

A first step towards a consensus static *in vitro* model for simulating full-term infant digestion



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ABSTRACT

In vitro alternatives to clinical trials are used for studying human food digestion. For simulating infant digestion, only a few models, lacking physiological relevance, are available. Thanks to an extensive literature review of the *in vivo* infant digestive conditions, a gastrointestinal static *in vitro* model was developed for infants born at term and aged 28 days. The model was applied to the digestion of a commercial infant formula. Kinetics of digestion, as well as the structural evolution, were compared with those obtained while submitting the same formula to the adult international consensus protocol of *in vitro* static digestion. The kinetics of proteolysis and lipolysis differed according to the physiological stage resulting mainly from the reduced level of enzymes and bile salts, as well as the higher gastric pH in the infant model. This *in vitro* static model of infant digestion is of interest for scientists, food or pharmaceutical manufacturers.

1. Introduction

The study of digestion is difficult to conduct in humans as *in vivo* trials imply technical, ethical and financial constraints. *In vitro* alternatives thus need to be developed for a better understanding of digestive kinetics. *In vitro* models allow the screening of various foods, before conducting *in vivo* animal or human trials on a limited number of food matrices. Whereas an international consensus has recently been found for mimicking digestion at the adult stage with an *in vitro* static model (Minekus et al., 2014), there is no physiologically-relevant harmonized model of *in vitro* static digestion at the infant stage (Shani-Levi et al., 2017). Previous reviews have compiled the physiological data available about infant digestive conditions (Abrahamse et al., 2012; Bourlieu et al., 2014), underlining the immaturity of the infant digestive system compared to the adult one. This immaturity concerns both enzymatic (type of enzymes and level of activity) and non-enzymatic (milk-based diet, frequency of feeding, bile salts concentrations) parameters.

The importance of the early infancy period in terms of nutrition and of preprogramming concept is now widely acknowledged. Using adequate *in vitro* digestion tools is a priority for optimizing infant formula. The quantity of recent publications presenting results of *in vitro* infant digestion is a good illustration of the interest towards this tool, such as reviewed recently (Shani-Levi et al., 2017). Digestion models can be

static (Dupont et al., 2010), semi-dynamic (Amara et al., 2014; Bourlieu et al., 2015) or dynamic (Blanquet et al., 2004; de Oliveira et al., 2016). Focusing on static models, a recent review has highlighted the variability in the parameters used to mimic infant digestion (Shani-Levi et al., 2017). In particular, the enzymatic parameters are scarcely expressed in international units (I.U.) and experimental enzyme characterization is most of the time omitted, which makes the comparison of the results among studies difficult. Thus, a detailed *in vitro* digestion model based on *in vivo* digestive parameters of neonates is needed. In this context, the aim of the present study was to firstly propose an *in vitro* static digestion model for the full-term newborn (28 days of life, gestation age from 38 to 42 wk) with parameters relying on *in vivo* data published in the literature, and secondly to compare this infant model with the adult international consensus model in regards to the digestive kinetics of the same food, *i.e.* a commercial infant formula.

2. Materials & methods

2.1. Meal

A commercial liquid infant formula for infants from birth to 6 months was bought in a local supermarket. The formula was composed of 1.18 g of proteins (ratio casein/whey protein w/w: 30/70) and

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3.45 g of lipids (43% of saturated fatty acids) for 100 ml of formula, as measured by the Kjeldhal method and the acido-butyrometric method, respectively. A content of 7.5 g of carbohydrates for 100 ml of formula was labelled.

2.2. Chemicals and enzymes

Materials were all standard analytical grade. Chemicals, enzymes and bile were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Pepsin (Sigma P6887) and pancreatin (Sigma, P7545 8XUSP) were of porcine origin while bile (Sigma B8631) was of bovine origin. Rabbit gastric extract (RGE) was provided by Lipolytech (Marseille, France). Enzyme activities were determined as described in the Electronic Supplementary Information of Minekus et al. (2014).

2.3. In vitro digestion protocol

2.3.1. Infant model

The infant gastrointestinal static *in vitro* model was set up in order to mimic as close as possible the digestive conditions of full-term infants. Parameters were defined thanks to the extensive literature review of the *in vivo* infant digestive conditions previously conducted within our laboratory (Bourlieu et al., 2014).

The infant food is liquid and its time of residence in the mouth is short. Considering this, the oral phase was omitted, such as suggested for the adult international consensus (Minekus et al., 2014). The static *in vitro* digestion model proposed here included two consecutive steps: a gastric and an intestinal phase. The full-term infant digestive parameters of the present model are summarized in Fig. 1.

2.3.1.1. Gastric phase. Parameters (meal to secretions ratio, pH) were determined based on the infant gastric conditions occurring at the emptying half-time, assumed to be more representative than the final time point. As described by Bourlieu et al. (2014), a gastric emptying half-time of 78 min has been reported for infant formula. The meal to secretions ratio was based on the simulation of secretion flows in the dynamic digestion model DIDGI® validated for infant formula digestion (Ménard et al., 2014), where the mean flow rate of secretions was fixed at 0.53 ml/min. At 78 min after the start of the meal ingestion, the ratio (v/v) of meal to secretions was 63 to 37.

Compilation of data measuring gastric pH in infants allowed the determination of a linear regression describing the gastric acidification curve: $\text{pH} = -0.015 \cdot \text{time (min)} + 6.52$, as previously described (Bourlieu et al., 2014). Considering the gastric emptying half-time of 78 min, gastric pH in the static model was set up at 5.3.

Based on postprandial enzyme activities determined in infant gastric aspirates, average values of 63 U of pepsin and 4.5 U of lipase per ml of gastric content and per kg of body weight of infant (Armand et al., 1996; Roman et al., 2007) were found. Thus, considering the mean body weight of a one-month old infant of 4.25 kg (de Oliveira et al., 2016), enzyme activities were set up at 268 U/ml of gastric content for pepsin and 19 U/ml of gastric content for lipase. Pepsin and gastric lipase were added as rabbit gastric extract (RGE). Rabbit gastric lipase presents 85% of sequence homology as compared to the human one (Roussel et al., 1999). The added amount of RGE covered 100% of the pepsin activity and 110% of the lipase activity required (21 U/ml).

Gastric fluid composition was based on a study on 30 full-term infants reported by Hyde (1968). The simulated gastric fluid (SGF) was composed of sodium chloride and potassium chloride fixed at 94 and 13 mM, respectively, and adjusted to pH 5.3 with HCl 1 M. After 60 min of gastric digestion, the pH was increased to 7 by addition of NaOH 1 M in order to stop gastric enzyme activities before further intestinal digestion.

2.3.1.2. Intestinal phase. The previous vial containing the 60 min gastric phase was adjusted to the intestinal pH of 6.6 using HCl 1 M. As for the gastric phase, the meal to secretions ratio for the intestinal phase was determined at 78 min of digestion using the simulation of secretion flows (bile, pancreatin and sodium bicarbonate) in the dynamic digestion model DIDGI® (Ménard et al., 2014), where the overall mean secretion flow rate was 0.85 ml/min. Thus, at 78 min of digestion, the ratio (v/v) meal to total secretion (gastric and intestinal) for the intestinal phase was 39 to 61. More precisely, the total volume of the intestinal phase was composed of 39% of meal, 23% of gastric secretion and 38% of intestinal secretion.

The simulated intestinal fluid (SIF), based on the characterization of duodenal fluid of 1-week-old full-term infants (Zoppi, Andreotti, Pajno-Ferrara, Bellini, & Gaburro, 1973), was composed of 164 mM of sodium chloride, 10 mM of potassium chloride and 85 mM of sodium bicarbonate and adjusted at pH 7. Calcium chloride was added separately before the beginning of the intestinal phase at a concentration of 3 mM within the volume of the intestinal fluid (Zoppi et al., 1973). Bovine bile extract was added to a final content of 3.1 mM of bile salts, which corresponds to the average postprandial value determined in duodenal aspirates of eight 2-week-old infants (Signer, Murphy, Edkins, & Anderson, 1974).

The added amount of pancreatin covered the intestinal lipase activity required of 90 U/ml of intestinal content (Norman, Strandvik, & Ojamae, 1972) and the trypsin activity needed i.e. 16 U/ml of intestinal content, which was consistent with previously reviewed *in*

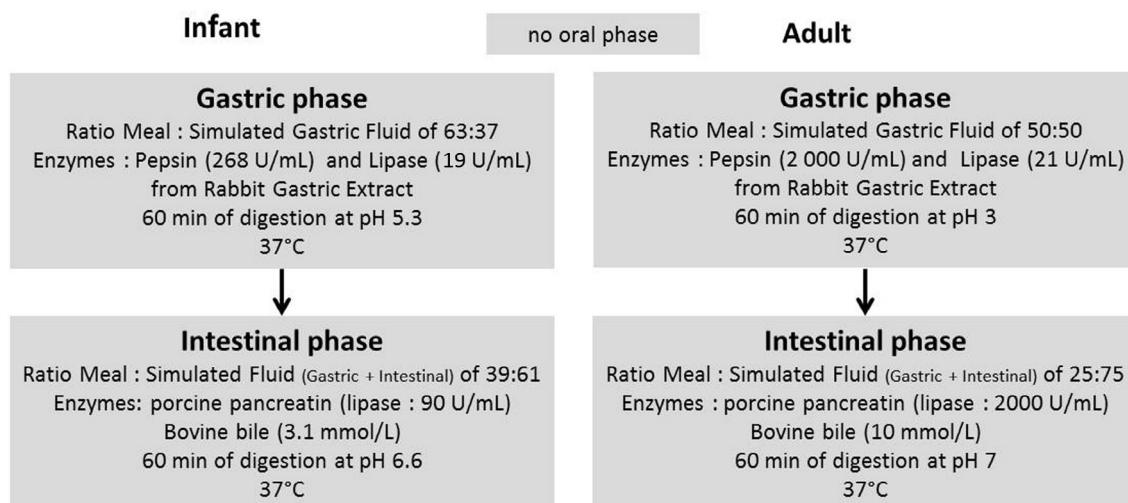


Fig. 1. Infant and adult digestive conditions used in the static *in vitro* digestion models.

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