochemical assays with human aspirates (yet to be published). However, these and other numerous scientific publications focus on IVD systems designed for evaluating the digestive fate of foods and oral formulations in the adult alimentary canal.

During a dedicated workshop held by the European Federation of Food Science and Technology (EFFoST) in Athens on November 2015, we found that current physiological literature offers professionals additional opportunities to recreate the unique and specific gastro-intestinal (GI) functions of other human populations, such as infants, the elderly and more. Such intriguing possibilities would open new opportunities to study and develop foods and oral formulations better tailored to the needs of such specific populations. Based on the pooled and accumulated experience of the INFOGEST network, it was decided to help a systematic and responsible orchestration of relevant global efforts, maximize synergisms between researchers and harmonize efforts to develop new IVD models. Thus, this paper provides a look into the current state of the art and paves possible future paths for developments, all with the aim of ensuring adequate and fruitful endeavors and outputs to the food and health community.

1.2 Current status of adult *in vitro* digestion (IVD) models

In vitro digestion models were initially developed to serve as research tools to characterize and clarify the structural and biochemical changes of food components under physiological conditions, caused by alimentary enzymes, GI motility and by the colonic microbiota. In principle, IVD models of the upper GI need to overcome the shortcomings of *in vivo* trials (*i.e.* ethical constraints, low throughput, control over subjects and reproducibility) and account for the most bio-relevant anatomical and physiological considerations mirroring the mouth, stomach, small and large intestine lumen and gut lining. In fact and in spite of their limitations, IVD models are particularly suited for investigating the luminal physiochemical changes in food, matters of bioaccessibility and some aspects of bioavailability.

Historically, efforts to develop IVD models began in the early 1990's with pioneering works to develop reliable, robust, reproducible and bio-relevant tools like the multi-compartmental GI model developed by TNO in the Netherlands(Minekus, Marteau, Havenaar, & Huisintveld, 1995) or the three stage continuous fermentation systems recreating the human colon(Macfarlane, Macfarlane, & Gibson, 1998; Molly, Woestyne, & Verstraete, 1993). Since, the field has boomed with numerous IVD models, ranging from simple static mono-compartmental models to computer-controlled multi-compartmental dynamic IVD models, as reviewed by others (Glahn, Wien, VanCampen, & Miller, 1996; Guerra, et al., 2012; Hur, et al., 2011; McClements & Li, 2010; Payne, et al., 2012; Yoo & Chen, 2006). Recent studies even raised the possibility of using human GI aspirates in IVD models (Ulleberg, et al., 2011) or coupling IVD models with human cell cultures of Caco-2 epithelial cells or Caco-2 co-cultures with HT-29 mucus producing cells (Emmanuelle Deat, et al., 2009; E. Deat, et al., 2009; Vors, et al., 2012). Yet, the low accessibility and stability of human aspirates and the complexity of coupling IVD research with cell cultures, challenge the wide spread use of highly bio-relevant alternatives over simple protocols currently used in IVD models. Further, *in vitro* cell culture systems have been coupled to some IVD models to enable investigating questions of cellular uptake and brush border enzymatic breakdown, which better elucidate the bioavailability of specific substances(Emmanuelle Deat, et al., 2009; E. Deat, et al., 2009; Manione, et al., 2015; Vors, et al., 2012).

Concomitantly, various efforts reported to develop and apply sophisticated IVD models that are intended to be more realistic, encompassing various aspects of digestion dynamics (e.g. physiological acid secretion and gastric emptying), mass transport

phenomena (*i.e*. absorption and diffusion) and rheological aspects (*i.e*. mixing) (Blanquet, et al., 2004; Dekkers, Kolodziejczyk, Acquistapace, Engmann, & Wooster, 2016; Kong & Singh, 2010a; Levi & Lesmes, 2014; Mercuri, Lo Curto, Wickham, Craig, & Barker, 2008; Shani-Levi, Levi-Tal, & Lesmes, 2013; Tharakan, Norton, Fryer, & Bakalis, 2010; Yoo & Chen, 2006). To date, both advanced and simple IVD models have be used to investigate a variety of systems. Examples include investigations of simple high purity protein solutions, multi-component model systems like emulsions and even more real foods, like dairy gels and pasta. These and other investigations have significantly advanced our understanding of the interplay between food ingredients, food products and the alimentary canal of healthy adults. Such insights include not just understanding of food breakdown but also its impact on gastro-intestinal functions, e.g. gastric emptying and intestinal motility, as detailed by others (Grundy, et al., 2016; Houghton, Hickson, & Read, 1987; Meyer, Elashoff, & Lake, 1999; Sarosiek, et al., 2010).

Identified research needs. Despite the various hectic activity in the field of understanding food's digestion in adults, there is still much room for further advancements and breaching of current gaps in knowledge and capabilities. The various discussions held during the Athens workshop identified that amongst future advancements in the field, research should include efforts: [I] To improve the bio-relevance of luminal composition and dynamics (e.g. pH profiles and use of gastric lipases); [II] To validate and/or correlate IVD data with *in vivo* findings; [III] To recreate the 3D micro-architecture of the intestinal lining and mucosa through co-cultures (e.g. Caco-2 and HT29 cell lines, grown on various scaffolds); and [IV] To develop predictive

in silico models. All of these topics were enthusiastically discussed in separate work groups during the workshop and are expected to bring up further scientific publications.

1.3 Rationale and approach for extending IVD models

Advancements in the field of food science and technology need to address numerous challenges that humanity is and will be facing in the 21 century(Floros, et al., 2010). These challenges will include feeding the growing and ageing world population, better and sustainable use of natural resources as well as improving our ability to exploit foods' potential to prevent diseases and maintain health promote wellness. In this respect, personalized or tailored nutrition seem highly promising and challenging strategies (Joost, et al., 2007; Qi, 2014; Zeevi, et al., 2015). Such endeavors will require bridging manufacturing capabilities and product engineering to meet the specific needs of the consumer. Based on the demonstrated success in the field of infant formula development, IVD models harbor great potential to facilitate relevant developments of foods tailored to thespecific GI capabilities of specific human starta such as elderly people, pregnant women, patients of various Inflammatory Bowel Diseases (IBD) and even diabetics.

Thus, this paper outlines the considerations and parameters needed for development of new IVD models as well as an overview of some of the new and emerging IVD models and the relevant physiological information, all with the aim of stimulating adequate and fruitful endeavors and outputs to the food and health community. Adapting the consensus INFOGEST protocol scheme, a basic static model is suggested to comprise of an oral, gastric and intestinal phase(Minekus, et al., 2014). Each phase should address the composition of the relevant simulated fluid (ionic and enzymatic composition), the time of processing and the nature of the bolus/chyme (liquid, semi-solid or solid and dilution

ratio with the bodily secretions). The selection of the quantitative aspects for the operational parameters should rely on information gathered from the most relevant human studies with good statistical power (*i.e*. avoiding studies with less than 10 subjects as a rule of thumb). In circumstances where no human data can be found, developers should either make their best effort to rationally approximate the values or attempt to determine them directly as a part of a human trial. Thus, any new IVD model should clearly define its parameters, justify their selection and support it with relevant references.

2. Practical considerations for developing IVD models

Human GI physiology is a complicated semi-continuous set of bioreactors that are intertwined with the hematological, hormonal and nervous systems and change during life(L R Johnson, 2007; Remond, et al., 2015; Tortora & Derrickson, 2011). This highly complex nature of the GI limits the ability to recreate its entire functions in an *in vitro* model. However, many aspects of luminal digestion can be mirrored in IVD models using reliable and detailed information on the digestive system that can be found in the scientific literature. Therefore, it is imperative to understand the limitations of each model and ensure they do not collide with the research hypotheses. To this end, it is also imperative to be aware of and address the key anatomical and physiological parameters of the relevant GI organs.

The process of food digestion is an orchestrated series of bioprocessing operations that involve the breakdown of food, the release of nutrients, their uptake or downstream fermentation before their ultimate removal from the body through defecation. During digestion in the upper GI, food structure is broken down in the mouth, stomach and small

intestine through complex reactions and interactions involving chemical and mechanical processes (Ferrua & Singh, 2010; L R Johnson, 2007; Sensoy, 2014). Therefore, the following section discuss the most critical physiological parameters that are essential for *in vitro* digestion models.

2.1. Oral phase

Oral processing involves mastication and mechanical breakdown of food into a soft mass, termed bolus which is a mixture of processed food and saliva(DeSesso & Jacobson, 2001). This short phase is detrimental to the sensorial perception of food and can be viewed as a coarse mechanical processing step with little chemical changes(Aken, Vingerhoeds, & Wijk, 2011; van Vliet, van Aken, de Jongh, & Hamer, 2009). This first step of digestion involves mixing food with salivary fluid that contains about 99% water in addition to various electrolytes and proteins, including enzymes such as amylase(Aps & Martens, 2005; Rantonen, 2003). Saliva is continuously secreted into the oral cavity by parasympathetic control. While resting, the flow rate is about 0.5 ml/min; but upon stimulation, the secretion increases 3 to 4- fold with maximal flow rates of 10 ml/min(AC, 1991b). Healthy adults will produce 500–1500 ml saliva per day(Aps & Martens, 2005). Salivary fluid composition depends on the flow rate: at higher flow rates, sodium, calcium, chloride, bicarbonate, amylase increase while phosphate and mucin concentrations decrease, and the potassium concentrations show little change. Salivary pH values also fluctuate between fasted to fed state with values of 6.2-7.4 to 7.4-7.6, respectively(C. H. M. Versantvoort, Van de Kamp, E. and Rompelberg, C.J.M., 2004). The key salivary enzyme is α-amylase that hydrolyzes starch and related α-(1,4)-linked polysaccharides(Nagler & Hershkovich, 2005; Shern, Fox, & Li, 1993). Mucin is also an

important component of saliva with studies indicating it to induce emulsion flocculation(Sarkar, Goh, & Singh, 2009, 2010; Singh & Ye, 2013; Vingerhoeds, Silletti, de Groot, Schipper, & van Aken, 2009). Yet, commercial mucins are partially hydrolyzed mixtures of mammalian mucins which limit their bio-relevance when applied in IVD models. In addition, there is some debate on the possible existence and activity of lingual lipase with a report indicating lingual lipase is active between pH 2-6.4, indicating that this enzyme is active from the mouth to the small intestine(Hamosh, 1994).

2.2 Gastric phase

Following bolus formation in the oral phase, the stomach further processes the bolus into a semi-solid chyme within four distinct regions: cardiac, fundic, body and the pyloric regions (Ferrua & Singh, 2010; Kong & Singh, 2010b). Gastric juice comprises of hydrochloric acid, enzymes (pepsin and gastric lipase), various electrolytes, mucus, intrinsic factor and hormones with approximately 2 L of gastric juice secreted daily and 0.7 L secreted after a typical meal(Kopf-Bolanz, et al., 2012; Seeley, Stephens, & Tate, 1992). Parietal cells lining the stomach wall are responsible for the secretion of hydrochloric acid into the gastric lumen and bicarbonate into the bloodstream. The activity of these cells is responsible for the unique pH of the stomach which dynamically changes during digestion from 1.5-2.0 in the fasted state to 3.0-7.0 in the fed state. Gastric acidity induces protein denaturation and precipitation, hydrolytic reactions (e.g. breakdown of starch) and significantly reduces bacterial counts in the gastric lumen. The post-prandial pH rise in the stomach is attributed to the buffering capacity of the ingested food and the Parietal cells generate a pH gradient that over the course of time reverts luminal pH back to the fasted state values. The pH profiles depend on age and clinical

conditions of the consumer (**Table 1**) and can have various ramifications to the properties of ingested food systems, such as emulsions and gels(Dekkers, et al., 2016; Shani-Levi, et al., 2013). Gastric lipolysis and proteolysis are tightly linked(AC, 1991b; Sams, Paume, Giallo, & Carriere, 2016). The key gastric proteolytic enzyme, pepsin, is activated from its precursor pepsinogen (secreted by chief cells) via acid hydrolysis. The activated enzyme, which is also equated with commercial porcine pepsin, has a wide range of activity with optimal activity at pH 2 and inactivate just above pH 6.5(Johnston, Dettmar, Bishwokarma, Lively, & Koufman, 2007). Pepsin is a non-specific protease and therefore hydrolyses itself (a reaction termed auto-pepsinolysis) and other enzymes present in the lumen. Another gastric enzyme is gastric lipase which is also activated by the acidic environment in the stomach(Sams, et al., 2016). Gastric lipase presents *sn*-3 regiospecificity thus it hydrolyses triglycerides into *sn*-1,2diglycerides and one free fatty acid, pancreatic triglyceride lipase colipase dependent which is *sn*-1,3 regioselective lipase (Miled, et al., 2000). However, commercial gastric lipase is hard to find and is currently neglected in many IVD models, thought it initiates lipolysis and release free fatty acids which activate pancreatic triglyceride lipase.

The pyloric sphincter controls gastric emptying into the small intestine and is affected by three major factors: volume of the meal, its osmotic pressure and caloric content. Approximately 2 kcal/per minute are delivered through the pylorus to the duodenum(Campbell, 2015; Sams, et al., 2016). Furthermore, gastric emptying has been well described by the Elashoff equation (Elashoff, Reedy, & Meyer, 1982).

2.3 Small intestinal phase

Gastric chyme is gradually emptied into the small intestine, where most of the chemical breakdown and absorption occur mediated by auxiliary secretions of the liver, gall bladder, pancreas and intestinal epithelia. Chyme entering from the stomach to the small intestine are neutralized using bicarbonate and the pH increases from 2 to 6.2 in the duodenum, which is the first segment of the small intestine(Kalantzi, et al., 2006). The main degradation of food starts in the duodenum into which about 1.2-1.5 L of pancreatic juice is secreted daily(L.R. Johnson, 2007). The jejunum and ileum are the later sections of the small intestine where digestion and absorption are completed before indigested fractions are pushed into the colon. Due to the anatomical complexity of the small intestine one cannot easily find data on food digestion in these segments. The pancreatic juice contains a mixture of enzymes, proenzymes, protease inhibitors, sodium bicarbonate and other electrolytes that are secreted in parallel and gradually over the course of 3-4 hours, depending on the meal ingested. The pancreatic secretions contain a variety of enzymes in their pro-enzyme forms and include protrypsin, prochymotrypsin, proelastase, procarboxypeptidases, pancreatic lipase and α-amylase in addition to ribonuclease and deoxyribonuclease(Boivin, Lanspa, Zinsmeister, Go, & Dimagno, 1990; Keller & Layer, 2005). Currently, IVD models make use of ill-defined mixtures of pancreatin or concoct enzyme mixtures mainly containing trypsin and α-chymotrypsin. Every day, the human liver produces about 0.6-1.0 L of bile, which are stored in the gallbladder(Seeley, et al., 1992). Bile acids are steroid acids composed mainly from taurocholic acid, glycocholic acid, taurochenodeoxycholic acid and glycochenodeoxycholic acid which are equal in concentration(Hofmann, 1999). In addition to enzymes delivered into the lumen,

enzymes located in the epithelial brush border contribute to the further digestion of food. The brush border enzymes include glycosidases (dextrinase, glucoamylase), peptidases (aminopeptidase, carboxypeptidase, dipeptidase) and phophatases(Holmes & Lobley, 1989). Altogether, the functions of the auxiliary organs in the fasted and fed conditions are stimuli responsive and are mainly affected by the composition of the ingested meal and the physical state of the consumer. Unfortunately, a wide range of enzyme outputs and activities (units) are reported due to different focus of the studies; type of diet (calories), specific nutrient (lipid, carbohydrate, protein or minerals) or non-nutrients (pharmaceuticals, drugs etc.) and physical properties of the meal(Armand, et al., 1996; O'Keefe, et al., 2003). Further, there are inconsistencies in data on the pancreatic enzyme activities due to the differences in biochemical assays used to characterize these secretions: ranging from use of natural or synthetic standards such as casein, BAEE, TAME, BTEE, measurement modes (potentiometric, colorimetric, spectrophotometric), calculation methods up to the definition of enzymatic units of activity. Examples for values found in literature are summarized in **Table 1**. Mechanically, the small intestine has a segmented nature of pushing chyme further down the GI and this segmentation motion was recently shown to be critical for luminal mixing and mass transfer (Tharakan, et al., 2010).

As denoted, the small intestine is the major site of absorption of small molecules, which can occur passively through diffusion or actively through various transporter systems in the gut wall(L R Johnson, 2007). Further processing of materials can then take place within epithelia, e.g. lipid packing into chylomicrons. Transit time through the small intestine varies according to the diet caloric density; the rheological/mechanical

properties (e.g. viscosity or gelling) and with consumer parameters, such as age and health (e.g. 2h for healthy adult and 3h for infant(Blanquet, et al., 2004)).

2.4 Large intestinal phase

Undigested and un-absorbed foodstuffs and bodily secretions transit into the large intestine through the ileocecal valve. In this last part of the human GI tract, water and electrolytes are re-absorbed and bacterial fermentation of fiber and un-digestible food components occurs before bulk material is excreted(Moran & Jackson, 1992). The colon is increasingly recognized for its milieu of bacteria, fungi, protozoa and archaea and rich metabolic activity equated to that of the human liver(Nardone & Malfertheiner, 2011; O'Hara & Shanahan, 2006; Olszewska & Jagusztyn-Krynicka, 2012; Turnbaugh, et al., 2007). Recent studies of the human colon microbiome have established various links between nutrition, the microbiome and health with evidence that microbiomes are affected by age, gender, diet, culture, geography and various physiological/pathological states(Albenberg & Wu, 2014; D'Argenio, et al., 2013; Flint, 2012; Holscher, et al., 2015; Olszewska & Jagusztyn-Krynicka, 2012). Therefore, it is no surprise that the field of IVD models of the human colon are also a vibrant field, as reviewed by others(Payne, et al., 2012). In essence, the colon hosts immense bacterial counts in three distinct loci: the proximal, transverse and distal colon, which vary in their steady state pH with values of 5.8, 6.2 and 6.8, respectively with transit times of 12-36h. Metabolically, the microbiome is highly active with both glycolytic and proteolytic activities noted and about 90% of fermented indigestible polysaccharides being metabolized into short-chain fatty acids. In addition, the mucosa lining of the colon is a major site for passive absorption of small metabolites and close interactions with the immune system(Clemente, Ursell, Parfrey, & Knight, 2012).

3. IVD models for specific populations

3.1. Infants

The functionality of human gastro intestinal tract (GIT) develops in the first year of life with newborns (< 28 days of life) and infants up to six months possessing an immature digestive system compared to older infants (>6 months) or the fully mature GI of an adult (**Figure 1**). Moreover, prematurity affects strongly the digestive capabilities, with decreased GI functionality in preterm babies compared to full-term newborns(J Bruce, 2012; D. Kelly & Coutts, 2000; E. J. Kelly & Newell, 1994; Ménard, Monfils, & Tremblay, 1995). In fact, there are various differences between infants and adults mainly in some digestive enzymes and a relatively elevated gastric pH (3.5-6.5), as exhaustively reviewed(Abrahamse, et al., 2012; Bourlieu, et al., 2014; T. T. Nguyen, Bhandari, Cichero, & Prakash, 2015a, 2015b). Briefly, infant digestion process neglects oral phase due to liquid meals rapidly transiting through the oral cavity (5-10 sec). Small stomach storage capacity, affecting meal frequency, transit and volume, increases quickly during the first month of life from 10-20 mL up to 90-150 mL per meal(Abrahamse, et al., 2012; Bourlieu, et al., 2014). Infant fasting gastric pH is less acidic than of an adult (respectively 4-5 vs. 2 in the fasted state) which may change gastric proteolysis, as optimal activity of pepsin is 1.5-2.2(Henderson, Hamosh, Armand, Mehta, & Hamosh, 1998; Li-Chan & Nakai, 1989; Schlamowitz & Peterson, 1959). Reduced pepsin secretion in newborns, 10-20% from adult levels, is another physiological reason explaining the limited gastric proteolysis (15%) reported for infants(Bourlieu, et al., 2014; Didier Dupont, et al., 2010; Romano, Giosafatto, Masi, & Mariniello, 2015). Pepsin secretion increases with postnatal age and is more immature in preterm infants(AC, 1991a).

In respect to intestinal digestion, proteolysis in infants has similar pH and trypsin concentrations as those in the intestine of adults, whereas chymotrypsins and carboxypeptidases-B just account for about 10% to 60% of the activity found in adults(Edginton & Fotaki, 2010; Lebenthal & Lee, 1980). Regarding lipid digestion, gastric lipase activity and output are similar in preterm(Roman, et al., 2007), full-term infants and adults(Armand, et al., 1996; Sarles, Moreau, & Verger, 1992). However, pancreatic lipases do vary between infants and adults with pancreatic triglyceride lipase (PTL) being the dominant intestinal lipolytic enzyme in adults while PTL-related protein 2 and bile salt-stimulated lipase are the key lipases in infants(Lindquist & Hernell, 2010). In light of the high fat diet of infants(Hamosh, 2006), human breast milk contains endogenous lipase (bile salt-stimulated lipase mainly, 3.6-5.3 U/mL of milk) that compensates for the low amount of pancreatic lipases (5-10% the concentration found in adults) and low concentration of bile salts (50% of adult values)(Lebenthal, Lee, & Heitlinger, 1983). Regarding carbohydrate digestion, scarce data suggests low values of pancreatic amylase are found in the GI of infants aged less than 6 months. Thus, carbohydrate digestion in infants is believed to be highly facilitated by swallowed salivary α-amylase (at birth average of 10 % of the adult level but highly variable(Christian, Edwards, & Weaver, 1999; Sevenhuysen, Holodinsky, & Dawes, 1984)) or mammary α-amylase. In addition, reports also indicate the infant GI performs carbohydrate digestion through lactase, sucrose-isomaltase and glucoamylase (with activities of ~50% above that of adults)(Bourlieu, et al., 2014; T. T. Nguyen, et al., 2015a).

Another important step in infant GI maturation is colonic colonization of the infant gut with microbiota, which begins at birth and is an important player in the maturation and education of the immune system. Development of the infant microbiota is characterized by rapid and large changes in microbial abundance, diversity and composition, until around 3 years of age when the microbiota becomes adult-like(Matamoros, Gras-Leguen, Le Vacon, Potel, & de La Cochetiere, 2013). Introduction of solid foods into the infant diet leads to a marked shift in microbial composition with an increase in clostridial species and a decrease in *Bifidobacterium* and *Enterobacteriaceae*. Many factors may influence the development of the gut microbiota in infants, such as mode of delivery, type of maternal diet, geographical location and consumption of antibiotics(Arrieta, Stiemsma, Amenyogbe, Brown, & Finlay, 2014).

Based on the current physiological knowledge of the infant GI, various static and dynamic IVD models have been applied by researchers(Blanquet, et al., 2004; de Oliveira, et al., 2015; D. Dupont, et al., 2010; Roussel C, 2016; Shani-Levi, et al., 2013). Yet, the development of a harmonized static infant IVD is needed. One of the most formidable challenges in this respect is the clear definition of the consumer being recreated since digestive parameters are highly affected by gestational and postnatal age. For instance preterm newborns compared to full-terms of same age have higher gastric pH resulting from more frequent feeding, lower pepsin activity (10% of adult activity at four weeks vs. 30% in full-terms), faster gastric emptying, more limited gallbladder contraction index, lower concentration of electrolytes in pancreatic fluid, no amylase secretion and lower global pancreatic activity(Bourlieu, et al., 2014).

To date, several studies have depicted static infant IVD models applied for studying various aspects of protein and lipid digestion. These various models are summarized in **Table 2**. As can be noted, various discrepancies are found in these models and include discrepancies in gastric pH, ill-defined enzymatic proteolytic activity of enzymes and large variance in experimental duration. For example, the enzyme activity was most of the time not checked experimentally or based on the supplier's general characteristics, which hampered experiment replication in other laboratory. After an estimation of the pepsin units per mL of milk, a very large range of values was observed, ranging from 4 to 18563 U/mL of milk (**Table 2**). An *in vivo* study by Armand et al. (1996) reported an average postprandial value of 63 U/mL of gastric content/kg of bodyweight of preterm infants, which would correspond to 425 U/mL of milk for a term newborn of 4.25 kg and a meal to secretion ratio of 63:39 v/v(Armand, et al., 1996). In respect to the intestinal phase, pH was homogeneous (6.5 -7.5), but duration varied largely from 5 to 120 min and the meal proportion in the total volume varied from 25 up to 76 %. After an estimation of pancreatin content within each model, a factor of 30 between the maximum and the minimum values was found across models, which remains lower than that for pepsin (a 4500 fold difference). Bile salts, arising from a porcine or bovine bile extract or from purified bile salts, were estimated to vary by a factor of 10 across models.

In all models presented in **Table 2**, no clear definition of the infant stage was given, except for Fogleman et al. (2012)(Fogleman, Cohen, Sakamoto, & Allen, 2012), who aimed to mimic preterm infant digestion. Further, there are some dynamic IVD models described in recent literature(Blanquet, et al., 2004; Ménard, et al., 1995; Menard, et al., 2014; Shani-Levi, et al., 2013) ,(de Oliveira, et al., 2015; Havenaar, et al., 2013; Roussel

C, 2016). In essence, these models try to recreate some of the dynamic aspects of digestion, e.g. gastric pH profiles post meal ingestion and gastric emptying rates. One of these has even been validated against *in vivo* data of proteolysis kinetics obtained in piglets(Menard, et al., 2014). The TIM model developed by TNO (Netherlands) was adapted to simulate the GI of newborns, infants and toddlers (0–1, 1–6, and 6–24 months of age, respectively) after ingestion of various types of food (formula milk, milk and cereals) and validated for these three age groups against published pharmacokinetic data on paracetamol(Havenaar, et al., 2013). However, in this study, not all GI parameters applied to this commercial IVD model have been made publicly available. The same model has been very recently adapted to mimic, based *on in vivo* data, the gastric and small intestinal conditions of infant from 6 months to 2 years(Roussel C, 2016). Some dynamic colonic models have also been developed(C Cinquin, Le Blay, Fliss, & Lacroix, 2004; Cécile Cinquin, Le Blay, Fliss, & Lacroix, 2006a, 2006b). The composition and diversity of the bacterial community, as well as its metabolism, was found to be well correlated with those found *in vivo* in infant feces.

Altogether, infant IVD models are increasing in their applicability to food research, however, the variances and discrepancies found in current infant IVD model call for future efforts to better define a simple, harmonized and consensus infant static IVD model, such as that obtained for an adult IVD model(Minekus, et al., 2014) as well as sophisticated dynamic IVD models. All of these should be developed with a rationale similar to that applied by the infant formula industry, *i.e.* focusing on specific and defined target populations such as stage one for 0-3 months, stage 2 for 3-6 months etc.

3.2. Elderly

Elderly nutrition, pharmacology and overall health care have been identified as one of the rising global challenges(UN, 2013). Ageing is typically accompanied by a milieu of changes including substantiated alterations and deterioration of gut functions, such as secretion of digestive fluids and enzymes, saliva, GIT contractions and chyme passage rates(Di Francesco, et al., 2005; Feldman, Cryer, McArthur, Huet, & Lee, 1996; Laugier, Bernard, Berthezene, & Dupuy, 1991; Nagler & Hershkovich, 2005; Russell, et al., 1993; Salles, 2007; Vellas, et al., 1988). Due to the irreversible nature of the changes in GIT functions, there is a growing need to deepen our understanding of foods' digestive fate in the elderly GI. This would facilitate rational design of foods to accommodate elderly physiological capabilities, improve nutrient bioaccessibility and bioavailability and help combat elderly malnutrition. Despite comprehensive knowledge on the GI deterioration with age and its ramifications to elderly malnutrition(Remond, et al., 2015), there are scant IVD models of the elderly GI found in literature. One most recent study assessed the antioxidant capacity of a milk protein matrix in aged women, both *in vitro* and *in vivo*(Power-Grant, et al., 2016). However, the target population of the study focused solely on women in the ages of 50-70. In relation to IVD models recreating elderly digestive conditions, two recent studies have been identified to apply *in vivo* data to the modeling parameters (Denis, et al., 2016; Levi & Lesmes, 2014). The first reports the set-up of a dynamic gastro-intestinal elderly (> 70 years old) model based on commercial bioreactors with details on all the parameters used and the rationale of their selection(Levi & Lesmes, 2014). The second, reports an adaptation of the TNO gastrointestinal model (TIM) to the specific digestive conditions of the elderly (> 65 years old) and is used to study meat protein dynamic digestion(Denis, et al., 2016). A

summary of the digestive conditions applied in these models are given in **Figure 2,** also summarizes conditions of the elderly population at the colon. Similar to the field of infant IVD modelling, elderly digestion models require not only harmonization but also validation and clearer definition of the elderly being studied.

3.3. Developing IVDs for humans with GI disorders

In light of the centrality of the GI system in human health and disease, various studies present information on human GI disorders. These are defined as diseases and/or conditions that interfere with the intake, digestion, and/or absorption of nutrients, causing various clinical symptoms and are broadly defined as maldigestion. Physiologically, the spectrum and underlying causes of GI disorders is immense from such conditions causing discomfort (e.g. lactose malabsorption) to those compromising health (e.g. pancreatic insufficiency in cystic fibrosis patients). All in all, these conditions arise from altered GI functions which lead to various effects on the disintegration, breakdown and uptake of nutrients and consequently on health(Högenhauer, 2010). Some common factors that interfere with food digestion and related disorders, infections and surgical procedures linked to them are summarized in **Table 3**. In respect to food breakdown and bioaccessibility, many of the situations described may be mirrored using IVD models, as such conditions have been found to arise from variance and abnormalities in digestive parameters such as changes in gastric/intestinal pH, secretion of digestive juices and transit times. Other disorders such as food allergies, autoimmune disorders (celiac sprue), Crohn's disease, obesity or diabetes are linked to interferences with the absorption and/or metabolism of nutrients from the food(Nolan, Johnston, & Walters, 2012), hence IVD models for such conditions require much more sophistication in their *in vitro* recreation,

492 if at all feasible. Yet, efforts to develop IVD models for specific strata of the population
493 would offer useful tools not only in the development of new tailored foods but also
494 improving relevant nutritional guidelines.
495 One example for such a potential novel IVD model is for the community of Cystic
496 Fibrosis (CF) patients that has over 35,000 cases registered in Europe(Colombo &
497 Littlewood, 2011). At least 85% of CF patients have pancreatic insufficiency of lipases,
498 resulting in fat malabsorption and binding patients to the use of pancreatic enzyme
499 supplements. Armand et al. (2004) studied the effect of diet on gastric lipase levels and
500 fat digestion in children with CF and reported that gastric lipase was high in cystic
501 fibrosis patients maintained on fat-rich diets(Armand, et al., 2004). Further, Gelfond et al.
502 (2013) measured the intestinal pH and GI transit profiles in CF patients(Gelfond, Ma,
503 Semler, & Borowitz, 2013). Based on this and other *in vivo* reports, the development of
504 an IVD model of a CF patient need to focus on the unique secretion of pancreatic fluid
505 and bile, both critical parameters in lipid digestion. Analytical studies show a 3.8-fold
506 higher content of glycoconjugates than tauroconjugates in human aspirates(Brodlie, et al.,
507 2015). Thus, artificial bile should reflect composition and imbalances between tauro- and
508 glycol-conjugates isomers and bile concentration should be low to reflect the decreased
509 bile secretion (1mM). In respect to enzymatic activity, CF patients are pancreatic
510 insufficient when pancreas function is below 10% than that of a healthy adult. Then, the
511 pancreatine activity in a CF model should be 10-fold lower than that considered in
512 healthy adults.
513 Another potential IVD model to be developed is that of Gastric Bypass (GBP) patients
514 (bariatric surgery patients). GBP surgery is one of the most common and effective

515 treatments for morbid obesity but can also be used to address conditions such as type 2
516 diabetes or hypertension. Available physiological literature data on the digestive process
517 are limited to indirect, with postprandial serum or urine measurements or scintigraphy
518 evaluation of gastric emptying. Gastric emptying is reported to be very rapid for liquids,
519 based on D-xylose in serum, from 18.6±6.9 min prior to GBP to 7.9±2.7 min after
520 GBP(Wang, et al., 2012). Extremely rapid pouch emptying was reported for water vs.
521 whey proteins vs. olive oil as preloads (30 min) for a liquid glucose drink (t_{50} 3.8±0.9 vs.
522 4.1±0.6 vs. 3.6±0.5 min, respectively) and for a solid beef patty meal (1.6±0.7 vs. 1.1±0.6
523 vs. 1.3±0.5 min, respectively) (N. Q. Nguyen, et al., 2016). Bojsen-Moller et al. (2015)
524 (Bojsen-Moller, et al., 2015) observed accelerated caseinate digestion and amino acid
525 absorption (C^{13} leucine), resulting in faster and higher but more transient postprandial
526 elevation of plasma amino acids. Overall, the incidence of a dumping syndrome, defined
527 as a rapid gastric emptying, is also elevated after GBP(Horowitz, Collins, Harding, &
528 Shearman, 1985).
529 Sleeve Gastrectomy (SG), whereby the stomach duodenum connection remains intact but
530 the volume of the stomach is drastically reduced, has been also been used as an option for
531 surgical treatment of obesity. In such patients, gastric emptying half times (t_{50}) were
532 reported to be drastically reduced for both liquids and solids food (SG vs. control group:
533 34.9±24.6 vs. 13.6±11.9 min for water and 78±15.01 vs. 38.3±18.77 min for solids [egg
534 sandwich])(Horowitz, et al., 1985). The growing body of evidence on the ramifications of
535 GBP procedures on GI function could enable the development of a relevant IVD model.
536 Such a model would require a short gastric phase between 30 and 60 minutes, probably
537 coupled with a higher pH of 3.5-4.0 compared to the pH 3.0 used in an adult IVD model.

However, without luminal data, only estimates are possible. Yet, a comprehensive effort should be done to mine the literature or conduct *in vivo* experiments to determine enzymatic activity of pepsin and pancreatic enzymes as well as bile compositions.

The examples of CF and GBP patients are only two possibilities for novel IVD models that can be developed and subsequently validated. Other GI conditions and abnormalities can be recreated in IVD models pending relevant *in vivo* data is collected or found in scientific literature. These stress out the potential of expanding the horizons of IVD models based on the rationale exploitation of medical research.

4. Conclusion

The current modern food production system is complex, dynamic and constantly strives to fabricate safe and nutritious food products and solutions. Amongst the various efforts, researchers and manufacturers seek to rationally process, structure and formulate foods towards healthier outcomes for the consumer. These include development of food delivery systems for protection of bioactives ingredients added to food, controlling and targeting their release in the human gastrointestinal tract and affecting various dimensions of consumer well-being, e.g. shaping the colon microbiome or inducing satiety and satiation. All of these efforts rely on understanding the underlying principles guiding food's digestive fate. An understanding, which can be significantly advanced thanks to the soaring number of studies using in vitro and in vivo digestion models.

As part of the food-health revolution and evolution of food manufacturing towards tailored and personalized foods, the potential of IVD models could be maximized when extended to recreate various strata of the human population. The development of IVD models should rely on better and extensive understanding of *in vivo* digestion conditions

561 in different groups of the population but would offer better opportunities to develop
562 relevant products with high bioefficacy. Evidently, such novel tools for food and
563 nutritional research would necessitate adequate standardization and validation to ensure
564 synchronization of efforts and success. Such efforts would also greatly benefit from the
565 deposition and gathering of relevant information in a database where food and health care
566 professionals could upload *in vivo* data or *in vivo in vitro* correlations and put together
567 pieces of puzzles needed in the development of new IVD models. In light of the concern
568 over rising prevalence of chronic diseases and challenges in feeding the world, nutritional
569 management of health and disease prevention are challenges at the footsteps of dedicated
570 professionals. The authors of this paper hope that it will stimulate relevant progress in the
571 field and help orchestrate global efforts towards the shared goal of advancing food
572 science and technology.
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Table and Figure Captions

Table 1. Physiological characterization of human gastro-intestinal fluids.

Table 2. Literature review of the proposed *in vitro* static models for infant gastro-intestinal digestion.

Table 3. Factors interfering with food digestion and related disorders.

Figure 1. Summary of the developing digestive physiology in the human infant. HGL- Human Gastric Lipase, PTL- pancreatic triglyceride lipase, BSSL-bile salt-stimulated lipase, PTLRP2- pancreatic triglyceride lipase-related protein 2.

Figure 2. Summary of the developing digestive physiology in the elderly.

Table and Figure

Table 1

	Fasted	Fed	Suggested References
Oral phase pH Fluid output Transit time of meal	 6; 6.2 – 7.4 0.5 mL/h	 7 ; 7.4-7.6 10 mL per meal 10 sec – 2 min	 Guyton A.C. 1991(AC, 1991a) Versantvoorf C.H.M. 2004(C. H. M. Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips, 2005) Guerra A. 2012(Guerra, et al., 2012)
Gastric phase pH Gastric lipase Transit time of meal	 1.5 - 2.0 120 - 130 U/mL	 3.0 – 7.0 15 min – 3 hours	 Dressman J.B.1986(Dressman, et al., 1990) Sams L. 2016(Sams, et al., 2016) Guerra A. 2012(Guerra, et al., 2012)
Small intestine pH Bicarbonate secretion Total fluid output Total proteolytic activity Trypsin Output activity Chymotrypsin Output activity Amylase output activity Lipase Bile Surface tension Transit time	 5.4-6.5 15-27 mM/h 118 mL/h 5.6-25.4 U/mL 1-4.5mM 32.3mN/m	 5.5-7.5 9000/24h 212/mL 200-300/h 50-100-500 U/mL 33-77 IU/mL 70-150 U/mL bw/15min 500-1000 (U/mL) 97-450 (IU/mL) 3000-6000 (U/mL) 100-400 (U/mL) 234-524 (IU/mL) 5.8-39 uM/mL TDC[a], GC[b], GCDC[c], GDC[d] 2.2-11.2mM 28mN/m 2-5h	 Dressman JB. 1990(Dressman, et al., 1990) Ekmekcioglu C. 2002(Ekmekcioglu, 2002) Ulleberg E. 2011(Ulleberg, et al., 2011)
Large intestine pH Bacterial load Short chain fatty acids Total fluid vol Transit time		 6.4-7.0 $1x10^{11}$-10^{12} CFU/g material 125-139 mM 187 ml 12-24h	 Payne A.N. (Payne, et al., 2012)

595 [a] Taurodeoxycholate; [b] Glycocholate; [c] Glycochenodeoxycholate; [d] Glycodeoxycholate

596 **Table 2**

Refs	Meal	Gastric phase							Intestinal phase					
		pH	Duration (min)	Meal : secretion ratio (v/v)	Lipase content (/mL of meal)	Pepsin[b] content	Pepsin[c] (U/mL of meal)	Molar ratio pepsin / meal	pH	Duration (min)	Meal : secretion ratio (v/v)	Enzyme(s) used[c]	Pancreatin equivalent (mg/mL of meal)	Bile[d] (mg/mL of meal)
Chatterton et al., 2004(Chatterton, Rasmussen, Heegaard, Sorensen, & Petersen, 2004)	Human milk	2, 3, 3.5, 4, 5 or 6.5	60	99 : 1	supernatant of gastric juice from 2 neonates		-	-	no duodenal phase					
Dupont et al., 2010(D. Dupont, et al., 2010)	Purified proteins	3	60	85 : 15	-	22.75 U/mg of protein	273[f]	0.0042	6.5	30	76 : 24	Porcine trypsin : 3.45 U/mg of protein Bovine chymotrypsin : 0.04 U/mg of protein	5.91[f,h]	1.32[i]
Fogleman et al., 2013(Fogleman, et al., 2012)	Human milk	5	120	66 : 34	42.5 mg	2.5 mg/ml of milk	7500	0.3317	7	120	40 : 60	Pancreatin : 2 mg/mL of SDF[g]	0.63	3.75

Lueamsaisuk et al., 2014(Lueamsaisuk, Lentle, MacGibbon, Matia-Merino, & Golding, 2014) Lueamsaisuk et al., 2015(Lueamsaisuk, Lentle, MacGibbon, Matia-Merino, & Golding, 2015)	Infant formula	2, 3.5, 4.5 or 5.5	120	20 : 50	40 U (*Rhizopus Oryzae* lipase)	4.5 mg/ml of SGF[e] (800-2500 U /mg)	18563	0.2829	No duodenal phase					
Prakash et al., 2014(Prakash, Ma, & Bhandari, 2014)	Infant formula	1.5	60	50 : 50	-	3.2 mg/ml of SGF	9600	0.1463	7	120	25 : 75	Pancreatin : 1.6 mg/ml of digesta	3.2	10

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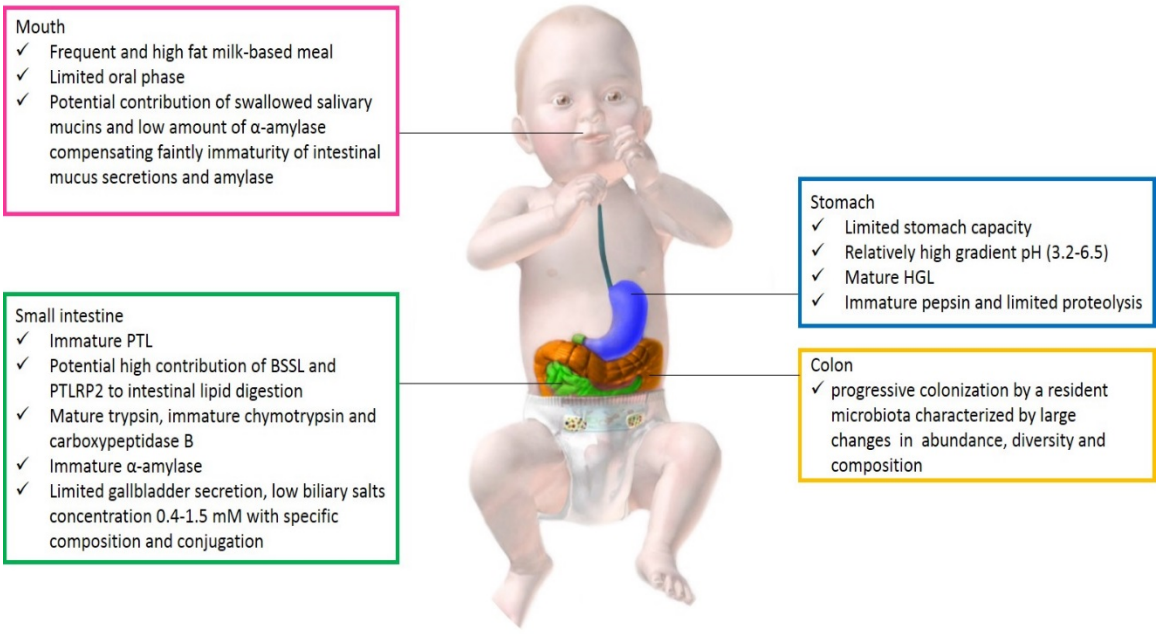
Refs	**Meal**	**Gastric phase**	**Intestinal phase**

		pH	Duration (min)	Meal : secretion ratio (v/v)	Lipase content (/mL of meal)	Pepsin[b] content	Pepsin[c] (U/mL of meal)	Molar ratio pepsin / meal	pH	Duration (min)	Meal : secretion ratio (v/v)	Enzyme(s) used[c]	Pancreatin equivalent (mg/mL of meal)	Bile[d] (mg/ mL of meal)
Wada & Lonnerdal, 2014(Wada & Loennerdal, 2014) Wada & Lonnerdal, 2015(Wada & Loennerdal, 2015)	Defatted bovine milk Defatted human milk	4	15	-	-	0.08 mg /mg of protein	2880[f]	0.020 0.044	7	5	-	Pancreatin: protein ratio of 1:62.5	0.19[f]	-
Dall'Asta et al., 2015(Dall' Asta, et al., 2015)	Human milk	4.5	35	15 : 9	-	0.013 mg/ml of milk	4.0	0.0001	7.5	120	15 : 20	Porcine pancreatin : 9 mg/mL of SDF	3.6	6 (bovine bile)
N-Guyen et al., 2015(T. T. Nguyen, et al., 2015a)	Infant formula	4	As detailed for Dupont et al., 2010											
Liu et al., 2016(Liu, et al., 2016)	Milk protein concentrate	3	60	50 : 50	-	113.8 U/ml of SGF	113.8	0.0008	6.5	60	25 : 75	Bovine trypsin : 8.6 U/ml of SDF	2.46[h]	4[i]

Table 3

Causes of maldigestion	Related diseases	Impact
Digestive enzyme deficiency	Chronic pancreatitis, cystic fibrosis, pancreatic carcinoma	Hydrolysis of proteins, carbohydrates and fats
Digestive enzyme inactivation by excess of HCl	Zollinger-Ellison syndrome	
Dissynchrony of enzyme release and inadequate mixing	Hyperthyroidism, post billroth ii procedure (gastrojejunostomy), gastric bypass	
Diminished bile salt synthesis	Cirrhosis	Fat solubilisation Fat soluble vitamins absorbtion
Impared bile secretion	Cystic fibrosis, chronic cholestasis	
Increased bile salt loss	Ileal disease or resection	
Bile salt de-conjugation	Bacterial: overgrowth	
Bacterial consumption of nutrients	Bacterial overgrowth associated to B12 deficiency	Bioavailability of specific nutrients
Reduced gastric acid	Atrophic gastritis associated to B12 deficiency	
Reduced intrinsic factor	Pernicious anemia associated to B12 deficiency	
Cofactors deficiency	Gastric surgery	

610 **Figure 1**



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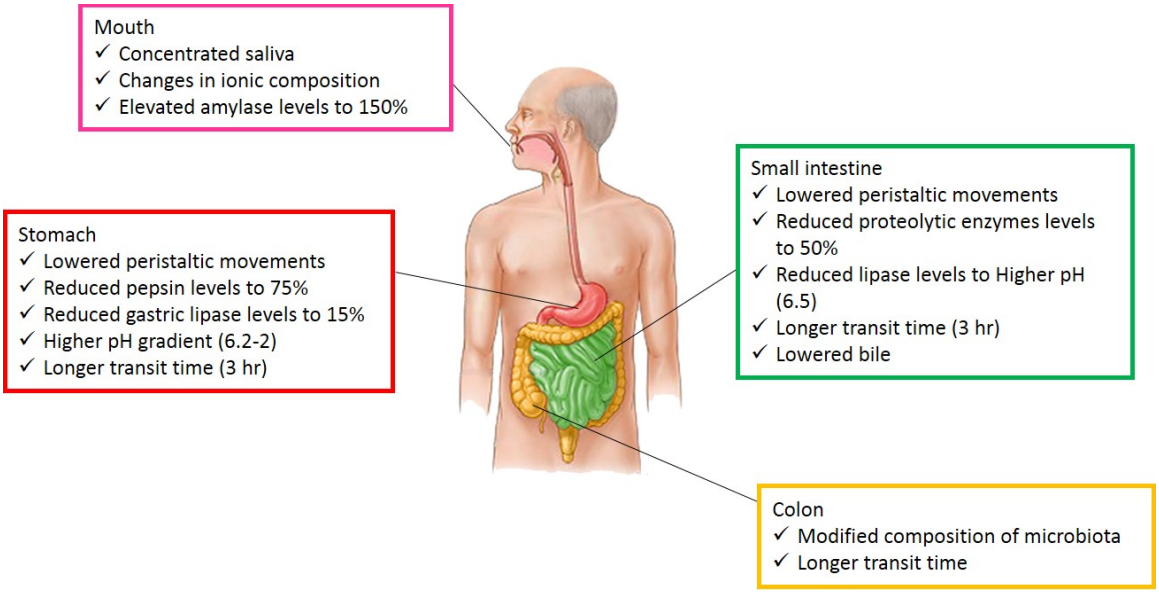
612 **Figure 2**



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