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

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34

35 **Abstract**

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

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

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

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

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

## 58 1. Introduction

### 59 1.1 *In vitro* models for food research

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

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

## 92 1.2 Current status of adult *in vitro* digestion (IVD) models

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

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

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

126 phenomena (*i.e.* absorption and diffusion) and rheological aspects (*i.e.* mixing) (Blanquet,  
127 et al., 2004; Dekkers, Kolodziejczyk, Acquistapace, Engmann, & Wooster, 2016; Kong  
128 & Singh, 2010a; Levi & Lesmes, 2014; Mercuri, Lo Curto, Wickham, Craig, & Barker,  
129 2008; Shani-Levi, Levi-Tal, & Lesmes, 2013; Tharakan, Norton, Fryer, & Bakalis, 2010;  
130 Yoo & Chen, 2006). To date, both advanced and simple IVD models have been used to  
131 investigate a variety of systems. Examples include investigations of simple high purity  
132 protein solutions, multi-component model systems like emulsions and even more real  
133 foods, like dairy gels and pasta. These and other investigations have significantly  
134 advanced our understanding of the interplay between food ingredients, food products and  
135 the alimentary canal of healthy adults. Such insights include not just understanding of  
136 food breakdown but also its impact on gastro-intestinal functions, e.g. gastric emptying  
137 and intestinal motility, as detailed by others (Grundy, et al., 2016; Houghton, Hickson, &  
138 Read, 1987; Meyer, Elashoff, & Lake, 1999; Sarosiek, et al., 2010).

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



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

### 150 1.3 Rationale and approach for extending IVD models

151 Advancements in the field of food science and technology need to address numerous  
152 challenges that humanity is and will be facing in the 21 century (Floros, et al., 2010).

153 These challenges will include feeding the growing and ageing world population, better  
154 and sustainable use of natural resources as well as improving our ability to exploit foods'

155 potential to prevent diseases and maintain health promote wellness. In this respect,  
156 personalized or tailored nutrition seem highly promising and challenging strategies

157 (Joost, et al., 2007; Qi, 2014; Zeevi, et al., 2015). Such endeavors will require bridging  
158 manufacturing capabilities and product engineering to meet the specific needs of the

159 consumer. Based on the demonstrated success in the field of infant formula development,  
160 IVD models harbor great potential to facilitate relevant developments of foods tailored to

161 the specific GI capabilities of specific human starta such as elderly people, pregnant  
162 women, patients of various Inflammatory Bowel Diseases (IBD) and even diabetics.

163 Thus, this paper outlines the considerations and parameters needed for development of  
164 new IVD models as well as an overview of some of the new and emerging IVD models

165 and the relevant physiological information, all with the aim of stimulating adequate and  
166 fruitful endeavors and outputs to the food and health community. Adapting the consensus

167 INFOGEST protocol scheme, a basic static model is suggested to comprise of an oral,  
168 gastric and intestinal phase (Minekus, et al., 2014). Each phase should address the

169 composition of the relevant simulated fluid (ionic and enzymatic composition), the time  
170 of processing and the nature of the bolus/chyme (liquid, semi-solid or solid and dilution

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

## 179 **2. Practical considerations for developing IVD models**

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

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

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

## 198 2.1. Oral phase

199 Oral processing involves mastication and mechanical breakdown of food into a soft mass,  
200 termed bolus which is a mixture of processed food and saliva(DeSesso & Jacobson,  
201 2001). This short phase is detrimental to the sensorial perception of food and can be  
202 viewed as a coarse mechanical processing step with little chemical changes(Aken,  
203 Vingerhoeds, & Wijk, 2011; van Vliet, van Aken, de Jongh, & Hamer, 2009). This first  
204 step of digestion involves mixing food with salivary fluid that contains about 99% water  
205 in addition to various electrolytes and proteins, including enzymes such as amylase(Aps  
206 & Martens, 2005; Rantonen, 2003). Saliva is continuously secreted into the oral cavity by  
207 parasympathetic control. While resting, the flow rate is about 0.5 ml/min; but upon  
208 stimulation, the secretion increases 3 to 4- fold with maximal flow rates of 10  
209 ml/min(AC, 1991b). Healthy adults will produce 500–1500 ml saliva per day(Aps &  
210 Martens, 2005). Salivary fluid composition depends on the flow rate: at higher flow rates,  
211 sodium, calcium, chloride, bicarbonate, amylase increase while phosphate and mucin  
212 concentrations decrease, and the potassium concentrations show little change. Salivary  
213 pH values also fluctuate between fasted to fed state with values of 6.2-7.4 to 7.4-7.6,  
214 respectively(C. H. M. Versantvoort, Van de Kamp, E. and Rompelberg, C.J.M., 2004).  
215 The key salivary enzyme is  $\alpha$ -amylase that hydrolyzes starch and related  $\alpha$ -(1,4)-linked  
216 polysaccharides(Nagler & Hershkovich, 2005; Shern, Fox, & Li, 1993). Mucin is also an

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

## 224 2.2 Gastric phase

225 Following bolus formation in the oral phase, the stomach further processes the bolus into  
226 a semi-solid chyme within four distinct regions: cardiac, fundic, body and the pyloric  
227 regions (Ferrua & Singh, 2010; Kong & Singh, 2010b). Gastric juice comprises of  
228 hydrochloric acid, enzymes (pepsin and gastric lipase), various electrolytes, mucus,  
229 intrinsic factor and hormones with approximately 2 L of gastric juice secreted daily and  
230 0.7 L secreted after a typical meal(Kopf-Bolanz, et al., 2012; Seeley, Stephens, & Tate,  
231 1992). Parietal cells lining the stomach wall are responsible for the secretion of  
232 hydrochloric acid into the gastric lumen and bicarbonate into the bloodstream. The  
233 activity of these cells is responsible for the unique pH of the stomach which dynamically  
234 changes during digestion from 1.5-2.0 in the fasted state to 3.0-7.0 in the fed state.  
235 Gastric acidity induces protein denaturation and precipitation, hydrolytic reactions (e.g.  
236 breakdown of starch) and significantly reduces bacterial counts in the gastric lumen. The  
237 post-prandial pH rise in the stomach is attributed to the buffering capacity of the ingested  
238 food and the Parietal cells generate a pH gradient that over the course of time reverts  
239 luminal pH back to the fasted state values. The pH profiles depend on age and clinical

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

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

261

## 262 2.3 Small intestinal phase

263 Gastric chyme is gradually emptied into the small intestine, where most of the chemical  
264 breakdown and absorption occur mediated by auxiliary secretions of the liver, gall  
265 bladder, pancreas and intestinal epithelia. Chyme entering from the stomach to the small  
266 intestine are neutralized using bicarbonate and the pH increases from 2 to 6.2 in the  
267 duodenum, which is the first segment of the small intestine(Kalantzi, et al., 2006). The  
268 main degradation of food starts in the duodenum into which about 1.2-1.5 L of pancreatic  
269 juice is secreted daily(L.R. Johnson, 2007). The jejunum and ileum are the later sections  
270 of the small intestine where digestion and absorption are completed before indigested  
271 fractions are pushed into the colon. Due to the anatomical complexity of the small  
272 intestine one cannot easily find data on food digestion in these segments. The pancreatic  
273 juice contains a mixture of enzymes, proenzymes, protease inhibitors, sodium bicarbonate  
274 and other electrolytes that are secreted in parallel and gradually over the course of 3-4  
275 hours, depending on the meal ingested. The pancreatic secretions contain a variety of  
276 enzymes in their pro-enzyme forms and include protrypsin, prochymotrypsin, proelastase,  
277 procarboxypeptidases, pancreatic lipase and  $\alpha$ -amylase in addition to ribonuclease and  
278 deoxyribonuclease(Boivin, Lanspa, Zinsmeister, Go, & Dimagno, 1990; Keller & Layer,  
279 2005). Currently, IVD models make use of ill-defined mixtures of pancreatin or concoct  
280 enzyme mixtures mainly containing trypsin and  $\alpha$ -chymotrypsin. Every day, the human  
281 liver produces about 0.6-1.0 L of bile, which are stored in the gallbladder(Seeley, et al.,  
282 1992). Bile acids are steroid acids composed mainly from taurocholic acid, glycocholic  
283 acid, taurochenodeoxycholic acid and glycochenodeoxycholic acid which are equal in  
284 concentration(Hofmann, 1999). In addition to enzymes delivered into the lumen,

285 enzymes located in the epithelial brush border contribute to the further digestion of food.  
286 The brush border enzymes include glycosidases (dextrinase, glucoamylase), peptidases  
287 (aminopeptidase, carboxypeptidase, dipeptidase) and phosphatases (Holmes & Lobley,  
288 1989). Altogether, the functions of the auxiliary organs in the fasted and fed conditions  
289 are stimuli responsive and are mainly affected by the composition of the ingested meal  
290 and the physical state of the consumer. Unfortunately, a wide range of enzyme outputs  
291 and activities (units) are reported due to different focus of the studies; type of diet  
292 (calories), specific nutrient (lipid, carbohydrate, protein or minerals) or non-nutrients  
293 (pharmaceuticals, drugs etc.) and physical properties of the meal (Armand, et al., 1996;  
294 O'Keefe, et al., 2003). Further, there are inconsistencies in data on the pancreatic enzyme  
295 activities due to the differences in biochemical assays used to characterize these  
296 secretions: ranging from use of natural or synthetic standards such as casein, BAEE,  
297 TAME, BTEE, measurement modes (potentiometric, colorimetric, spectrophotometric),  
298 calculation methods up to the definition of enzymatic units of activity. Examples for  
299 values found in literature are summarized in **Table 1**. Mechanically, the small intestine  
300 has a segmented nature of pushing chyme further down the GI and this segmentation  
301 motion was recently shown to be critical for luminal mixing and mass transfer (Tharakan,  
302 et al., 2010).

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

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

#### 310 2.4 Large intestinal phase

311 Undigested and un-absorbed foodstuffs and bodily secretions transit into the large  
312 intestine through the ileocecal valve. In this last part of the human GI tract, water and  
313 electrolytes are re-absorbed and bacterial fermentation of fiber and un-digestible food  
314 components occurs before bulk material is excreted(Moran & Jackson, 1992). The colon  
315 is increasingly recognized for its milieu of bacteria, fungi, protozoa and archaea and rich  
316 metabolic activity equated to that of the human liver(Nardone & Malfertheiner, 2011;  
317 O'Hara & Shanahan, 2006; Olszewska & Jagusztyn-Krynicka, 2012; Turnbaugh, et al.,  
318 2007). Recent studies of the human colon microbiome have established various links  
319 between nutrition, the microbiome and health with evidence that microbiomes are  
320 affected by age, gender, diet, culture, geography and various physiological/pathological  
321 states(Albenberg & Wu, 2014; D'Argenio, et al., 2013; Flint, 2012; Holscher, et al., 2015;  
322 Olszewska & Jagusztyn-Krynicka, 2012). Therefore, it is no surprise that the field of IVD  
323 models of the human colon are also a vibrant field, as reviewed by others(Payne, et al.,  
324 2012). In essence, the colon hosts immense bacterial counts in three distinct loci: the  
325 proximal, transverse and distal colon, which vary in their steady state pH with values of  
326 5.8, 6.2 and 6.8, respectively with transit times of 12-36h. Metabolically, the microbiome  
327 is highly active with both glycolytic and proteolytic activities noted and about 90% of  
328 fermented indigestible polysaccharides being metabolized into short-chain fatty acids. In  
329 addition, the mucosa lining of the colon is a major site for passive absorption of small  
330 metabolites and close interactions with the immune system(Clemente, Ursell, Parfrey, &  
331 Knight, 2012).



### 332 **3. IVD models for specific populations**

#### 333 3.1. Infants

334 The functionality of human gastro intestinal tract (GIT) develops in the first year of life  
335 with newborns (< 28 days of life) and infants up to six months possessing an immature  
336 digestive system compared to older infants (>6 months) or the fully mature GI of an adult  
337 (**Figure 1**). Moreover, prematurity affects strongly the digestive capabilities, with  
338 decreased GI functionality in preterm babies compared to full-term newborns(J Bruce,  
339 2012; D. Kelly & Coutts, 2000; E. J. Kelly & Newell, 1994; Ménard, Monfils, &  
340 Tremblay, 1995). In fact, there are various differences between infants and adults mainly  
341 in some digestive enzymes and a relatively elevated gastric pH (3.5-6.5), as exhaustively  
342 reviewed(Abrahamse, et al., 2012; Bourlieu, et al., 2014; T. T. Nguyen, Bhandari,  
343 Cichero, & Prakash, 2015a, 2015b). Briefly, infant digestion process neglects oral phase  
344 due to liquid meals rapidly transiting through the oral cavity (5-10 sec). Small stomach  
345 storage capacity, affecting meal frequency, transit and volume, increases quickly during  
346 the first month of life from 10-20 mL up to 90-150 mL per meal(Abrahamse, et al., 2012;  
347 Bourlieu, et al., 2014). Infant fasting gastric pH is less acidic than of an adult  
348 (respectively 4-5 vs. 2 in the fasted state) which may change gastric proteolysis, as  
349 optimal activity of pepsin is 1.5-2.2(Henderson, Hamosh, Armand, Mehta, & Hamosh,  
350 1998; Li-Chan & Nakai, 1989; Schlamowitz & Peterson, 1959). Reduced pepsin  
351 secretion in newborns, 10-20% from adult levels, is another physiological reason  
352 explaining the limited gastric proteolysis (15%) reported for infants(Bourlieu, et al.,  
353 2014; Didier Dupont, et al., 2010; Romano, Giosafatto, Masi, & Mariniello, 2015).  
354 Pepsin secretion increases with postnatal age and is more immature in preterm  
355 infants(AC, 1991a).

356 In respect to intestinal digestion, proteolysis in infants has similar pH and trypsin  
357 concentrations as those in the intestine of adults, whereas chymotrypsins and  
358 carboxypeptidases-B just account for about 10% to 60% of the activity found in  
359 adults (Edginton & Fotaki, 2010; Lebenthal & Lee, 1980). Regarding lipid digestion,  
360 gastric lipase activity and output are similar in preterm (Roman, et al., 2007), full-term  
361 infants and adults (Armand, et al., 1996; Sarles, Moreau, & Verger, 1992). However,  
362 pancreatic lipases do vary between infants and adults with pancreatic triglyceride lipase  
363 (PTL) being the dominant intestinal lipolytic enzyme in adults while PTL-related protein  
364 2 and bile salt-stimulated lipase are the key lipases in infants (Lindquist & Hernell, 2010).  
365 In light of the high fat diet of infants (Hamosh, 2006), human breast milk contains  
366 endogenous lipase (bile salt-stimulated lipase mainly, 3.6-5.3 U/mL of milk) that  
367 compensates for the low amount of pancreatic lipases (5-10% the concentration found in  
368 adults) and low concentration of bile salts (50% of adult values) (Lebenthal, Lee, &  
369 Heitlinger, 1983). Regarding carbohydrate digestion, scarce data suggests low values of  
370 pancreatic amylase are found in the GI of infants aged less than 6 months. Thus,  
371 carbohydrate digestion in infants is believed to be highly facilitated by swallowed  
372 salivary  $\alpha$ -amylase (at birth average of 10 % of the adult level but highly  
373 variable (Christian, Edwards, & Weaver, 1999; Sevenhuysen, Holodinsky, & Dawes,  
374 1984)) or mammary  $\alpha$ -amylase. In addition, reports also indicate the infant GI performs  
375 carbohydrate digestion through lactase, sucrose-isomaltase and glucoamylase (with  
376 activities of ~50% above that of adults) (Bourlieu, et al., 2014; T. T. Nguyen, et al.,  
377 2015a).

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

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

400 To date, several studies have depicted static infant IVD models applied for studying  
401 various aspects of protein and lipid digestion. These various models are summarized in  
402 **Table 2**. As can be noted, various discrepancies are found in these models and include  
403 discrepancies in gastric pH, ill-defined enzymatic proteolytic activity of enzymes and  
404 large variance in experimental duration. For example, the enzyme activity was most of  
405 the time not checked experimentally or based on the supplier's general characteristics,  
406 which hampered experiment replication in other laboratory. After an estimation of the  
407 pepsin units per mL of milk, a very large range of values was observed, ranging from 4 to  
408 18563 U/mL of milk (**Table 2**). An *in vivo* study by Armand et al. (1996) reported an  
409 average postprandial value of 63 U/mL of gastric content/kg of bodyweight of preterm  
410 infants, which would correspond to 425 U/mL of milk for a term newborn of 4.25 kg and  
411 a meal to secretion ratio of 63:39 v/v(Armand, et al., 1996). In respect to the intestinal  
412 phase, pH was homogeneous (6.5 -7.5), but duration varied largely from 5 to 120 min and  
413 the meal proportion in the total volume varied from 25 up to 76 %. After an estimation of  
414 pancreatin content within each model, a factor of 30 between the maximum and the  
415 minimum values was found across models, which remains lower than that for pepsin (a  
416 4500 fold difference). Bile salts, arising from a porcine or bovine bile extract or from  
417 purified bile salts, were estimated to vary by a factor of 10 across models.

418 In all models presented in **Table 2**, no clear definition of the infant stage was given,  
419 except for Fogleman et al. (2012)(Fogleman, Cohen, Sakamoto, & Allen, 2012), who  
420 aimed to mimic preterm infant digestion. Further, there are some dynamic IVD models  
421 described in recent literature(Blanquet, et al., 2004; Ménard, et al., 1995; Menard, et al.,  
422 2014; Shani-Levi, et al., 2013) (de Oliveira, et al., 2015; Havenaar, et al., 2013; Roussel

423 C, 2016). In essence, these models try to recreate some of the dynamic aspects of  
424 digestion, e.g. gastric pH profiles post meal ingestion and gastric emptying rates. One of  
425 these has even been validated against *in vivo* data of proteolysis kinetics obtained in  
426 piglets(Menard, et al., 2014). The TIM model developed by TNO (Netherlands) was  
427 adapted to simulate the GI of newborns, infants and toddlers (0–1, 1–6, and 6–24 months  
428 of age, respectively) after ingestion of various types of food (formula milk, milk and  
429 cereals) and validated for these three age groups against published pharmacokinetic data  
430 on paracetamol(Havenaar, et al., 2013). However, in this study, not all GI parameters  
431 applied to this commercial IVD model have been made publicly available. The same  
432 model has been very recently adapted to mimic, based *on in vivo* data, the gastric and  
433 small intestinal conditions of infant from 6 months to 2 years(Roussel C, 2016). Some  
434 dynamic colonic models have also been developed(C Cinquin, Le Blay, Fliss, & Lacroix,  
435 2004; Cécile Cinquin, Le Blay, Fliss, & Lacroix, 2006a, 2006b). The composition and  
436 diversity of the bacterial community, as well as its metabolism, was found to be well  
437 correlated with those found *in vivo* in infant feces.

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

### 445 3.2. Elderly

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

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

### 473 3.3. Developing IVDs for humans with GI disorders

474 In light of the centrality of the GI system in human health and disease, various studies  
475 present information on human GI disorders. These are defined as diseases and/or  
476 conditions that interfere with the intake, digestion, and/or absorption of nutrients, causing  
477 various clinical symptoms and are broadly defined as maldigestion. Physiologically, the  
478 spectrum and underlying causes of GI disorders is immense from such conditions causing  
479 discomfort (e.g. lactose malabsorption) to those compromising health (e.g. pancreatic  
480 insufficiency in cystic fibrosis patients). All in all, these conditions arise from altered GI  
481 functions which lead to various effects on the disintegration, breakdown and uptake of  
482 nutrients and consequently on health (Högenhauer, 2010). Some common factors that  
483 interfere with food digestion and related disorders, infections and surgical procedures  
484 linked to them are summarized in **Table 3**. In respect to food breakdown and  
485 bioaccessibility, many of the situations described may be mirrored using IVD models, as  
486 such conditions have been found to arise from variance and abnormalities in digestive  
487 parameters such as changes in gastric/intestinal pH, secretion of digestive juices and  
488 transit times. Other disorders such as food allergies, autoimmune disorders (celiac sprue),  
489 Crohn's disease, obesity or diabetes are linked to interferences with the absorption and/or  
490 metabolism of nutrients from the food (Nolan, Johnston, & Walters, 2012), hence IVD  
491 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 (Brodie, 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 pancreatic 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  $18.6 \pm 6.9$  min prior to GBP to  $7.9 \pm 2.7$  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 ( $t_{50}$   $3.8 \pm 0.9$  vs.  
522  $4.1 \pm 0.6$  vs.  $3.6 \pm 0.5$  min, respectively) and for a solid beef patty meal ( $1.6 \pm 0.7$  vs.  $1.1 \pm 0.6$   
523 vs.  $1.3 \pm 0.5$  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  $34.9 \pm 24.6$  vs.  $13.6 \pm 11.9$  min for water and  $78 \pm 15.01$  vs.  $38.3 \pm 18.77$  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.

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

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

#### 546 **4. Conclusion**

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

557 As part of the food-health revolution and evolution of food manufacturing towards  
558 tailored and personalized foods, the potential of IVD models could be maximized when  
559 extended to recreate various strata of the human population. The development of IVD  
560 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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579 **Table and Figure Captions**

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

581

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

584

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

586

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

590

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

592

593 **Table and Figure**594 **Table 1**

	<b>Fasted</b>	<b>Fed</b>	<b>Suggested References</b>
<b>Oral phase</b> 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)
<b>Gastric phase</b> 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)
<b>Small intestine</b> 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 <sup>a</sup> , GC <sup>b</sup> , GCDC <sup>c</sup> , GDC <sup>d</sup>  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)
<b>Large intestine</b> pH Bacterial load  Short chain fatty acids Total fluid vol Transit time		6.4-7.0 1x10 <sup>11</sup> -10 <sup>12</sup> CFU/g material 125-139 mM 187 ml 12-24h	Payne A.N. (Payne, et al., 2012)

595 <sup>a</sup>Taurodeoxycholate; <sup>b</sup>Glycocholate; <sup>c</sup>Glycochenodeoxycholate; <sup>d</sup>Glycodeoxycholate

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596 **Table 2**

Refs	Meal	Gastric phase							Intestinal phase					
		pH	Duration (min)	Meal : secretion ratio (v/v)	Lipase content (/mL of meal)	Pepsin <sup>b</sup> content	Pepsin <sup>c</sup> (U/mL of meal)	Molar ratio pepsin / meal	pH	Duration (min)	Meal : secretion ratio (v/v)	Enzyme(s) used <sup>c</sup>	Pancreatin equivalent (mg/mL of meal)	Bile <sup>d</sup> (mg/mL of meal)
Chatterton et al., 2004(Chat terton, Rasmusse n, 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 <sup>t</sup>	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 <sup>t,h</sup>	1.32 <sup>t</sup>
Fogleman et al., 2013(Fogl eman, 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 <sup>g</sup>	0.63	3.75

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Levi, C. S., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet Diot, S., Bourlieu, C., Brodkorb, A., Cilla, A., Deglaire, A., Denis, S., Dupont, D., Heredia, A., Karakaya, S., Giosafatto, C. V. L., Mariniello, L., Martins, C., Ménard, O., El, S. N., Vegarud, G. E., Ulleberg, E., Lesmes, U. (2017). Extending in vitro digestion models to specific human populations:

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 ( <i>Rhizopus Oryzae</i> lipase)	4.5 mg/ml of SGF <sup>e</sup> (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
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Levi, C. S., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet Diot, S., Bourlieu, C., Brodkorb, A., Cilla, A., Deglaire, A., Denis, S., Dupont, D., Heredia, A., Karakaya, S., Giosafatto, C. V. L., Mariniello, L., Martins, C., Ménard, O., El, S. N., Vegarud, G. E., Ulleberg, E., Lesmes, U. (2017). Extending in vitro digestion models to specific human populations:



		pH	Duration (min)	Meal : secretion ratio (v/v)	Lipase content (/mL of meal)	Pepsin <sup>b</sup> content	Pepsin <sup>c</sup> (U/mL of meal)	Molar ratio pepsin / meal	pH	Duration (min)	Meal : secretion ratio (v/v)	Enzyme(s) used <sup>c</sup>	Pancreatin equivalent (mg/mL of meal)	Bile <sup>d</sup> (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 <sup>f</sup>	0.020 0.044	7	5	-	Pancreatin: protein ratio of 1:62.5	0.19 <sup>f</sup>	-
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 <sup>h</sup>	4 <sup>i</sup>

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Levi, C. S., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet Diot, S., Bourlieu, C., Brodkorb, A., Cilla, A., Deglaire, A., Denis, S., Dupont, D., Heredia, A., Karakaya, S., Giosafatto, C. V. L., Mariniello, L., Martins, C., Ménard, O., El, S. N., Vegarud, G. E., Ulleberg, E., Lesmes, U. (2017). Extending in vitro digestion models to specific human populations:

600 **Table 3**

<b>Causes of maldigestion</b>	<b>Related diseases</b>	<b>Impact</b>
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
Impaired bile secretion	Cystic fibrosis, chronic cholestasis	
Increased bile salt loss	Ileal disease or resection	
Bile salt de-conjugation	Bacterial: overgrowth	Bioavailability of specific nutrients
Bacterial consumption of nutrients	Bacterial overgrowth associated to B12 deficiency	
Reduced gastric acid	Atrophic gastritis associated to B12 deficiency	
Reduced intrinsic factor	Pernicious anemia associated to B12 deficiency	
Cofactors deficiency	Gastric surgery	

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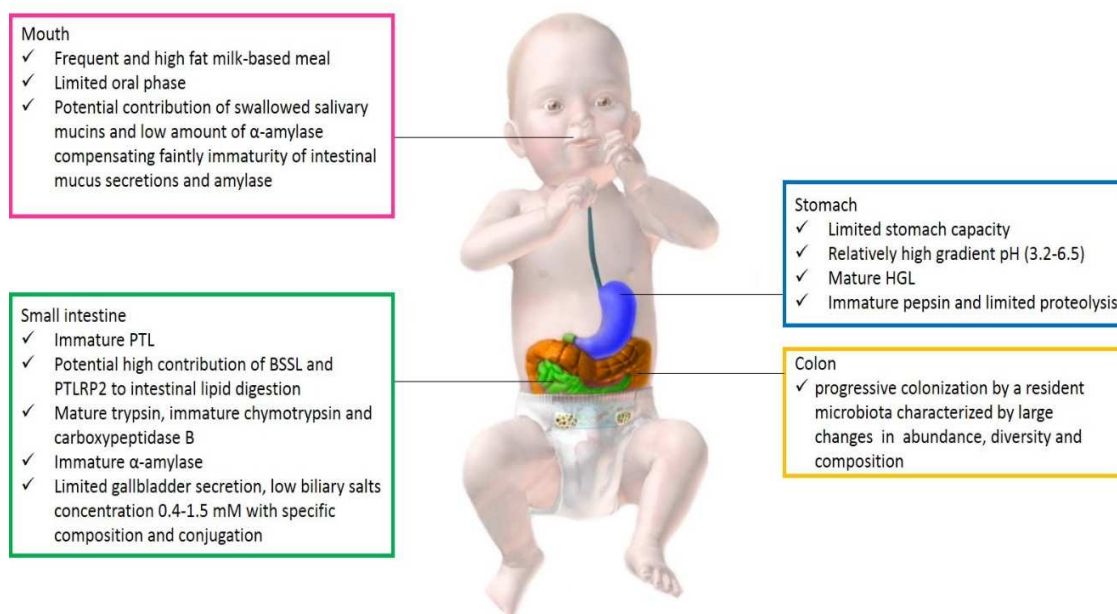
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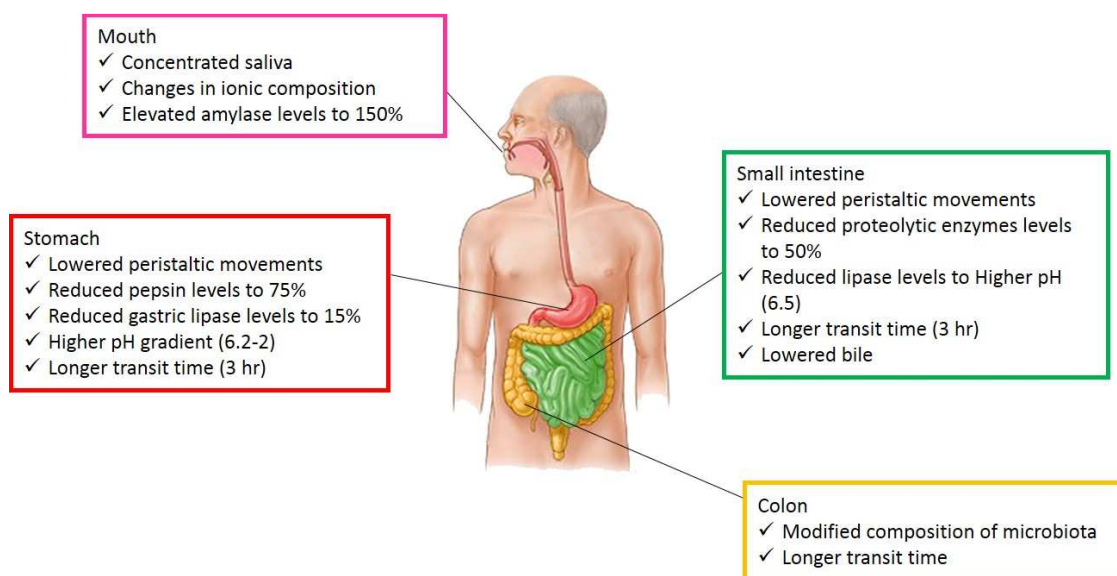
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610 **Figure 1**

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612 **Figure 2**

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615 **5. References**

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ACCEPTED MANUSCRIPT

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

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## Article Highlights

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- 7 • *In vitro* digestion models show great promise in food and nutritional research.  
8 • Paper outlines considerations for development of new *in vitro* digestion models.  
9 • Human studies elucidate the variance of digestive conditions.  
10 • Digestion models of infants, elderly and patients with GI disorders are reviewed.